SENSORS AND PROBES

Fishing for fluorescent proteins

The first fluorescent protein cloned from a vertebrate species is a promising tool for clinical diagnostics and research.

Spineless sea creatures such as jellyfish and coral have given scientists access to a sweeping palette of colorful fluorescent proteins for use in a diverse array of cell biology experiments. Until recently, however, such proteins appeared to be a feature unique to invertebrate species.

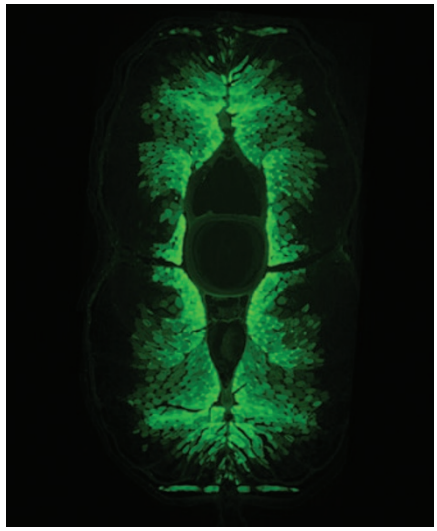
This explains Atsushi Miyawaki's surprise when he learned in 2006 of the existence of a novel fluorescent protein in the Japanese freshwater eel, known locally—and to sushi aficionados worldwide—as unagi. Miyawaki, a researcher specializing in bioimaging at the RIKEN Brain Science Institute in Wako, Japan, had attended a talk in which Seiichi Hayashi of Kagoshima University described a green fluorescent protein isolated from unagi muscle tissue. "After coming back to my laboratory, I bought eels at the fish market and tried just shining light on the muscle," he recalls. "I was astonished by the bright green fluorescence!"

Miyawaki and colleagues have now successfully cloned the gene encoding this protein, which they have termed UnaG, and their structural and functional analysis of UnaG has revealed several surprising and potentially useful characteristics. Unlike other fluorescent proteins that can essentially function autonomously, UnaG requires a cofactor to generate its distinctive green glow: bilirubin, an intermediate product of heme metabolism. Sequence analysis indicates that UnaG belongs to the family of fatty acid binding proteins (FABPs), which are involved in lipid transport or storage. Other FABPs feature shallow binding pockets and tend to exhibit broad target specificity, but UnaG recognizes bilirubin with surprisingly high affinity and specificity, gripping the ligand tightly in a network of hydrogen bonds. As a result, UnaG generates fluorescence almost immediately upon addition of bilirubin.

Heme breakdown gives rise to 'unconjugated' bilirubin, which subsequently binds to albumin and is transported to the liver, where it is enzymatically converted to 'conjugated' bilirubin. Unconjugated bilirubin is a potent antioxidant, and Miyawaki hypothesizes that UnaG may help eels to maintain stores of bilirubin in muscle tissue to protect against damage during periods of migration. "They need to swim a long distance, and so there must be some anaerobic oxidative stress," he says, and notes that by analogy, migratory birds and insects express other FABPs in their muscle to maintain energy stores. On the other hand, Miyawaki also says that "we haven't the slightest idea why it is fluorescent."

Nevertheless, this feature could make UnaG a valuable clinical tool. Moderate levels of unconjugated bilirubin appear to confer some protection against medical conditions associated with oxidative damage, such as cardiovascular disease or diabetes. However, a spike in bloodstream levels of unconjugated bilirubin can be toxic, causing jaundice or the neurological disorder kernicterus in infants and young children. Miyawaki recalls learning to quantify bilirubin via indirect, low-sensitivity assays as a medical student, and he notes that remarkably little has changed since then. "Methods for measuring bilirubin have shown almost no evolution over the last 100 or so years," he says. In comparison, Miyawaki's team found that UnaG can glom onto all of the unconjugated bilirubin in a blood sample within roughly 10 minutes, yielding a quantitative fluorescent signal with 1,000-fold greater sensitivity than existing assays.

UnaG might have basic research applications as well. Unlike GFP and its relatives, UnaG can become fluorescent in the absence of oxygen, which could prove useful in certain *in vivo* imaging contexts. Furthermore, bilirubin readily permeates cell membranes, which means it can be applied as an exogenous 'switch' to activate UnaG fluorescence in organisms that do not naturally produce bilirubin, such as yeast and microbes.



The UnaG protein expressed in the muscle of the Japanese freshwater eel generates bright green fluorescence. Reprinted with permission from *Cell*.

Moving forward, Miyawaki's priorities are twofold. The first goal will be to achieve a more robust understanding of UnaG function, which will require careful investigation of the dynamics of bilirubin metabolism and distribution within eel muscle. In parallel, he and his team are using protein engineering to optimize UnaG for clinical and basic research applications, and they have already generated an UnaG variant with more stable fluorescence. Now they are aiming to optimize its binding affinity and to design variants that can also recognize conjugated bilirubin, in the hope of devising a robust and sensitive clinical assay. "If we could measure bilirubin concentrations very precisely, we might be able to compare the measurements for many physiological conditions and understand bilirubin dynamics within our body," says Miyawaki.

Michael Eisenstein

RESEARCH PAPERS

Kumagai, A. *et al.* A bilirubin-inducible fluorescent protein from eel muscle. *Cell* **153**, 1602–1611 (2013).