CHEMICAL BIOLOGY

Illuminating lipids

Visualization of choline-containing phospholipids in cells and *in vivo* is made possible by the metabolic incorporation of a choline analog with an alkyne handle for click chemistry–based labeling.

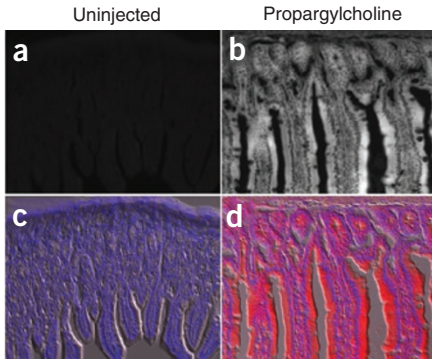
The Cu(I)-catalyzed cycloaddition reaction between an alkyne group and an azide group, better known as 'click chemistry,' has proven its utility in a multitude of applications in biology. Because alkynes and azides are not naturally found in biological molecules, click reactions are incredibly specific. A cell served an appropriate analog of a natural molecule containing a click chemistry handle will metabolically incorporate it into the proper macromolecule, allowing the handle to be specifically labeled with a probe.

Although such methods have been developed for labeling proteins, sugars and nucleic acids in cells, click chemistry has not been applied for labeling lipids. Adrian Salic of Harvard Medical School and his collaborators from Kansas State University now report such an approach for labeling and visualizing choline-containing phospholipids.

Salic, who has a strong interest in cell signaling, wanted to develop a method to visualize phospholipid transport within the cell. Having had previous success with developing click chemistry methods for labeling DNA and RNA, he decided to tackle lipids.

The typical approach for visualizing phospholipids is to synthesize a fluorophore-containing phospholipid molecule, add it to cells and allow it to passively partition into the cell membrane. Salic and his colleagues instead took advantage of metabolic labeling to target choline-containing phospholipids. Choline phospholipids are the most abundant type of phospholipid and are important in cell signaling as well as serving as a major structural component of membranes. The researchers synthesized a choline analog, propargylcholine, which contains an alkyne handle. Cells fed with propargylcholine efficiently incorporated it into phospholipids, which could then be labeled with a fluorophore bearing an azide group.

Salic notes that this approach will allow them to visualize lipids with higher spatial and temporal resolution than the typical method. Such a visualization approach is also complementary to biochemical analysis methods such as organelle fractionation followed by mass spectrometry. With the metabolic labeling approach, "There's the potential for interrogating the entire biosynthetic pathway, which starts with your building block and ends with a complex lipid, distributed within the cell or organism," explains Salic. "So in that respect there are no other comparable methods to visualize lipids." In addition to fluorescence labeling, the researchers can also click on gold nanoparticles for immuno-electron microscopy studies.



Visualizing choline phospholipids *in vivo*: after injection of mice with propargylcholine, strong labeling of the intestines was observed. (**a**,**b**) Staining with fluorescent azide, shown in grayscale. (**c**,**d**) Staining with fluorescent azide (red) overlaid with DNA stain (blue). Reprinted with permission from the National Academy of Sciences, USA.

The researchers used this approach to visualize choline phospholipids in cultured cells as well as in organs from mice injected with propargylcholine. In cells, they observed propargylcholine incorporation into all classes of choline phospholipids with high efficiency, and distribution to the plasma, nuclear and mitochondrial membranes. In mice, they observed strong staining of the intestine, kidney, liver and spleen.

Salic's group is now working to extend the method to other classes of lipids, in addition to using it to address yet unanswered questions about lipid cell biology. "We hope to investigate issues of how lipids are transported about the cell, which before was impossible," he says.

Allison Doerr

RESEARCH PAPERS

Jao, C.Y. *et al.* Metabolic labeling and direct imaging of choline phospholipids *in vivo*. *Proc. Natl. Acad. Sci. USA* **106**, 15332–15337 (2009).