to interact with a diversity of proteins including Gα subunits. Although Ballon et al. showed in the study that Gα subunits do not compete for the binding of Sst2 to the cytoplasmic tail of Ste2, it remains to be determined if other proteins that interact with the cytoplasmic tail influence Sst2 binding. Finally, it is known that upon receptor activation, Sst2 and other RGS proteins may be phosphorylated. Whether this phosphorylation affects interactions with receptors also remains to be determined. Clearly, answering these questions will have significant impact on understanding the functional specificity of RGS proteins as well as the roles in signal transduction of other DEP domain-containing proteins.

Songhai Chen[1] and Heidi E. Hamm[1]
[1] Department of Pharmacology
Vanderbilt University Medical Center
Nashville, Tennessee 37232

Selected Reading

Abramow-Newerly, M., Roy, A.A., Nunn, C., and Chidiac, P. (2006). Cell. Signal. *18*, 579–591.

Ballon, D.R., Flanary, P.L., Gladue, D.P., Konopka, J.B., Dohlman, H.G., and Thorner, J. (2006). Cell *126*, 1079–1093.

Civera, C., Simon, B., Stier, G., Sattler, M., and Macias, M.J. (2005). Proteins *58*, 354–366.

Hamm, H.E. (1998). J. Biol. Chem. *273*, 669–672.

He, W., Cowan, C.W., and Wensel, T.G. (1998). Neuron *20*, 95–102.

Kovoor, A., Seyffarth, P., Ebert, J., Barghshoon, S., Chen, C.K., Schwarz, S., Axelrod, J.D., Cheyette, B.N., Simon, M.I., Lester, H.A., and Schwarz, J. (2005). J. Neurosci. *25*, 2157–2165.

Malbon, C.C., and Wang, H.Y. (2006). Curr. Top. Dev. Biol. *72*, 153–166.

Martemyanov, K.A., Hopp, J.A., and Arshavsky, V.Y. (2003). Neuron *38*, 857–862.

Ponting, C.P., and Bork, P. (1996). Trends Biochem. Sci. *21*, 245–246.

Wong, H.C., Mao, J., Nguyen, J.T., Srinivas, S., Zhang, W., Liu, B., Li, L., Wu, D., and Zheng, J. (2000). Nat. Struct. Biol. *7*, 1178–1184.

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PALM Reading: Seeing the Future of Cell Biology at Higher Resolution

The inherent resolution limit of the light microscope has been a limiting factor in investigations of many fields of cell biology. A recent paper in *Science* by Betzig and coworkers describes a new method that can push the limit significantly lower.

Answers to many of the remaining mysteries in biology lie hidden somewhere between the cellular and the molecular scales. This is the domain of self-organizing supramolecular structures and molecular nanomachines that play roles in almost all of a cell's basic functions. Examples of these structures are kinetochores, vesicle budding sites at the plasma membrane and on trafficking organelles, cell adhesion sites, leading edges of migrating cells, and neuronal synapses. We still don't understand these structures and processes very well. Therefore, advances that would open new windows into this world are likely to give us important novel insights into cell biological mechanisms.

This world of tiny molecular machines is just below the resolution limit of the light microscope. Light microscopes can resolve two objects that are separated by about 200 nm, but images of objects that are closer than that are blurred into one fuzzy blob. The potential rewards for lowering this limit have motivated lots of work to try to develop methods to improve the resolution of light microscopy. Different approaches have been developed that can offer significant improvements in resolution (Betzig and Trautman, 1992; Donnert et al., 2006; Gustafsson, 2000, 2005; Willig et al., 2006).

In a recent issue of *Science,* Eric Betzig and his colleagues described a new super-resolution light microscopy method (Betzig et al., 2006). Their new method, photoactivated localization microscopy (PALM), is based on an innovative combination of two previously developed approaches: nanometer-resolution localization of single molecules and use of photoactivatable fluorescent proteins.

It turns out that the resolution limit of the light microscope does not actually prevent acquisition of spatial information below the limit of ~200 nm. It simply means that if two objects are closer than the resolution limit, they will appear as one object. However, it is possible to estimate the location of an isolated object with much higher accuracy, sometimes down to an accuracy of a few nanometers. This approach has been widely used in studies of single fluorescent molecules in vitro. When a solution of a fluorescently labeled protein is sufficiently dilute, the protein molecules can be detected as individual, clearly separated objects with a light microscope. Although each fluorescent molecule appears as a spot with a diameter of a couple of hundred nanometers, much larger than the actual molecule, the center of that spot, and the location of the molecule, can be estimated with high accuracy. This has been used, for example, for tracking nanometer movements of motor proteins (see e.g., Yildiz et al., 2003).

However, in cells, proteins are usually very concentrated, and thus single molecules cannot be resolved spatially. The method of Betzig et al. (2006) uses another dimension to separate individual molecules, namely time. To do this they use photoactivatable fluorescent proteins (PA-FP; Patterson and Lippincott-Schwartz, 2002; Lukyanov et al., 2005). A PA-FP fused to a protein of interest is expressed in cultured

mammalian cells. The PA-FPs are initially in their inactive, nonfluorescent state, but they become fluorescent when illuminated by a pulse of short wavelength light. The trick is to keep the intensity of the activating light sufficiently low so that only a small subset of PA-FPs in the sample is activated. When the subset is small enough, the activated molecules can be imaged and resolved as well-separated, single molecules. The activation pulse is followed by longer wavelength excitation light and image acquisition using a high-sensitivity CCD camera. This imaging step is continued until the activated molecules are photobleached by the excitation light. Then the sample is illuminated with a new pulse of short wavelength light to activate a new subset of single molecules, and a new image is acquired. These cycles of activation, image acquisition and photobleaching are continued until most of the molecules in the sample have been imaged. Finally, the localizations of the single molecules are calculated from the set of images collected from the sample. The standard deviations of the localization estimates are used to create new, sharper and more precisely localized spots to represent the single molecules. The spots from each of the images are then combined to form the final, much sharper image of the distribution of the protein in the sample. PALM has reached resolutions down to ~2–25 nm.

There are some features that limit the versatility of PALM—at least in its present incarnation. First, it takes several hours to go through the cycles of photoactivation and image acquisition to collect all of the data needed to generate a single high-resolution image. This necessitates the use of fixed specimens, which precludes PALM's use for imaging of live cells. Second, single fluorescent molecules are not easy to visualize even with the most sensitive cameras. To overcome this problem, the authors used total internal reflection fluorescence microscopy (TIRF), an imaging technique that provides a very high signal-to-noise ratio for detection of single molecules. However, TIRF microscopy can only be used for imaging structures very close to the microscope coverslip, such as cell surface structures or ultrathin sections. These limitations could potentially be circumvented in the future, and the authors suggest possible ways to develop a faster version of PALM capable of imaging three-dimensional samples. Importantly, the authors are also determined to develop a "two-color" PALM, which would be a significant improvement, allowing one to compare localizations of pairs of fluorescently tagged proteins at unprecedented resolution. This would seem to be a relatively straightforward development, as the authors have already used two differently colored PA-FPs in "single-color" experiments.

Immunoelectron microscopy has been traditionally used to localize proteins at resolution levels below light microscopy. Compared to immunoelectron microscopy, PALM requires much simpler sample preparation. This is especially true when PALM is used for looking at cell surface structures. Also, the specificity and sensitivity of fluorescent labeling with FPs is very high compared to immunoelectron microscopy methods.

Marko Kaksonen[1] and David G. Drubin[1]
[1] Department of Molecular and Cell Biology
University of California, Berkeley
16 Barker Hall
Berkeley, California 94720

Selected Reading

Betzig, E., and Trautman, J.K. (1992). Science *257*, 189–195.

Betzig, E., Patterson, G.H., Sougrat, R., Lindwasser, O.W., Olenych, S., Bonifacino, J.S., Davidson, M.W., Lippincott-Schwartz, J., and Hess, H.F. (2006). Science, in press. Published online August 10, 2006. 10.1126/science.1127344.

Donnert, G., Keller, J., Medda, R., Andrei, M.A., Rizzoli, S.O., Luhrmann, R., Jahn, R., Eggeling, C., and Hell, S.W. (2006). Proc. Natl. Acad. Sci. USA *103*, 11440–11445.

Gustafsson, M.G. (2000). J. Microsc. *198*, 82–87.

Gustafsson, M.G. (2005). Proc. Natl. Acad. Sci. USA *102*, 13081–13086.

Lukyanov, K.A., Chudakov, D.M., Lukyanov, S., and Verkhusha, V.V. (2005). Nat. Rev. Mol. Cell Biol. *6*, 885–891.

Patterson, G.H., and Lippincott-Schwartz, J. (2002). Science *297*, 1873–1877.

Willig, K.I., Rizzoli, S.O., Westphal, V., Jahn, R., and Hell, S.W. (2006). Nature *440*, 935–939.

Yildiz, A., Forkey, J.N., McKinney, S.A., Ha, T., Goldman, Y.E., and Selvin, P.R. (2003). Science *300*, 2061–2065.