Splitting fluorescent protein helps image clusters in live cells

December 26 2009

Half a protein is better than none, and in this case, it's way better than a whole one. A Rice University lab has discovered that dividing a particular fluorescent protein and using it as a tag is handy for analyzing the workings of live cells, particularly in the way they employ iron-sulfur clusters.

Iron and sulfur in just the right amounts are critical to good health. They're in the food people eat and vitamins they take every day, but having too much or too little in the cells can cause serious problems.

Iron-sulfur clusters are molecules with as few as four atoms. They are manufactured and regulated by proteins in living cells, and their role is a fairly recent field of study for researchers interested in Friedreich's ataxia, sideroblastic anemia and myopathy, diseases caused by defects in proteins. But until now, there's been no way to look at such "metalloclusters" in living cells.

Jonathan Silberg, an assistant professor of biochemistry and cell biology at Rice, has been studying the mysteries of these molecules for years. He has come up with a way to see what they're doing in living cells. Silberg and his team published a paper in the December edition of Chemistry & Biology that details a new technique for imaging clusters that involves attaching them, through an intermediary, to fluorescent fragments of protein.

That intermediary is a human protein called GRX2, a glutaredoxin that

helps cells deal with oxidative damage on other proteins. Its activity can be switched off in test tubes by association with an iron-sulfur cluster. The team had already proved that GRX2 would still bond with iron-sulfur clusters even when tagged with a green fluorescent protein; this makes it useful for in vitro studies, but the fluorescence wasn't strong enough to be seen in living cells.

However, attaching fragments of a yellow fluorescent protein called Venus to monomers (single molecules) of GRX2 worked quite well. When injected into living cells, the tagged monomers find and use iron-sulfur clusters as a kind of bridge and bond with each other. That brings the Venus fragments close enough to each other to light up sufficiently to be seen through a microscope.

"If we need an iron-sulfur cluster to get fluorescence, then we have a reporter for those clusters in living cells," Silberg said. The custom proteins can be used to analyze cells for signs of diseases involving iron-sulfur irregularities.

"That's why I'm really excited about this. This is a screen that will allow fundamental biology that nobody can do right now," he said. "And it has high potential for helping us find real treatments for disease."

Silberg said iron and sulfur were present in Earth's primordial stew even before there was oxygen. "The atmosphere was anaerobic when life evolved, and iron and sulfur were plentiful. These metalloclusters are easy to build, so you can imagine that if the chemistry's simple and the molecules are around, proteins will evolve to do a lot of chemistry using iron-sulfur clusters.

"Then photosynthetic organisms evolved and started to produce oxygen. Iron is very easily oxidized, so aerobic organisms evolved all this machinery to protect it, to repair it. That's the machinery we're

studying."

Measuring clusters in live cells is a breakthrough of great interest to the American Heart Association, which partly funded the study. "They gave us money to build more tools," Silberg said. "They're interested in Friedreich's ataxia (which can lead to heart disease), but they also want to know if we can develop ways to image other proteins with metalloclusters."

In this study, he said, "We actually answered a fundamental biological question -- that glutaredoxins associate using metalloclusters in vivo. No one's ever showed that in living human cells."

Refining the tools has high priority in Silberg's lab now, but in the long term, he sees potential for the technology to study the roots of aging itself. Iron is toxic to the body if not managed properly, he said, and since oxidation appears to be central to aging, studies of the process tend to draw a lot of interest.

"Will people age faster because their iron-sulfur cluster assembly is different? To me, the answer is decades out, but it's a very interesting question. How will subtle differences in oxidative stress affect aging?

"It's getting more tantalizing now that there are direct links between defects in iron-sulfur cluster assembly and nuclear genome stability," he said. "It's no longer, 'Oh, mitochondrial oxidative stress is connected somehow to nuclear mutations.' There's evidence that iron-sulfur cluster assembly defects in the mitochondria can be that connection."

The paper's authors are Silberg; Rice postdoctoral researchers Ryan McGuire and Kevin Hoff, who is also affiliated with the California Institute of Technology; Rice graduate student Peter Nguyen; and Stephanie Culler, a postdoctoral scholar, and Christina Smolke, an

assistant professor of chemical engineering, both at CalTech.

Provided by Rice University