Protocol

Activating Photoactivatable Proteins with Laser Light to Visualize Membrane Systems and Membrane Traffic in Living Cells

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, such as the confocal laser-scanning microscope (CLSM). This protocol describes the steps for activating one of the first photoactivatable proteins, PA-GFP.

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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, unactivated and stably or transiently transfected with PA-GFP or similar protein(s)

PA-GFP was first described by Patterson and Lippincott-Schwartz (2002).

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). A protocol is also available for* ***Time-Lapse Imaging of Membrane Traffic in Living Cells*** *(Snapp and Lajoie 2011b).*

METHOD

1. In stably or transiently transfected unactivated cells, excite the unactivated protein with low-intensity laser light (i.e., 1% transmission 413-nm Enterprise II ion laser or 5 μW) from a 405

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

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Cite this article as *Cold Spring Harbor Protoc*; 2011; doi:10.1101/pdb.prot066571

or 413-nm laser and alternately excited with low-intensity 488-nm laser excitation (i.e., 1 μW or 1% transmission of a 40-mW argon laser) and visualized with a long-pass 505-nm filter for both excitation tracks. Fluorescence should be at background levels with 488-nm excitation.

2. Photoactivate the cell or region of interest (ROI) by rapidly irradiating it with high-intensity ultraviolet (UV) laser light (i.e., 1 mW of 413-nm laser light) as if performing a photobleaching experiment (see **Photobleaching Regions of Living Cells to Monitor Membrane Traffic** [Snapp and Lajoie 2011c]).

   The number of photoactivating iterations and the amount of laser transmission must be determined empirically for each microscope system. A typical protocol is one photobleach iteration of 5% transmission of 413-nm laser light at scan speed 8 and zoom 3 using a 63× oil numerical aperture (NA) 1.4 objective on a Zeiss 510 microscope.

3. Excite the sample with the 488-nm laser. After photoactivation, there should be a 10- to 100-fold increase in fluorescence.

   Photoactivation is also possible with a mercury arc lamp, but it will lack specificity for particular cells or structures. In this approach, cells can be imaged before activation with excitation for GFP, exposed to the arc lamp for an experimentally determined period of time with a cyan fluorescent protein (CFP) excitation filter set, and then imaged again with typical imaging conditions for GFP on a CLSM.

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

Patterson GH, Lippincott-Schwartz J. 2002. A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* **297:** 1873–1877.

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