Fluorescent protein research shines brilliantly

Red fluorescent protein allows dual-wavelength microscopy

A new red fluorescent protein (RFP) enables some literally brilliant research: Through genetic engineering, researchers at the Technical University of Munich in Garching, Germany, and at Max Planck Institute of Biochemistry in Martinsried, Germany, have created an enhanced monomeric, or single-unit, version of an RFP that is very bright in live cells. Dubbed mRFPmars, the protein can be combined with GFPs for dual-wavelength fluorescence microscopy.

This tandem has already proved useful in visualizing the filamentous actin structures and microtubules that make up the cellular skeleton in *Dictyostelium* cells. Investigators are now using the duo to monitor cytoskeletal proteins during cell movement and division.

The scientists are doing experiments in collaboration with Kurt Anderson at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, using total internal reflection fluorescence microscopy to analyze cytoskeletal dynamics, according to research team member Annette Müller-Taubenberger. She's a senior scientist in Günther Gerisch's cell dynamics group at the Max Planck Institute of Biochemistry. The researchers reported on their new fluorescent protein in the Nov. 5 issue of *FEBS Letters*.

According to Müller-Taubenberger, the development arose because investigators confronted a problem. One red fluorescent protein, DsRed, didn't work in *Dictyostelium*, where only immature, green fluorescent precursors were detectable. The group tried a well-known DsRed mutation, mRFP1. A stable monomer that matured in an hour, mRFP1 did produce red fluorescence in labeled cells. However, the output was dim, making it impossible to acquire images with high resolution in time and space. The fluorescence was also too weak to allow double labeling and total internal reflection fluorescence microscopy.

Enhanced monomeric RFP

The researchers, therefore, set out to create an enhanced monomeric RFP. Monomeric fluorescent proteins have the least functional impact when fused to a target protein, so they ensure that what is tracked after fusion is most representative of what goes on before.

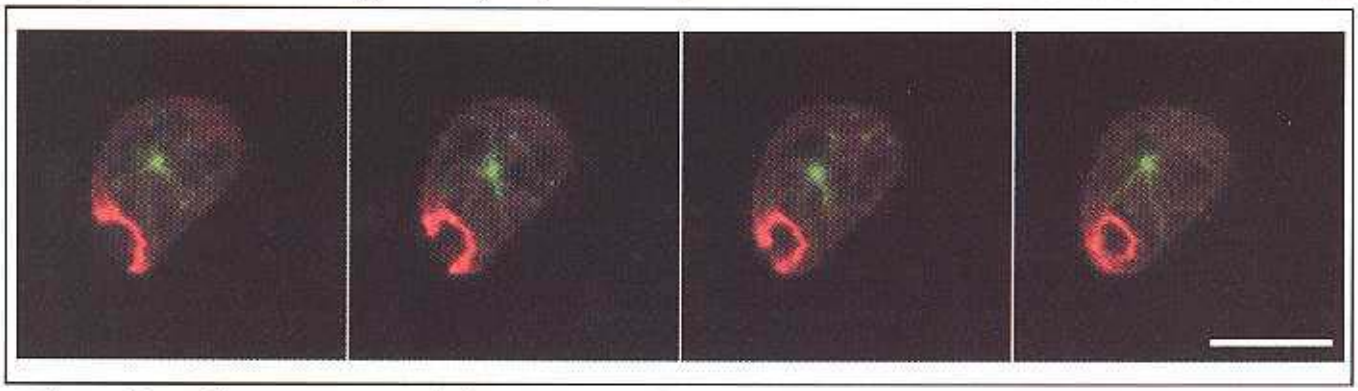
The collaborators started with an RFP sequence from Roger Tsien of the University of California, San Diego, and combined it with mutations in another DsRed screen. Markus Fischer with the Technical University of Munich made a synthetic gene comprising two sets of mutations: one that made the protein monomeric and one that enhanced its fluorescence in living cells. The researchers also optimized the codons, or genetic coding sequence, of the new protein.

Müller Taubenberger said the manipulation and optimization were not exceptionally difficult. "The important thing was to show that it really worked nicely in living cells."

After characterizing the spectroscopic properties of their protein, the researchers grew cultures of *Dictyostelium* in which the protein was fused to targets of interest. They then used confocal laser scanning microscopy to observe the cells that expressed the new protein. When taking fluorescence spectra, they used a confocal microscope from Carl Zeiss AG of Jena, Germany.

To demonstrate the usefulness of mRFPmars, the researchers selected three targets that paralleled previous GFP-fusion research. The first, LimEAcoil, is a probe with a great affinity for the filamentous actin network structures in the cytoskeleton. The second, ABD120, prefers the actin-filament binding domain of *Dictyostelium* filamin, but also binds to filamentous actin. The third, α-tubulin, is incorporated into microtubules over their entire length.

After being introduced into living cells, all three fusion proteins showed an emission maximum around 610 nm when excited by either 488- or 543-nm wavelength lasers. The emission spectra, the researchers said, was very similar to the mRFPmars spectra from the purified protein. There was some emission in the



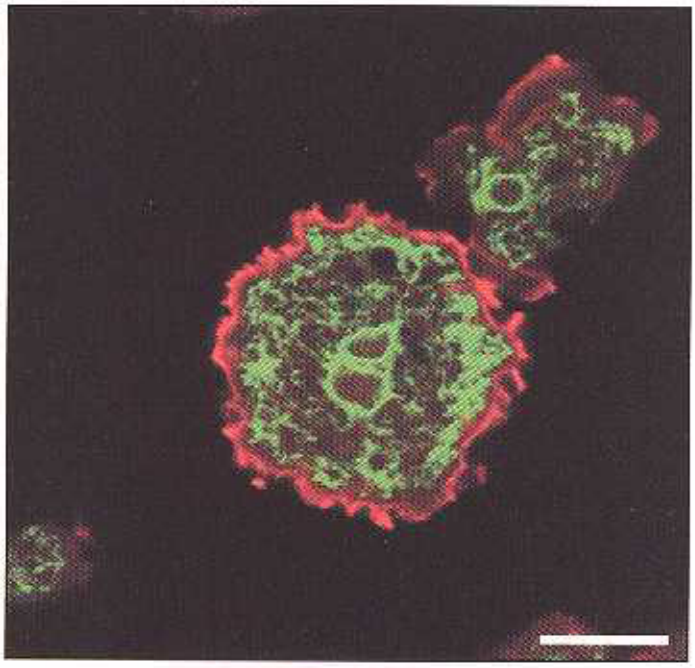
A Dictyostelium cell expresses GFP-α-tubulin (green) to visualize microtubules and mRFPmars-LimEAcoil (red) to show filamentous actin structures. Consecutive frames (5-s time interval) show the formation of a macropinocytic cup. The bar corresponds to 10 μm.

green, with a peak around 520 nm, but this, they noted, was minimal. The relatively weak green peak made the new RFP suitable for use in combination with GFPs.

Watching cells feed

The group tested the utility of mRFPmars for dual labeling in a series of experiments with various GFP-tagged proteins. After fusion, the GFPs highlighted particular parts of the cells, while the new RFP illuminated actin structures in the cytoskeleton. This arrangement, the scientists reported, allowed them to study the involvement of cytoskeletal proteins in organelle dynamics, endocytosis and mitosis.

They also combined mRFPmars with green fluorescent Alexa 488 dextran. Wild type *Dictyostelia* are phagocytes, sweeping up bacteria to feed and grow. They also ingest nutrients via fluid uptake in a process known as macropinocytosis. The actin in the cytoskeleton plays a part in this procedure. By using mRFPmars-ABD120 with Alexa 488, the researchers could follow the macropinocytic progression and the intake of fluid.



Dictyostelium *cells expressing mRFPmars-imΔcoil (red) to visualize the actin cytoskeleton, combined with calnexin-GFP (green) to label the endoplasmic reticulum, enable dual-wavelength microscopy.*

Although the investigators have demonstrated the use of mRFPmars only in *Dictyostelia*, the protein could be useful in other organisms. Müller-Taubenberger said that quite a bit of interest has been expressed in the new RFP but, so far, none of the commercial variety.

She noted that work has begun to optimize the protein for other systems, including mammalian cells. This research may involve developing species-specific versions of mRFP; to do this, the German researchers are collaborating with other groups. These efforts are ongoing but not yet definitive. "Our results are promising," Müller-Taubenberger said. "But the experiments are under way, and it is too early to say anything conclusive." □

Hank Hogan

Surgery on a small scale

Picosecond laser pulses ablate microtubules

Sometimes you learn more by subtraction than addition. That's the idea behind laser cell surgery, in which researchers selectively disable part of a cell to study that area's role in the overall cell.

Now a team of investigators from the Irvine and San Diego campuses of the University of California has demonstrated controlled ablation of microtubules in living cells using a picosecond laser.

Combining green fluorescent protein variants fused to microtubule proteins allowed the researchers to selectively remove cellular elements with greater precision than had been possible.

"This ultimately could result in a better understanding of how cells carry out their various functions," said Michael Berns, a biomedical engineering and cell biology professor in the Beckman Laser Institute at the university in Irvine. The group published its results in the December issue of *Biophysical Journal*.

Microtubules are long protein polymers that are part of a cell's cytoskeletal system. They're responsible for cell movement, including the beating of flagella and the alignment and separation of chromosomes during cell division.

To study the role that microtubules and organelles play in a cell, researchers can remove organelles or part of them and see what processes are disrupted in the cell. This surgery must be done in such a way that only the intended target is affected. Now that laser pulse widths have moved into the picosecond and femtosecond range, researchers can perform microsurgery and destroy a precise section of the cell with little or no collateral damage.

This confinement, Berns noted, is likely because of the nonlinear nature of interaction of the laser with the cell. The nonlinearity ensures that the impact drops off sharply with distance from the focal point.

However, many possible targets in the cell are at or below the limit of resolution of a light microscope. So while it's possible to zap a given spot, it's hard to be sure that the laser is aimed at the right point. One solution, proposed several years ago, was to fuse fluorescent proteins to a protein known to be in the organelle of interest. The fluorescence could then