Components of Calcium Signaling in Autophagy

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BACKGROUND

- Autophagy is a process that degrades unwanted cellular components to maintain homeostasis. It is upregulated under many conditions, notably cellular nutrient depletion, or "starvation" (Fig. 1).

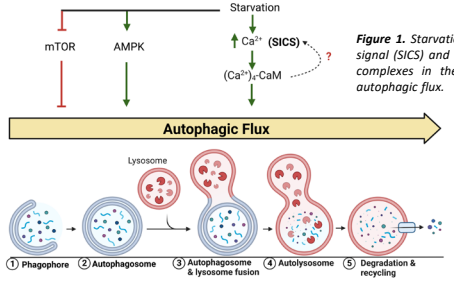

Figure 1. *Starvation-induced Ca^{2+} signal (SICS) and Ca^{2+}-calmodulin complexes in the regulation of autophagic flux.*

- Starvation, mimicked experimentally by removal of extracellular amino acids, triggers an increase in intracellular Ca^{2+} (SICS, *s*tarvation-*i*nduced *C*a^{2+} *s*ignal) that is predicted to initiate many Ca^{2+}-dependent activities of autophagy. However, the components and mechanisms of SICS are unknown.
- Calmodulin (CaM) is the ubiquitous transducer of intracellular Ca^{2+} signals. We recently showed that CaM is critical for the regulation of autophagy under basal and starved condition (*Giles et al., FEBS J 2022, doi: 10.1111/FEBS.16432*). However, it is unknown if CaM itself regulates SICS.
- The lysosome is the location for cargo degradation and is also a Ca^{2+} storage site, in addition to the ER as the main Ca^{2+} store. The *t*ransient *r*eceptor *p*otential *m*ucolipin *1* (TRPML1) is an important Ca^{2+} release channels on the lysosome. However, how Ca^{2+} release via the TRPML1 participates in autophagy and general Ca^{2+} signaling is unknown.

HYPOTHESIS

We hypothesized that CaM directly regulates SICS and the TRPML1-mediated Ca^{2+} signal.

METHODS & RESULTS

Starvation-Induced Ca^{2+} Signal and Effect of CaM buffering

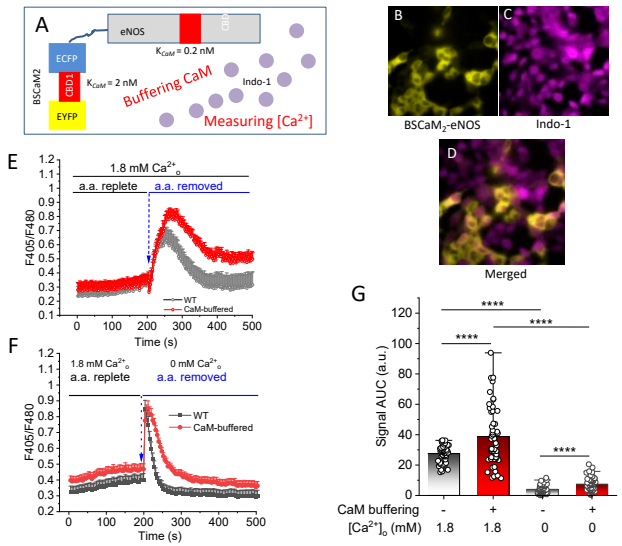

Figure 2. Components of Starvation-Induced Ca^{2+} Signal and Effects of CaM buffering.
A*, Paradigm for molecular buffering of CaM and for measurement of starvation-induced Ca^{2+} signal in the same cells.* ***B-D****, Fluorescent images of BSCaM$_2$-eNOS (B), indo-1 (C), and merged image (D).* ***E & F****, Average Ca^{2+} response time courses in WT and CaM-buffered cells before and after removal of amino acids in equimolar Ca^{2+} medium (E) or absence of extracellular Ca^{2+} (F). 100 μM L-NAME was present throughout.* ***G****, Areas under the curve of the Ca^{2+} signals in WT vs CaM-buffered cells from E and F, respectively. n = 45 – 51 cells for each type from 4 independent experiments; ****, p<0.0001, two-sample t test.*

Effects of lysosomal TRPML1 activation on intracellular Ca^{2+} in normal and CaM-buffered condition

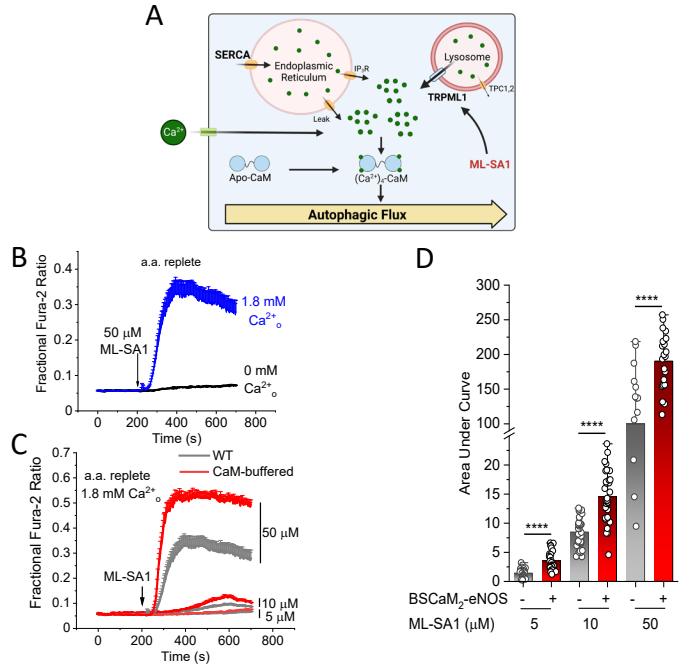

Figure 3. Effects of TRPML1 activation on intracellular Ca^{2+} in normal and CaM-buffered conditions.
A*, Potential sources of Ca^{2+} involved in response to starvation. SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; TPC1/2, two-pore Ca^{2+} channel 1, 2; ML-SA1, TRPML1 agonist.* ***B****, Average time courses of WT HEK293 cells in amino acid-replete medium containing 100 μM L-NAME were treated with ML-SA1 in the presence or absence of extracellular Ca^{2+}. WT and CaM-buffered cells were identified as in Fig. 2B-D.*
C*, Average time courses of intracellular Ca^{2+} simultaneously measured in co-cultured WT (gray) and CaM-buffered (red) HEK293 cells treated with the specified ML-SA1 doses in amino acid-replete, Ca^{2+}-containing media.* ***D****, AUC values of the responses from corresponding groups of cells in C. n = 21 – 33 cells for each ML-SA1 dose. ****, p < 0.0001, two-sample t test.*

Relation between ER and TRPML1-releasable Ca^{2+} pools

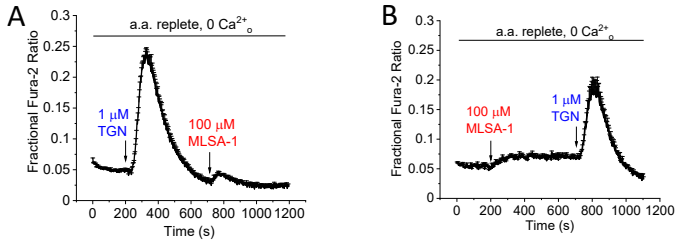

Figure 4. Comparison of Ca^{2+} released by TRPML1 activation before and after ER Ca^{2+} depletion. *A-B. Average intracellular Ca^{2+} time courses of HEK293 cells treated in a.a.-replete, Ca^{2+}-free medium with the specified doses of SERCA inhibitor thapsigargin (TGN) or TRPML1 agonist ML-SA1 in the specified orders. Note the differences in shape and amplitude of the TGN- and ML-SA1-induced signals in A & B. n = 20 – 22 cells.*

Effects of graded CaM buffering in TRPML1-mediated Ca^{2+} release

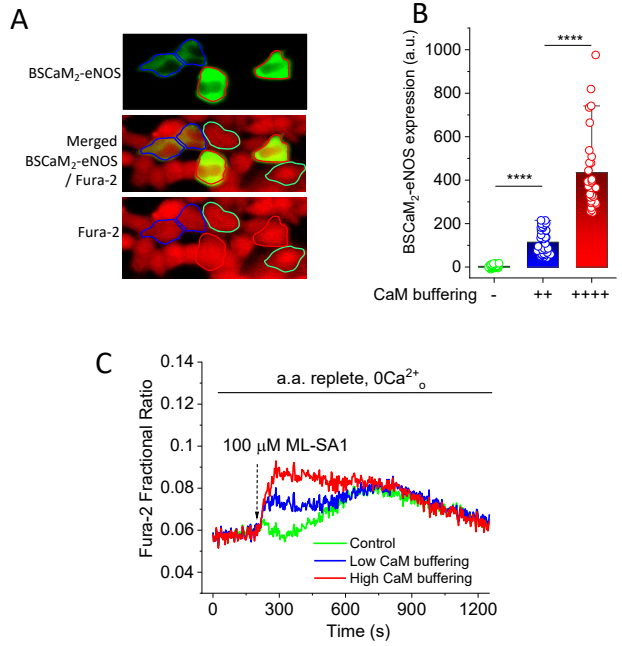

Figure 5. Effects of graded CaM buffering on TRPML1-mediated Ca^{2+} release from lysosomes.
Mixed WT and CaM-buffered cells were loaded with fura-2/AM, followed by TRPML1 activation with 100 μM ML-SA1 in a.a.-replete, Ca^{2+}-free medium containing 100 μM L-NAME. ***A-C****, Post-imaging analysis to identify graded CaM buffering and its effect on ML-SA1-induced Ca^{2+} release. Equal numbers of cells with low and high CaM buffering and non-buffering were visually identified using fluorescence of BSCaM$_2$-eNOS (A, upper panel) and fura-2 (A, middle/low panels), respectively. Recorded BSCaM$_2$ fluorescence intensities of the selected groups were subjected to statistical analysis to first confirm graded buffering (B) and then contrasted with the corresponding average time courses of Ca^{2+} release in response to ML-SA1 (C). Note the increasing in the ML-SA1-stimulated Ca^{2+} release signals as CaM buffering increases. n = 13 - 15 cells in each group; ****, p < 0.0001.*

SUMMARY & CONCLUSIONS

- Starvation-induced Ca^{2+} signal (SICS) consists of both organellar Ca^{2+} release and extracellular Ca^{2+} entry. Molecular buffering of CaM is associated with increases in both components.
- Activation of TRPML1 triggers a small Ca^{2+} release signal and a large Ca^{2+} entry signal that are higher with CaM-buffered cells at all doses tested. The mechanism of this Ca^{2+} entry signal is unclear.
- There is connection between the ER Ca^{2+} pool and the lysosomal Ca^{2+} pool releasable via TRPML1.
- Increasing buffering of CaM is associated with increases in lysosomal Ca^{2+} release via TRPML1, suggesting that CaM may have an inhibitory effect on TRPML1.
- The mechanisms whereby acute starvation triggers SICS components and whereby CaM regulates TRPML1 are under investigation.

ACKNOWLEDGMENTS

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