Bilirubin glow

By C. Simone Fishburn, Senior Editor

Bilirubin tests in jaundiced babies are commonplace in maternity wards but involve multiple steps and vary in reliability. **RIKEN Brain Science Institute** researchers have now found that a fluorescent protein identified in eels potentially binds bilirubin and might provide a simpler and more reliable diagnostic.¹ Convincing hospitals to invest in a new detection device, however, might turn out to be a bigger challenge than developing the assay.

In searching for the source of the green glow in Japanese unagi eels (*Anguilla japonica*), the RIKEN researchers pinpointed a protein called UnaG. UnaG is the first fluorescent protein identified in a vertebrate—previous ones were found in jellyfish or bacteria.

Because cloned UnaG did not fluoresce on its own in bacteria, the researchers looked for and found a cofactor in the eel's blood that was necessary to trigger fluorescence. That cofactor surprisingly turned out to be bilirubin, a key clinical biomarker of liver function.

When UnaG binds bilirubin it triggers a molecular switch that makes it fluoresce green. The fluorescence intensity was proportional to the concentration of bilirubin from 0–25 mg/dL, which spans the range of bilirubin levels found in healthy serum to those seen in bilirubinemia.

UnaG is the first example of a ligand-inducible fluorescent protein. All other known fluorogenic compounds are either constantly fluorescent or fluoresce following covalent binding to another molecule.

The team was led by Atsushi Miyawaki, deputy director of the RIKEN Brain Institute, and results were published in *Cell*.

In humans, bilirubin exists in an unconjugated (UC-BR) and a conjugated (C-BR) form. The sum of both components constitutes what is known as total serum bilirubin (TsB). UC-BR is the key parameter used by physicians to determine disease risk and treatment strategy in neonates.

UnaG binds specifically and with high affinity to UC-BR, which is a lipophilic product of heme metabolism that is transported via serum albumin to the liver. In healthy individuals, UC-BR binds glucuronide covalently in the liver, forming water-soluble C-BR that is excreted into bile. In the compromised liver, formation of C-BR can be impaired, which in turn causes buildup of UC-BR in the circulation to toxic levels.

In neonates, excess bilirubin resulting from inefficient breakdown of hemoglobin can lead to a form of brain damage termed kernicterus.

Treatment is often simple, most commonly using phototherapy, but early diagnosis is essential.

Bilirubin testing is performed routinely on newborn infants with jaundice and on older children and adults with liver disease, sickle cell disease or other forms of hemolytic anemia.

New assay, old instruments

Miyawaki thinks the direct measurement of UC-BR represents one of the advantages a UnaG-based assay would have over available techniques that employ roundabout methods of quantitation and are variable between laboratories.²

Most marketed bilirubin assays are based on a method first developed in 1916. The most widely used assay measures C-BR and TsB and calculates UC-BR by subtracting C-BR from TsB. The assay employs a reagent that changes color upon binding C-BR.

To provide a measure of TsB, a second compound called an accelerator is added to the reaction, which makes the initial reagent bind to all forms of bilirubin and results in a different measurable color change.

The principal manufacturers of these assays are **Roche's** Roche Diagnostics group, **Danaher Corp.** (previously Beckman Coulter) and **Siemens AG's** Siemens Diagnostics unit.

An alternative method is the Vitros assay from **Johnson & Johnson's** Ortho Clinical Diagnostics division. With this method, TsB and hemoglobin are measured

spectrophotometrically. C-BR and UC-BR concentrations are subsequently calculated from those two measurements.

Both techniques are considerably more complex than Miyawaki's assay, which can determine UC-BR levels by direct measurement and works with high sensitivity in both blood and serum.

Stanley Lo, an associate professor at **Medical College of Wisconsin** and associate director of clinical laboratories at **Children's Hospital of Wisconsin**, told *SciBX* that “something specific for unconjugated bilirubin would be very attractive for the newborn population.”

He added that current assays are complex but do fall within the 10% precision required by the **College of American Pathologists** (CAP) Chemistry Resource Committee.³ CAP, along with the **American Academy of Pediatrics**, issues guidelines for bilirubin testing.

Although Miyawaki's assay for direct, sensitive and reliable measurement of UC-BR could provide a superior test for bilirubin, one big hurdle is that few clinical laboratories are equipped with fluorometers.

According to Lo, requiring a new instrument for a single assay could present a significant obstacle to selling it to clinical labs. Hospitals are most open to new assays that do not involve additional investments, so without a wider need for fluorescence detection the assay is likely to encounter challenges in its adoption, he said.

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—Stanley Lo,
Medical College of Wisconsin

Nevertheless, Miyawaki is developing a fluorescence detector for use with the assay. He thinks the device will be simple and inexpensive to construct because it only will need single, fixed excitation and emission filters, a photomultiplier tube and an LED.

He also is performing mutagenesis studies on UnaG in search of a derivative that might bind C-BR with high specificity. The separate and precise measurement of C-BR and UC-BR could provide physicians with a better screening tool to help identify the source of a bilirubin-related liver dysfunction.

The products of the research have been patented by the RIKEN Brain Science Institute and are available for licensing. Miyawaki said he is in discussions with undisclosed companies to commercialize the assay.

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REFERENCES

1. Kumagai, A. *et al. Cell*; published online June 13, 2013; doi:10.1016/j.cell.2013.05.038
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2. Lo, S.F. *et al. Clin. Chem.* **50**, 190–194 (2004)
3. Lo, S.F. & Dumas, B.T. *Semin. Perinat.* **35**, 141–147 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

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RIKEN Brain Science Institute, Saitama, Japan
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
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