JCB Journal of Cell Biology

SPOTLIGHT

A new probe illuminates endo-lysosomal Ca^{2+} measurements: A role for vesicular IP_3 receptors?

Anthony J. Morgan[1]

In this issue, Calvo et al. (https://doi.org/10.1083/jcb.202410094) report a new bioluminescent Ca^{2+} probe (ELGA) targeted to acidic endo-lysosomes (ELs) to permit selective and dynamic recording of endo-lysosomal Ca^{2+} uptake and release. Ca^{2+} was not only released by canonical EL channels but, surprisingly, by IP_3 receptors.

Signal transduction pathways are constantly evolving into ever more complex pathways that intersect with one another, and one modality—that of Ca^{2+} signalling—is no exception. Having established ER-mediated Ca^{2+} release and Ca^{2+} entry across the plasma membrane as two dominant sources of Ca^{2+} signals, other internal organelles have gradually been welcomed into the Ca^{2+} network, either as Ca^{2+} sources or as Ca^{2+} sinks (or both). These newer players include mitochondria, peroxisomes, the Golgi, and endo-lysosomes (ELs) (as well as secretory vesicles) (1). In spite of their smaller physical size, these other Ca^{2+}-signalling organelles exert potent effects on cell biology and manifestly "punch above their weight".

Understanding when (and how) different Ca^{2+} sources are recruited and how they control Ca^{2+} fluxes across their membranes is a difficult enough task when in isolation, but the system is rendered all the more complex by the fact that these Ca^{2+} sources intersect or overlap with one another in two ways: (a) they can be active at the same time (so that cytosolic Ca^{2+} signals may be a complex summation of multiple Ca^{2+} stores); (b) Ca^{2+} released from one organelle can be transferred to a different neighboring organelle (particularly at membrane contact sites) to either "fill" the recipient compartment or to trigger yet more Ca^{2+} release from it (via Ca^{2+}-induced Ca^{2+} release). Tracking the ins and outs of Ca^{2+} within each compartment requires organelle-specific Ca^{2+} reporters targeted to their respective lumina, so that the dynamic changes in their Ca^{2+} content are optically isolated from their neighbors' content. For the ER and mitochondria, luminal reporters (fluorescent or luminescent) have been successfully optimized and exploited for decades and have unambiguously illuminated how complex organellar Ca^{2+} release/transfer events occur in (patho)-physiological conditions.

However, ELs have proven the *enfants terribles* among organelles because of their acidic and proteolytic lumina: low pH can crush a reporter's chromophore, or the Ca^{2+}-binding motif (or both), whereas proteolysis of genetically encoded Ca^{2+} indicators (GECIs) can affect targeting and activity. Given the dynamic interactions of ELs with sundry other compartments, the ability to explicitly isolate endo-lysosomal Ca^{2+} signals from those of other sources has been an imperative since the inception of the field. Although chemical reporters have reported Ca^{2+} fluxes in acidic vesicles (2), GECIs have had less success (with a few exceptions). Therefore, the recent paper led by the Alonso group excitingly showcases a new reporter for endo-lysosomal Ca^{2+}.

Calvo et al. (3) targeted a novel Ca^{2+} reporter to an endo-lysosomal sub-compartment by its fusion to the luminal tail of VAMP-7, a marker of late endosomes (and secretory vesicles). Instead of a calmodulin-based, purely fluorescent GECI, they used a bioluminescent reporter, the Ca^{2+}-binding aequorin protein fused to a GFP variant, which they named endo-lysosomal GFP-aequorin (ELGA). The new probe satisfied all the fundamental criteria of appropriate Ca^{2+} affinity, selective localization to the endo-lysosomal system, and a relative insensitivity to pH down to 6.0, which is crucial. The endo-lysosomal continuum of vesicles exhibits luminal pH varying from 6.5 to 4.5, so the new probe cannot, unfortunately, record from the most acidic lysosomal compartments, but it does report Ca^{2+} in a less acidic subpopulation. Hereafter, the authors referred to this ELGA-labelled compartment as "ELs," although their identity is slightly unclear when the markers they used are predominantly late-endosome/lysosome.

That aside, calibrating the ELGA signals in three different cell culture models confirmed that the resting $[Ca^{2+}]$ of these ELs was ~300–400 µM, in line with previous estimates of the lysosomal or ER $[Ca^{2+}]$ and ample to act as a *bona fide* Ca^{2+} store. As would be expected, known agonists of endo-lysosomal Ca^{2+}-permeable channels elicit sizeable Ca^{2+} release from the ELGA compartments. A huge and unique advantage of a luminal Ca^{2+} probe is that it can be used in permeabilized cells without the reporter (or Ca^{2+}) being diluted into the medium. Consequently, cell-impermeant agonists can be used, and NAADP, a dinucleotide messenger that activates TPC channels, evoked a robust Ca^{2+} release in permeabilized cells. This is remarkably one of the few examples where NAADP responses (notoriously fragile) have been preserved in permeabilized

[1]Department of Pharmacology, University of Oxford, Oxford, UK.

Correspondence to Anthony J. Morgan: anthony.morgan@pharm.ox.ac.uk.

mammalian cells. Similarly, agonists of TRPML1 channels (ML-SA1, or the lipid, $PI(3,5)P_2$) also evoked Ca^{2+} release from these vesicles but not (crucially) from the ER (as measured in parallel with an analogous ER probe). This reinforces that ELGA responses need not be due to ER signal contamination and that ELGA can report a different, non-ER compartment. This result is key for what follows below.

How mammalian ELs fill with Ca^{2+} is uncertain, and although the H^+ gradient and/or novel Ca^{2+}-transporters have been implicated, inhibition of the V-H^+-ATPase pump with bafilomycin A1 did not prevent Ca^{2+} refilling as measured by ELGA (suggesting that Ca^{2+}/H^+ exchange is not required). In contrast, the surprise was that endo-lysosomal Ca^{2+} refilling (at least in permeabilized cells) was not only ATP-dependent but also sensitive to inhibitors of the ER Ca^{2+} pumps (SERCA). One explanation could be that the ER transfers its Ca^{2+} to ELs (4) and that the SERCA inhibition is an indirect consequence of depleting the "refilling station". However, the authors could discount this explanation because inter-organelle Ca^{2+} transfer should be blocked with a fast Ca^{2+} chelator, but it did not affect endo-lysosomal refilling. This raises the possibility that SERCA is also present on acidic vesicles to fill them directly, and the authors showed a small degree of colocalization of SERCA and the endo-lysosomal marker LAMP2. This surprising conclusion is at odds with some lysosomal data in the literature, so perhaps the Ca^{2+} handling of this ELGA-labelled compartment differs from that of the later, more acidic lysosomes.

Another unexpected result was that these ELs appear to be decorated with functional IP_3 receptors (IP_3Rs), the canonical Ca^{2+} channels of the ER. ELGA measurements revealed robust Ca^{2+} release in response to IP_3 that was abolished in IP_3R-null cells. Using super-resolution imaging, endogenous IP_3Rs were suggested to have a small degree of overlap with different endo-lysosomal markers (TRPML1, TPC1, LAMP1, and LysoTracker) and with an ELGA variant. The idea that acidic vesicles contain IP_3Rs has some precedents (particularly in secretory vesicles), but the notion of their being on a sub-compartment of ELs is a daring suggestion; if this holds up, then IP_3-mediated Ca^{2+} release from non-ER (and non-Golgi) stores could represent a new paradigm in Ca^{2+} signalling.

In summary, the new endo-lysosomal ELGA probe is a new genetic Ca^{2+} reporter with modest pH insensitivity that can label and record Ca^{2+} fluxes in a sub-compartment of acidic vesicles. This compartment reassuringly bears many of the hallmarks of classical endo-lysosomal pharmacology and markers (e.g., responding to endo-lysosomal channel activation), but it surprisingly shares some of these features with the ER (i.e., IP_3Rs and SERCA). Although the skeptical may express understandable concerns about the ER contaminating the ELGA signals, the authors made strenuous efforts to discount this, and many data are compelling that this is not the case. How SERCA and IP_3Rs might traffic to ELs is an open question, but an important one to answer to bolster this unusual model. Will acidic-vesicle IP_3 signalling be a new avenue of research?

Acknowledgments

Author contributions: Anthony J. Morgan: conceptualization and writing — original draft.

Disclosures: The author has completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.

References

1. Peggion, C., et al. 2023. *Cell Calcium*. https://doi.org/10.1016/j.ceca.2023.102783
2. Narayanaswamy, N., et al. 2019. *Nat. Methods*. https://doi.org/10.1038/s41592-018-0232-7
3. Calvo, B., et al. 2025. *J. Cell Biol.* https://doi.org/10.1083/jcb.202410094
4. Garrity, A.G., et al. 2016. *Elife*. https://doi.org/10.7554/eLife.15887