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/THE FIRST WORD

Green Fluorescent Protein: The Next Generation

Last February, Martin Chalfie and his colleagues published their results on the use of green fluorescent protein (GFP), an accessory protein involved in the green light emission of the jellyfish, *Aequorea victoria*, as a genetic marker and protein tag (*Science* **263**:802-805, February 11,1994). Interest was immediate and widespread, because the jellyfish-lit system showed that it was possible to introduce fluorescent labeling to intact cells, tissues, and organisms, as well as sliced specimens, genetically. Nothing needs to be added from the outside. In addition, GFP-based systems use already-existing technologies and imaging methods—fluorescent microscopes and filters, fluorescent cell sorting, automated flow cytometers, and so on. Traditional fluorescence has always been a messy business. The labeling is done by purifying proteins and then conjugating them to fluorescein and rhodamine. Every experiment requires the purification of an antibody to the molecule in question, and the dye attachments are hard to control. In living cells, it is difficult to get the conjugates pipetted across the cell membrane. GFP proteins are small, bright, accurate, and now it seems, easy to manipulate.

Can GFP fluorescence be tailored to specific conditions? Can we get some more colors? The answer to both questions appears to be yes, based on the results of two recent papers on the next generation of GFP proteins. Roger Heim and his colleagues report in the *Proceedings of the U.S. National Academy of Science* (**91**:12501-12504, December 1994) on the development of shifted mutants to GFP, one of which fluoresces blue. And in this issue of *Bio/Technology* (p. 151), Simon Delagrave and his colleagues report on several red-shifted mutants to GFP.

In the *Bio/Technology* paper, Delagrave et al. describe using optimized combinatorial mutagenesis and digital imaging spectroscopy to create and examine a library of red-shifted GFP mutants. The spectrally shifted mutants were initially identified by their green luminescence, which was observed when they were excited with a 490 nm light, but which disappeared when they were excited at 410 nm. This contrasts with normal wild-type GFP, which excites at either wavelength.

In the Heim et al. work, GFP was mutagenized (random mutagenesis) and screened for variants with altered spectra. Three mutants were found with significant alterations in the ratio of the two main excitation peaks. A fourth mutant, P4, fluoresced bright blue, in contrast to the green of the wild type. The excitation and emission maxima were shifted by 14 and 60 nm, respectively, from those of the wild type.

Bioluminescence has not given up its secrets easily, and these recent efforts are the culmination of decades of work by many researchers. GFP-based recombinant genetic and protein identification systems are exciting because they will make some current, more cumbersome methods obsolete. It should be possible to substitute GFP variants for fluorescein and rhodamine tagging of interacting proteins. GFP systems do not require exogenously added substrates or cofactors, which gives them a leg up on luciferase-based systems. Detection of intracellular GFP requires only ultraviolet or blue light, so it is nontraumatic.

The spectrally separable GFP mutants described in Heim et al. and Delagrave et al. should make it easier to analyze gene expression cascades, monitor the effects of drugs, hormones, or toxins on the expression of proteins in living cells, and simultaneously track more than one protein in a living organism. It also offers the possibility of detecting gene induction before changes in the phenotype are apparent, which could be very useful for screening purposes. It is a high-tech ending to a story that began with what some might consider basic research ephemera—jellyfish and fireflies and fox glow.

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