Protocol

Photobleaching Regions of Living Cells to Monitor Membrane Traffic

Erik Lee Snapp and Patrick Lajoie

Eukaryotic cells are composed of an intricate system of internal membranes that are organized into different compartments—including the endoplasmic reticulum (ER), the nuclear envelope, the Golgi complex (GC), lysosomes, endosomes, caveolae, mitochondria, and peroxisomes—that perform specialized tasks within the cell. The localization and dynamics of intracellular compartments are now being studied in living cells because of the availability of green fluorescent protein (GFP)-fusion proteins and recent advances in fluorescent microscope imaging systems. This protocol outlines two methods for photobleaching living cells to monitor membrane traffic. The first method involves selective photobleaching using a confocal laser-scanning microscope (CLSM) that can bleach discrete selected regions of interest. As outlined in the second method, photobleaching can also be performed with older CLSMs that lack the capacity for selective photobleaching. In this case, photobleaching is accomplished by zooming into a small region of the cell and scanning with full laser power.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPE: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Cells expressing the desired GFP-fusion protein(s)

Imaging medium <R>

Equipment

CLSM

It is assumed that the investigator is familiar with the basic operation of a CLSM. A detailed discussion of the instrumentation and the imaging chamber can be found in ***Imaging of Membrane Systems and Membrane Traffic in Living Cells*** *(Snapp and Lajoie 2011a).*

Temperature control hardware

METHOD

1. Follow the imaging protocol described in **Time-Lapse Imaging of Membrane Traffic in Living Cells** (Snapp and Lajoie 2011b). Identify the cell of interest on the CLSM, and bring it to the

Adapted from *Live Cell Imaging*, 2nd edition (ed. Goldman et al.). CSHL Press, Cold Spring Harbor, NY, USA, 2010.

Cite this article as *Cold Spring Harbor Protoc*; 2011; doi:10.1101/pdb.prot066563

desired focus. Scan an image of the whole cell at the desired excitation light intensity, line averaging, zoom, etc. Modify the pinhole and detector gain for maximal fluorescence signal with no pixel saturation.

For selective photobleaching, follow Step 2. For photobleaching using an older CLSM, follow Steps 3–5.

Selective Photobleaching

2. Define a region of interest (ROI) for the photobleach, and empirically determine photobleaching conditions (i.e., scan speed, zoom, laser power, microscope objective, and the minimal number of laser iterations required for photobleaching) so that after photobleaching, the fluorescent signal of the photobleached ROI decreases to within background intensity levels.

   Typical bleaching conditions require a 100–1000-fold increase in laser power (decrease in attenuation) for 1–10 bleach iterations (roughly 0.1–2 sec) for many organelles. Suggested conditions for photobleaching enhanced GFP (EGFP) or enhanced yellow fluorescent protein (EYFP) with a 40-mW 488/514-nm argon or 25-mW argon laser are 45%–60% power with 100% transmission. Red fluorescent proteins (RFPs) can be photobleached with 100% transmission of a 543- or 561-nm laser. Enhanced cyan fluorescent protein (ECFP) variants can be photobleached with a 405- or 413-nm laser line. However, intense ultraviolet (UV) light can be phototoxic to cells, and UV photobleaching is not recommended.

Photobleaching Using an Older CLSM

3. Zoom into the smallest possible area at the highest zoom possible (usually zoom 8–32, depending on the microscope). At high zooms, the laser dwells longer on an ROI per line scan and, thus, will deliver more bleaching radiation.
4. Set the laser power at maximum, and remove all neutral-density filters from the path of the laser beam.
5. Scan (photobleach) the zoomed ROI.

RELATED INFORMATION

A protocol is also available for **Activating Photoactivatable Proteins with Laser Light to Visualize Membrane Systems and Membrane Traffic in Living Cells** (Snapp and Lajoie 2011c).

RECIPE

Imaging Medium

Phenol-red-free cell growth medium (e.g., RPMI or DMEM)
Fetal bovine serum (10%)
Glutamine (2 mM)
HEPES (25 mM, pH 7.4)

REFERENCES

Snapp EL, Lajoie P. 2011a. Imaging of membrane systems and membrane traffic in living cells. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top066548.

Snapp EL, Lajoie P. 2011b. Time-lapse imaging of membrane traffic in living cells. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot066555.

Snapp EL, Lajoie P. 2011c. Activating photoactivatable proteins with laser light to visualize membrane systems and membrane traffic in living cells. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot066571.