Cell culture, transfection and imaging

Authors: Marianna Leonzino[1], Andrés Guillén-Samander[1] and Pietro De Camilli[1]

[1]Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA

[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

Protocols for general preparation of cells for imaging and also for imaging experiments involving cellular hypotonic shock and cytosolic Ca^{2+} changes as performed in https://doi.org/10.1083/jcb.202010004

A. General preparation

1. COS-7 or HeLa (ATCC) cells were cultured at 37ºC and 5% CO2 in DMEM containing 10%FBS, 1mM sodium pyruvate, 100U/ml penicillin, 100mg/mL streptomycin and 2mM L-glutamine (all from Gibco).

Note: For general maintenance, when cells reached 80-90% confluency, they were deattached from the dish with Trypsin and diluted 1:20 in a new dish.

2. For imaging experiments, cells were seeded on glass-bottomed dishes (MatTek) at a concentration of $75x10^3$ cells per dish and transiently transfected after 6h using FuGene HD (Promega).
3. Cells were imaged 36-48 hours after transfection.
4. Just before imaging, the growth medium was removed and replaced with pre-warmed live-cell imaging solution (Life Technologies).
5. All live-cell imaging was performed at 37°C and 5% CO_2.
6. Spinning-disk confocal microscopy was performed using an Andor Dragonfly system equipped with a plan apochromat objective (63×, 1.4 NA, oil) and a Zyla scientific CMOS camera.
7. Cells to be imaged were identified by scanning the dish.

B. Hypotonic shock experiments

1. Once a field of view with transfected cells is found, acquisition is started (generally a rate of 12 frames/minutes was used).
2. Live-cell imaging solution was replaced with pre-warmed distilled water

Suggestion: Minimal volume of live cell imaging media is placed in the dish to allow removal with a pipette in one single step. On the contrary, maximal volume of distilled water is added to allow the highest dilution of leftover ions in the dish.

Warning: If media exchange is to be performed by hand, extreme care should be taken not to move the dish during the process. If substantial amount of live cell imaging media needs to be left in the dish to prevent movements during the aspiration procedure, a further media replacement step could be implemented to achieve maximal hypotonic shock.

C. Experiments evaluating cytosolic Ca^{2+} changes

Cytosolic Ca^{2+} was monitored by the intensity of the RFP genetically encoded Ca^{2+} indicator for optical imaging (R-GECO; plasmid was a gift from R. Campbell, University of Alberta, Edmonton, AB, Canada; Addgene catalog no. 45494)

1. Once a field of view with transfected cells is found, acquisition is started (generally a rate of 0.5 Hz was used)
2. To acutely increase cytosolic Ca^{2+}, Thapsigargin (Life Technologies) was added to a final concentration of 2 µM.
   Note: Live-cell imaging solution (Life Technologies) contains 1.8 mM Ca^{2+}
3. Cells were allowed to recover for 10 minutes.
4. To decrease cytosolic Ca^{2+}, EGTA and BAPTA-AM (Thermo Fisher Scientific) were added to the medium to a final concentration of 4 mM and 10 µM, respectively.
5. Cells were imaged for 10 more minutes, then acquisition was stopped.