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Cells Don Festive Holiday Colors

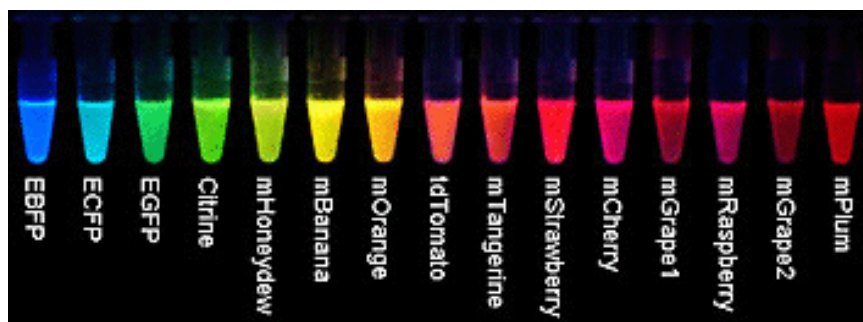


Image Title: Coming to a lab near you -- the 2004 color palette of fluorescent proteins. - Courtesy of Roger Y. Tsien

The latest holiday gifts being offered to the scientific community this season by scientists in the laboratory of Howard Hughes Medical Institute investigator Roger Y. Tsien come in a dazzling variety of hues—cherry, strawberry, tangerine, tomato, orange, banana and honeydew. The color spectrum would make Pantone proud.

No, Tsien's group is not giving out fruit baskets; the names describe vibrant new varieties of fluorescent protein that the researchers have created to tag cells and observe a range of cellular processes.

"One of the reasons we name the proteins after fruits is to remind people that there is no 'best fruit' in the grocery store."

— Roger Y. Tsien

By splicing the genes for the fluorescent proteins into specific genes in the cell, researchers can detect when those genes are switched on to produce

proteins. They can then use the telltale fluorescent colors to separate the cells visually. The availability of the new colors will enable scientists to track the effects of multiple genetic alterations in a single cell.

Tsien and his colleagues at the University of California, San Diego, published a research article describing the new fluorescent proteins in the December 2004 issue of the journal *Nature Biotechnology*. Lead author on the paper was HHMI predoctoral fellow Nathan C. Shaner in Tsien's laboratory. In separate studies, Tsien's team "borrowed" the immune system's machinery for generating antibody diversity and used it to evolve a new red fluorescent protein. (To read about those studies, please go to <http://www.hhmi.org/news/tsien.html>).

In addition to offering fluorescent proteins in a range of distinctive colors, Tsien's group has improved their design, creating proteins that are "monomers" that consist of only single protein units. Fluorescent proteins found in nature with yellow to red colors are invariably four-unit agglomerations that are often toxic or disruptive when fused to proteins that scientists hope to track.

"The analogy is that if you have a detective who is supposed to be tracking suspects, and that detective has to go around in groups of four and track four suspects at once, the suspects are likely to know something is up," said Tsien.

The latest collection of fluorescent proteins builds on the researchers' earlier success in reengineering a four-unit fluorescent protein isolated from a coral-like creature of the species *Discosoma*. From that multimeric protein, the researchers engineered a monomeric protein, called mRFP1, which still retained fluorescent properties. However, certain of these properties were still not optimal for a fluorescent marker.

Shaner, Tsien and their colleagues set out to improve mRFP1's fluorescent properties—making strategic mutations in the gene for the protein—to render it more useful as a biological marker. "Basically, we were trying to guess from the crystal structure of the protein or from past knowledge of mutations, where we might make useful mutations," said Tsien. "In the process of trying to fix these characteristics, we also discovered more colors," he said. Further adjustments to the genes pushed the fluorescence of some of the proteins to longer wavelengths, filling in gaps in the spectrum of colors, said Tsien.

In the *Nature Biotechnology* article, the researchers also reported a demonstration of the improved functionality of the new proteins. They fused the protein mCherry (the "m" stands for monomeric) to proteins that were part of the cell's microtubule transport system, and of the cell's structural cytoskeleton. In both cases, mCherry successfully labeled the cellular structures. However, in the case of the microtubule protein, mRFP1 did not label it properly.

The researchers also created a red protein "tdTomato" in which two subunits still stick to each other, but they have been permanently joined head to tail. The resulting monolithic unit has no tendency to aggregate further, yet it

fluoresces more brightly and resists fading better than true monomers. But in some cellular applications their larger molecular weight might interfere with cellular processes, said Tsien. And Tsien's team has engineered other fluorescent proteins that may be more or less sensitive to changes in acidity.

Tsien said such a variety of fluorescent proteins will enable scientists to have considerable choices in making such tradeoffs, "and one of the reasons we name the proteins after fruits is to remind people that there is no 'best fruit' in the grocery store," he said.

According to Tsien, the new assortment of fluorescent proteins will give researchers an easier way to track the effects of multiple genetic alterations in the same cells. "They can be applied to single cells, where you want to track different proteins, to different organelles, all the way up to whole animals," he said.

Future efforts will aim not only at developing monomeric proteins with new colors, said Tsien, but also working on those with more complicated properties, such as the ability to change color under different conditions. His ultimate goal, he said, is to broaden the range of tools that biologists have at their disposal to track genetically altered proteins and cells.