RESEARCH PROFILES

A metabolically labeled rat

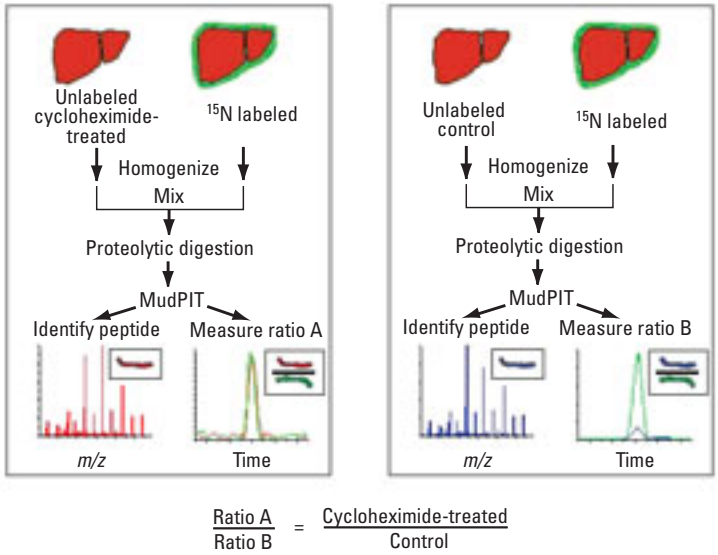
Proteomics researchers often label cultured cells, unicellular organisms, and more complex model organisms, such as the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, by feeding them isotopically labeled substances. But John Yates and colleagues at the Scripps Research Institute, the University of Colorado Health Sciences Center, and the University of Vermont have gone a step further. In this issue of *Analytical Chemistry* (pp 4951–4959), they have metabolically labeled a mammal, the rat *Rattus norvegicus*.

Christine Wu and Michael MacCoss, who were postdoctoral fellows in Yates's laboratory when the study was conducted, led the charge, which has resulted in the first report of a mammal labeled with ^{15}N. "It's been documented before with deuterium, but the animals don't do well with >20% deuterium—they die," says Wu. In contrast, ^{15}N-labeled rats appear healthy and have the same growth curve as normal rats.

According to Wu, an advantage of using ^{15}N instead of other common quantitative labeling procedures, such as isotope-coded affinity tag (ICAT) technology, is that ^{15}N is incorporated into nearly all the amino acids in every protein, whereas ICAT only labels cysteines. If a protein or a peptide fragment does not have a cysteine, it does not get labeled and it is not quantified by the ICAT method. She adds that chemical modification reactions are not as efficient as metabolic labeling.

Although labeling the rats was a fairly simple procedure, it was a little taxing. The researchers added ^{15}N-labeled algae in powder form to rat food that lacked protein. Therefore, all ingested proteins were labeled. Wu and MacCoss fed the rats every 6 h, which also allowed them to monitor the animals closely for any adverse reactions.

Wu and MacCoss used tissues from a labeled rat as an internal standard in a quantitative proteomics study. In that experiment, they fed ^{15}N-labeled food to a rat for 44 days, then spiked liver tissue from the rat into both control and cycloheximide-treated rat liver samples. They digested the proteins and analyzed the peptides by LC/MS/MS. The amount of each unlabeled peptide from both the control rat and the cycloheximide-treated rat was compared separately to its ^{15}N-labeled counterpart in a ratio. Taking the ratio of these two ratios for each peptide revealed changes in protein levels in response to cycloheximide. "This way, the internal standard cancels out," says Wu. "You've also removed a lot of the systematic error that's intrinsic to these [types of] experiments."



A schematic of the quantitative proteomics experiment with a cycloheximide-treated rat, a control rat, and an ^{15}N-labeled rat.

Although cycloheximide is a drug that terminates protein synthesis, the researchers observed an increase in the levels of many proteins. Wu explains that this is partly because they administered sublethal doses, which allowed some translation to occur. Also, the levels of proteins responsible for degradation may have decreased, causing the accumulation of some proteins.

Most of the proteins that changed in cycloheximide-treated rats were membrane-associated. The levels of proteins involved in metabolizing xenobiotics increased with cycloheximide exposure, which was expected because the liver rids the body of foreign substances. The levels of some chaperones that are involved in the quality control of protein folding also increased, possibly because some proteins may not have been fully synthesized when the rat was treated with cycloheximide, which could result in misfolding.

To validate the labeling method, three quality-control proteins were further studied by quantitative Western analysis. The results were similar to those obtained by metabolic labeling.

Until recently, one nagging limitation of the labeling procedure has been the cost of the ^{15}N-labeled algae. When the project began, the cost was high, though Wu says it was comparable to using ICAT methodology. Now, she reports that an algae supplier, Spectra Stable Isotopes, has significantly reduced the price, enabling the researchers to experiment with new ways of feeding the rats. "Before, we would take them out and feed them the powder four times a day. Now, [the algae] is made into pellets, [which are] put into the cage so the rats can have access to it all day long," Wu says.

Wu predicts that ^{15}N labeling will be widely adopted, and many researchers are already asking her for advice on the method. She says that ^{15}N labeling opens the door to many new experiments that examine changes in mammalian protein levels. In fact, Yates and Wu are already working on a project to study rat brain development using this labeling method. ■

—Katie Cottingham