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Investigating the interplay between PIKfyve and ClC-7 in lysosomal acidification and trafficking.

Xavier Leray, Anowarul Amin, Mary Weston, Joseph Mindell

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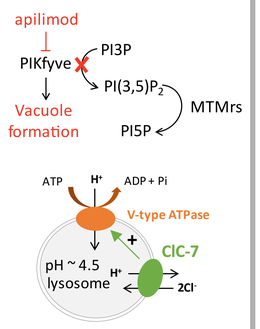
PIKfyve is a lipid kinase found in the membrane of late endosomes and lysosomes. It is responsible for the synthesis of the phospholipid PI(3,5)P2. PIKfyve inhibition quickly and drastically impairs late endosomal/lysosomal formation, leading to the generation of big vacuoles. Recently, knocking out lysosomal Cl^-/H^+ exchanger ClC-7 has been proposed to provide substantial resistance against this vacuole phenotype. Given that ClC-7 is suggested to play a role in lysosomal acidification, it raises the possibility that PIKfyve may regulate ClC-7 activity, in turn modulating lysosomal pH, and that endosomal/lysosomal pH could tune endocytic trafficking.
In this new study, we are investigating the interplay between PIKfyve and ClC-7 in lysosomal acidification and vacuole formation.

Open Questions

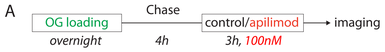
Is lysosomal/vacuolar pH altered under PIKfyve inhibition?
Is ClC-7 critical for this process?
Is lysosomal pH critical for vacuole formation?



1) PIKfyve inhibition leads to hyperacidic lysosomes

Apilimod is a PIKfyve inhibitor.
OG is a pH-sensitive ratiometric dye.

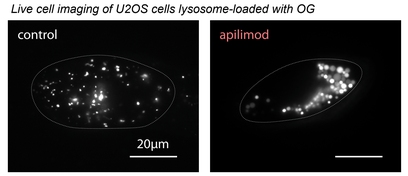
A



For method explanations, see the bottom left section of this poster.

First, Oregon Green 488 dextran (OG) is loaded into the endocytic pathway by fluid phase endocytosis. Then, OG is washed out from the media and a 4h incubation period let OG-containing endosomes to mature and eventually fuse with lysosomes.
Finally cells are incubated for 3h with the PIKfyve inhibitor apilimod before performing pH-ratiometric live cell imaging with an epifluorescence microscope.

B *Live cell imaging of U2OS cells lysosome-loaded with OG*



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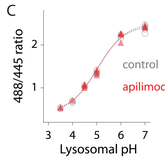
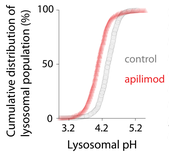


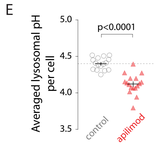
(C) Representative experiment. pH calibration curves for control and apilimod treated cells are similar. This control experiment indicates that pH measurements is not altered when done on big vacuoles instead of small lysosomes. pH measured in both conditions can thus be compared without bias.

D

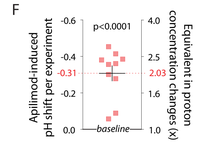


(D) Representative experiment. Each symbol represents the proportion of lysosomes having a pH value below the pH value represented in the abscissa axis. It first confirms that OG-containing vesicles in control condition are acidic endolysosomal compartments. It also indicates that hyperacidification is general and not restricted to a subset group, as shown by the global pH shift and shape conservation of the curve for apilimod condition compared to control.

E



F



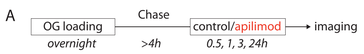
(E) Representative experiment. Each dot represents the averaged lysosomal pH of one cell.
(F) Each dot represents the apilimod-induced lysosomal pH shift measured in one experiment.

2) Does lysosomal pH alteration correlate with their increase in size?

Apilimod and WX8 are PIKfyve inhibitors.

Timecourse effect of apilimod on lysosomal pH of U2OS cells (representative experiment).

A



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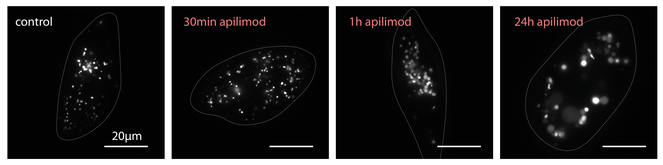

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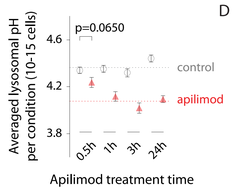

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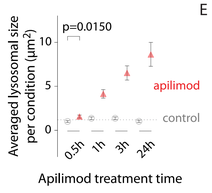

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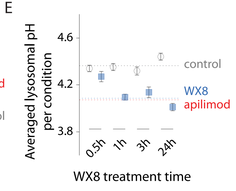


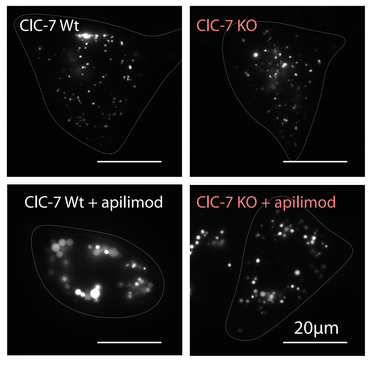
Lysosomal hyperacidification and increase in size does not correlate.

3) Is ClC-7 involved in hyperacidification?

A

B

C



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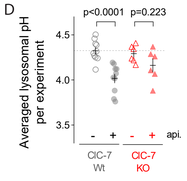

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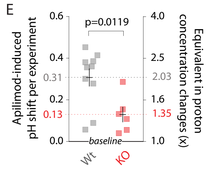
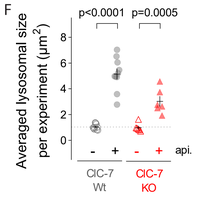


(D) Each dot represents average of cells lysosomal pH in one experiment.
(E) Each dot represents the apilimod-induced lysosomal pH shift measured in one experiment.
(F) Each dot represents average of cells lysosomal size in one experiment.

F



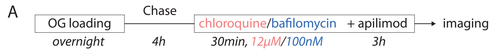
ClC-7
- is required for lysosomal hyperacidification
- participates but is not required for vacuolization.

4) Is lysosomal hyperacidification involved in vacuole formation?

Chloroquine is a lysosomotrophic agent. Bafilomycin-A1 is a v-ATPase inhibitor.

Effect of lysosomes pre-alkilinization on apilimod treatment.

A



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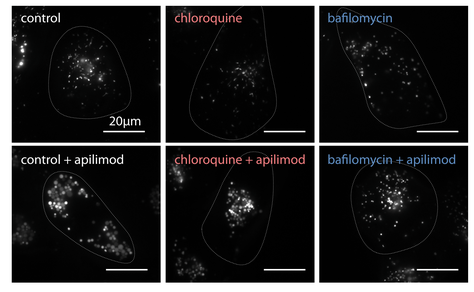

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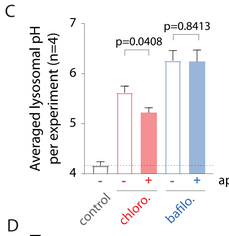

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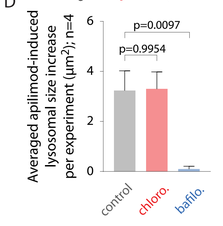
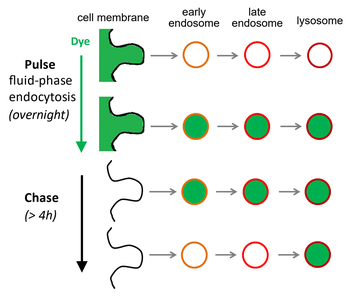


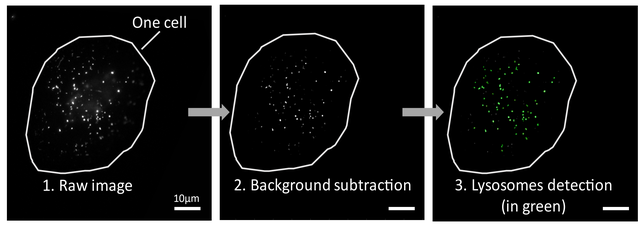
Lysosomal hyperacidification is not required for vacuolization.

How to "dye-load" lysosomes



How to extract lysosomal fluorescence

We use the software Slicer2D to detect fluorescent lysosomes and extract through image processing their size and fluorescent intensity at 488nm and 445nm.

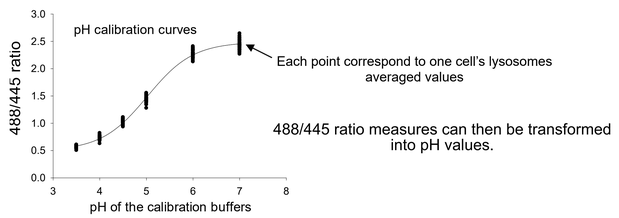


For each cell, the 488/445 ratio of each individual fluorescent lysosome is measured.

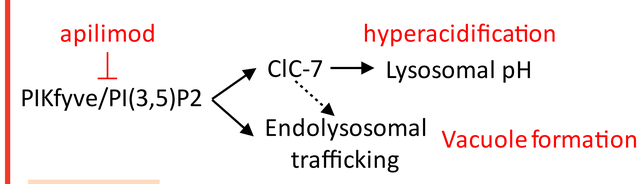
How to measure lysosomal pH

Oregon Green 488 Dextran is a pH ratiometric fluorescent dye.
The 488nm/445nm ratio will predictibly be affected by pH.

Monensin and Nigericin are ionophores that equilibrates pH between media, cytoplasm and lysosomes. Measuring 488/445 lysosomal ratio in pH calibrated buffers in presence of Monensin and Nigericin generates a pH calibration curves.



Model suported by our results



Conclusions

PIKfyve might regulate lysosomal pH through inhibition of ClC-7 activity. ClC-7 activity, but not lysosomal pH, might participate in endolysosomal trafficking.