Research Roundup

Handy tool for high-resolution microscopy

Conventional fluorescent microscopes normally reach their resolution limit at around 200 nm. Now, Eric Betzig (HHMI, Ashburn, VA), Harald Hess (NuQuest Research LLC, La Jolla, CA), and colleagues present a super-resolution microscopy technique capable of localizing individual fluorescent molecules at the 2–25-nm scale.

When two or more fluorescent proteins in a cell are less than 200 nm apart, their separate signals will be indistinguishable by the average fluorescent microscope and will appear as one bright blob. The new technique devised by Betzig et al., called photoactivatable localization microscopy (PALM), gets around this fundamental problem using two main tricks.

The first trick is to isolate molecules by viewing just a few at a time. This technique can be likened to asking a few scattered people in a crowded auditorium to stand up briefly, then another few people, and so on, until each person in the room has been momentarily distinguished from the seated mass. The second trick is to take tens of thousands of photos while this is going on and afterwards let the computer work out who was sitting where.

To distinguish individual fluorescently labeled proteins from the crowd inside a cell, the team used photoactivatable fluorescent proteins (PA-FPs) and activated just a few at a time. PA-FPs remain essentially invisible until they are stimulated with a particular wavelength of light (activation light). By delivering a brief, weak dose of activating light to the sample, only a few PA-FPs become fluorescent. These proteins are then viewed using light of a different wavelength (excitation light) until their fluorescence is completely bleached and they are no longer visible. The process is repeated with slowly increasing doses of activation light until all molecules have been activated, viewed, and bleached.

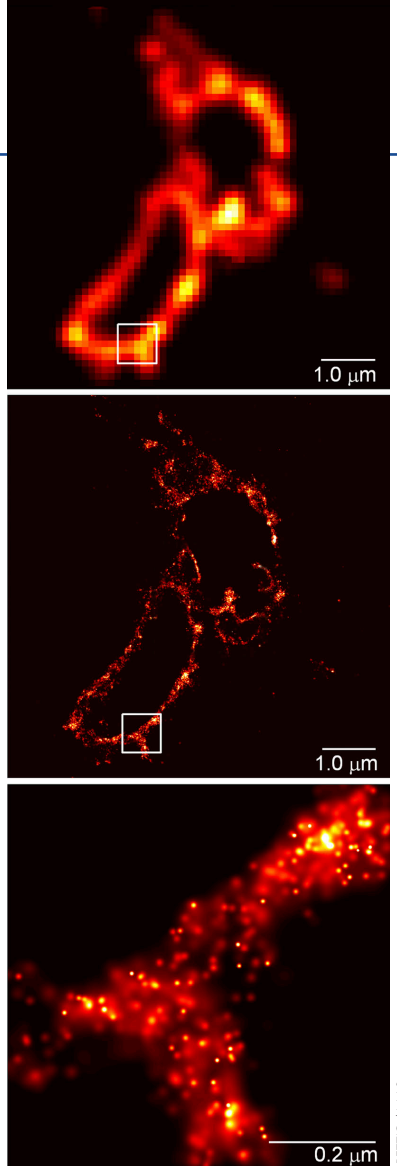
The process takes anywhere from 2–12 hours per sample and generates 10^4–10^5 images. The 2D images are then plotted against time. If one imagines the plot as a 3D stack of images, with time = 0 at the top, then the activation and subsequent bleaching of each molecule resembles a separate 3D blob. The computer then turns these blobs into well-defined dots by calculating their center point. If the stack of images were to be flattened into one picture without first converting blobs to dots, the resulting image would appear much like an ordinary, blurry fluorescent microscope image. Instead, the well-defined dots give the flattened final image a clear, highly resolved appearance.

Not all blobs are of equal brightness and clarity, of course, and the computer is able to recapitulate these variations in the final image using some nifty statistical calculations. As Betzig explains, the final image is "a probability density map, where brightness is proportional to the probability that a PA-FP molecule exists at a given location."

The PALM technique has been made possible thanks to the developments of both PA-FPs and total internal reflection fluorescence (TIRF) microscopy. In TIRF, directing light to a sample at an extremely oblique angle results in the illumination of only very thin sections. The benefit of PALM is far less background noise from out-of-focus light and cellular autofluorescence.

The technique is also possible due to a fortuitous characteristic of gold beads: they glow under the excitation light. Precisely localizing molecules at the nanometer scale is clearly incompatible with movement, but avoiding mechanical or thermal drift at this level over such long periods of time is virtually impossible. To get around this problem, the team added gold beads to their samples. "We just sprinkle them onto the sample like salt," says Betzig. During the image capturing, this gold-bead seasoning acts as a constantly glowing frame of reference. This simple solution is good news for cell biologists hoping to use the technique, since it circumvents the need for expensive temperature- and vibration-controlled devices.

The authors predict that PALM might eventually be used for localizing proteins in 3D. Currently, however, the technology is lacking. The common method of rendering 3D images of fixed cells or tissues, confocal microscopy, produces large amounts of out-of-focus light, leading to too much peripheral bleaching. "You'd lose all the information you want," explains Betzig. One other possibility might be to perform PALM on serial cryosections and then construct a 3D image retrospectively. Realigning the sections might be very difficult, however, as gold beads are too tough for microtomes and thus cannot be used as a physical reference.



PALM transforms normal fluorescent blur (top) into high-resolution images (middle and bottom).

Although the researchers have so far only looked at single-labeled proteins, the ultimate goal for PALM is to visualize two proteins in two colors at once. But Betzig explains that, presently, "the palette of photoactivated proteins is not all that broad. The success [of two-color PALM] will be dependent on our collaborators' coming up with better labeling techniques. We're close, we're really close," he says, "but we're not there yet." JCB

Reference: Betzig, E., et al. 2006. *Science*. doi:10.1126/science.1127344.

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An open or closed case for HMGA

Senescence turns a chromatin-activating protein towards the dark side. Upon senescence induction, high mobility group A (HMGA) protein helps to make silent heterochromatin, report Masashi Narita, Scott Lowe, and colleagues (Cold Spring Harbor Laboratory, NY).

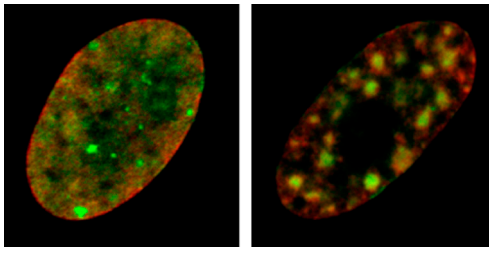
HMGA proteins promote open and active chromatin, are highly expressed in the early embryo, and can promote tumorigenesis. But the new results now show that HMGA is also found in senescent cells, which—in stark contrast to embryonic or tumor cells—no longer respond to mitogenic stimuli.

Senescent cells often form dense nuclear chromatin blobs called senescence-associated heterochromatic foci (SAHF), which the Lowe lab first described three years ago. While working to determine the chromatin components and epigenetic modifications that characterize SAHFs, they found that HMGA was upregulated in senescent cells. Its previously diffuse nuclear distribution in normal cells became a punctate pattern, as HMGA colocalized at SAHFs with the HP1 heterochromatin protein.

HMGA does not just passively associate with SAHFs; knock down of HMGA revealed that it is needed both to establish and maintain SAHFs. The transcriptional repression of multiple genes, including cell cycle factors, also depended on HMGA.

How can HMGA switch between such opposite roles? Evidence suggests that HMGA can be phosphorylated, acetylated, and methylated at specific amino acids. Acetylation, at least, has been shown to alter HMGA's transcriptional activity. It is therefore possible that, just as the chromosomal histones are modified to either condense or open chromatin, so too is HMGA. JCB

Reference: Narita, M., et al. 2006. *Cell.* 126:503–514.



LOWE/ELSEVIER

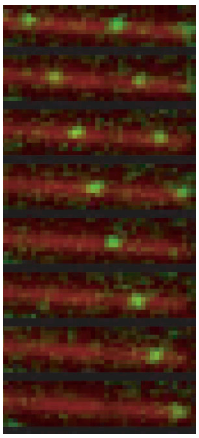
HMGA (red) colocalizes with HP1 (green) at SAHFs in senescent cells (right).

Microtubule munching

A yeast kinesin rapidly munches long microtubules, but its munching speed slows as the microtubules get shorter, report Vladimir Varga, Jonathon Howard, and colleagues (Max Planck Institute, Dresden, Germany). Along with Mohan Gupta, David Pellman, and colleagues (Harvard, Boston, MA), the team also shows that this kinesin-8, called Kip3p, paradoxically eats away at the growing ends of microtubules.

Microtubule length must be tightly controlled for cell functions as diverse as transport, motility, and division. In vitro studies by both groups showed that Kip3p binds all along the length of microtubules, but then motors along to accumulate at their plus ends, where it functions as a depolymerase.

Varga et al. found that Kip3p had a bigger appetite for longer microtubules, as these were devoured more rapidly than short microtubules. The authors suggest that longer microtubules bind more Kip3p and thus accumulate more depolymerase at their plus ends. The ends probably lose some Kip3p as they shorten, and shorter microtubules have less room to pick up more of the motor.



HOWARD/MACMILLAN

Kip3p (green) binds to microtubules (red) and motors along to their plus ends (right).

Plus-end accumulation of Kip3p was seen both in vitro and in cells. In the cell, however, the plus end is where growth and shrinkage occur. "The plus end is the place where the action is happening," says Varga. At this end, he says, there is probably, "a fight between polymerization and destabilizing proteins." He suggests that microtubules might grow from their plus ends until enough Kip3p accumulates to swing the battle in favor of shortening. JCB

References: Varga, V., et al. 2006. *Nat. Cell Biol.* doi:10.1038/ncb1462.
Gupta, M.L., et al. 2006. *Nat. Cell Biol.* doi:10.1038/ncb1457.

Live long and cancer-free

Mutations that prolong the life span of worms also suppress tumor formation, report Julie Pinkston, Cynthia Kenyon, and colleagues (University of California, San Francisco).

Mutations in the *gld-1* gene of *Caenorhabditis elegans* cause germ cells to proliferate uncontrollably, giving rise to lethal tumors. In most organisms, tumor susceptability increases with age, so Pinkston et al. were interested in how mutations that increase the life span of *C. elegans* might affect *gld-1* mutant worms.

Four different mutations that each promote longevity also suppressed tumorigenesis. When *gld-1* worms carried mutations in *daf-2, eat-2, isp-1,* or *clk-1* genes, the proliferation rate of germ line tumor cells was dramatically reduced. Remarkably, however, none of these mutations affected the proliferation rate of germ cells in worms without the *gld-1* mutation.

Mutations in *daf-2* interfere with insulin signaling, *eat-2* mutations restrict calorie intake, and *isp-1* and *clk-1* mutations impair mitochondrial activity. These apparently diverse routes may lead to both longevity and protection against cancer by limiting energy or nutrient availability to cells, says Kenyon.

In normal worms, the mutations do not cause nutrients to fall below levels necessary for cell division. In rapidly growing tumor cells, however, the demand for nutrients is higher. The mutations may then be sufficient to starve the tumor cells, inducing a stress response that ultimately shuts down the cell cycle machinery. JCB

Reference: Pinkston, J.M., et al. 2006. *Science.* 313:971–975.