RESEARCH BRIEFS

GFP chimeras monitor signal molecules

Fluorescent reporters of physiological indicators like calcium have been created by grafting various receptor proteins to green fluorescent protein (GFP; *Proc. Natl. Acad. Sci. USA* **96,** 11241–11246, 1999). "Whereas older GFP-based biosensors generally rely on energy transfer between two different GFP mutants, our new indicators offer the possibility of making single GFPs sensitive to physiologically relevant substrates," explains Geoffrey Baird, senior author on the paper. The findings were unexpected because GFP's rigid structure and complex chromophore did not seem amenable to such radical modifications. The researchers found a site that would tolerate such insertions by mutagenesis, and circular permutations that flipped two portions of the polypeptide around a central site. The spectra of these permutated GFP variants were essentially unchanged, suggesting that this site would tolerate large insertions. They went on to graft into this site several receptor proteins, including calmodulin. Results showed that the binding of calcium to the grafted receptors led to as much as a seven-fold increase in fluorescence, even in living cells. "Our work potentially allows one to make GFP-based biosensors for a wide variety of biologically relevant substrates, not just for calcium" says Baird. Further GFP derivatives are currently under development to sense other second messengers and signaling events.

Quenching biosensor

Researchers at Los Alamos National Laboratory (Los Alamos, NM) and the University of California (Los Angeles, CA) have developed a highly sensitive assay that could be used to detect a wide range of biological and non-biological compounds. The polymer, known as MPS-PPV, has been the focus of intense interest for its possible uses in light-emitting diodes and other electronic devices, but the new study (*Proc. Natl. Acad. Sci. USA* **96,** 12287–12292, 1999) puts the compound's unusual fluorescence characteristics to a different use. The scientists found that certain small molecules could bind to the polymer backbone in an aqueous solution and efficiently quench the compound's fluorescence. The team then conjugated biotin to the small-molecule quencher and demonstrated that avidin binding to the biotin efficiently sequesters the quencher and allows the MPS-PPV to fluoresce. "The performance and sensitivity we…have achieved are comparable to several different assay techniques such as ELISA [and] PCR," explains David Whitten, a researcher at Los Alamos and senior author on the paper. Unlike ELISA and PCR, the new technique is rapid and requires minimal equipment, making it well-suited to detecting biological weapons, hazardous chemicals, or antibodies in field or clinical settings.

Research Briefs written by Natalie DeWitt, Alan Dove, and Andrew Marshall .

β-secretase identified

Researchers at Amgen (Thousand Oak, CA) have cloned and characterized a β-secretase that could represent a potential target for Alzheimer's therapies (*Science* **286,** 735–741, 1999). Secretases are involved in the processing of amyloid precursor protein (APP) to produce amyloid β, which accumulates to form plaques. Until recently, many researchers had been attempting to find the secretase (termed BACE for beta-site APP-cleaving enzyme) using biochemical purification. In contrast, the Amgen team, headed by Martin Citron, used an expression cloning strategy to identify the protein. Overexpression of the BACE was shown to increase the amount of APP cleavage products in a manner consistent with β-secretase action. Antisense inhibition of BACE also decreased the amount of APP cleavage products in cell culture. Using cellular immunostaining and ELISA quantification, the researchers confirmed that the subcellular location and expression pattern of BACE corresponded with those predicted for β-secretase. According to Amgen, screening is already under way to identify a small molecule inhibitor of the protein. Meanwhile, Bart De Strooper and colleagues have used confocal microscopy to show (*J. Cell. Biol.* **147,** 277—294, 1999) that presenilin-1 and APP are located in the same places, adding to weight to the evidence that this protein might be the elusive cellular γ-secretase that prevents amyloid β formation.

Germline transmission of artificial chromosome

At a recent meeting in London (BioPartnering Europe, 16–18 October), Chromos Molecular Systems (Burnaby, BC) released preliminary data on a satellite DNA-based artificial chromosome (SAT-DAC) that allows researchers to insert virtually any gene into mammalian cells without the risk of disrupting other genes at the site of integration. The modified chromosome is stably inherited in a transgenic mouse system. While the work could have a broad range of agricultural and clinical applications, it also has bioethicists wringing their hands over the looming possibility of human germline gene therapy. The SAT-DAC technology relies on the ability of an acrocentric chromosome to amplify itself when foreign DNA is inserted. A unique feature of the Chromos system is that the resulting artificial chromosome can be separated from other mammalian chromosomes because of its higher A-T content. "There are other groups that have made artificial chromosomes, but they don't have a way of isolating them," explains Carl Perez, director of projects at Chromos. The company plans to use the technology for transgenic livestock and some types of human gene therapy, but Perez stresses that Chromos will not license SAT-DACs for human germline gene therapy.

BAC vaccine

Bacterial artificial chromosomes (BACs) have become a standard tool of genome research, but a multinational team of scientists has now demonstrated a new potential application for BACs—DNA-based vaccines. Until now, the limited size of vectors used for DNA vaccination has posed a significant problem, restricting the number of antigens that can be delivered to a single cell. In the new report (*Proc. Natl. Acad. Sci. USA* **96,** 12697–12702, 1999), the researchers demonstrate that a BAC carrying most of the genome of herpes simplex virus 1 (HSV-1) is an effective vaccine, producing both humoral and cell-mediated immunity in mice and protecting the animals from a lethal challenge with the virus. The vaccine, called a BAC-VAC, includes a replication-competent HSV-1 genome that contains a deletion in the genome packaging sequence. While the DNA is capable of carrying almost every step of a herpesvirus infection when introduced into mammalian cells, the deletion prevents the vector from producing viable virus particles. Because the vaccine mimics a normal infection, both arms of the immune system respond, and mice immunized with the BAC-VAC appear to gain lasting immunity to the virus.