

Up Front

Lessons From Lipids

Betting that genetics will help us understand fatty acids.

Fatty acids are intriguing molecules,” says researcher Marina Kniazeva. “We know that DNA stores information and proteins carry out specific functions, but what do fatty acids do in this scheme?” Kniazeva grapples with that question as a senior research specialist in the lab of Min Han, an HHMI investigator at the University of Colorado at Boulder.

Although his lab has focused on many other cell and developmental biology problems, Han and his colleagues recently backed their way into research on fatty acids, the building blocks of lipids. Searching for a gene mutation that causes a form of retinal degeneration in humans, Kniazeva, Han, and colleagues at other institutions collaborated to identify an enzyme that elongates fatty acid chains. The finding is the first to link a disruption in a fatty acid elongase directly to a disease state.

Kniazeva and Han realized that determining how a defect in this enzyme led to the retinal disease would take extensive molecular and genetic studies. They decided to apply the tools they know the best, genetics, to investigate how fatty acids are made and their functions regulated. “Little is known about why there are so many various fatty acids and why their proper levels are important. This is

still a wide-open area,” he says. “We decided to take a look.”

A DIFFERENT BREED

Fatty acids are made up of carbon chain backbones, which can be saturated (with no double bonds) or unsaturated (with double bonds that cause kinks in the chain)—traits that make them a different breed from, say, proteins. For one thing, the study of the oily, uncharged properties of fatty acids requires a special set of biochemistry skills and instruments. Lipids have different structures than proteins, and, unlike proteins, lipid structures do not necessarily hold information about their function.

Scientists know that some lipid molecules like cholesterol circulate throughout the body; others get incorporated into the zipper-like molecules of the cell membrane. One famous group, the omega-3 fatty acids found in certain kinds of fish, are thought to help prevent heart disease. Changes in fatty acid profiles have been linked to diseases such as cancer. And because lipids make up more than half the dry weight of our brains, the balance of various fatty acid levels is likely to be important in the central nervous system.

For the most part, though, researchers still don’t understand how the fatty acid composition of cells affects daily operations

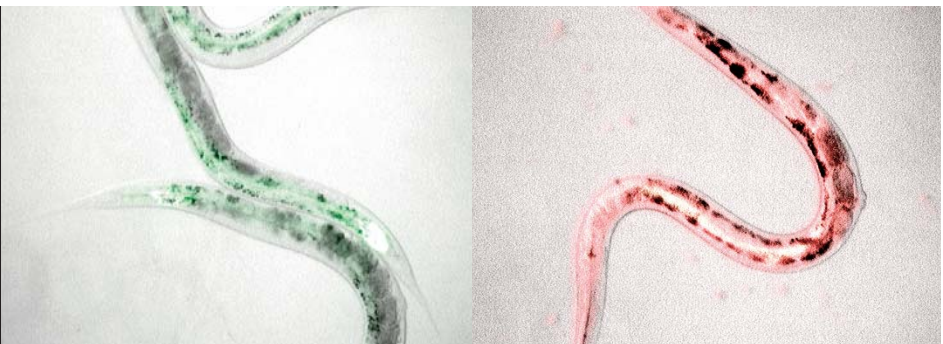


Min Han (left) and colleague Marina Kniazeva blend genetics, biochemistry, and genomic technology.

or how it might cause disease if that profile gets out of whack. And because fatty acids are not synthesized directly from DNA, as proteins are, studying them requires indirect genetic manipulation: Han’s lab can exploit the genes that make the enzymes that direct the synthesis of fatty acids, but not the fatty acids themselves.

“It’s a difficult field to start with,” says

Nematode Code *Min Han’s laboratory uses green fluorescent protein to localize tissues producing proteins of interest in the round-worm *C. elegans*. The branched-chain fatty-acid-making enzyme works in the gut as indicated by green color (left). Red lipophilic dye stains fat in the worm’s intestine (right), allowing researchers to visualize defects in lipid metabolism.*



RAY NG (TOP), MARINA KNAZEVA (BOTTOM)



Han, “and because we are not lipid people we need to learn a lot. And we will get a lot of scrutiny. So it’s a risky area for us to get into.”

But Han is a betting man. After middle school, he worked on a farm for four years in China. He crammed three years of high school into seven months of self-tutoring to get into Beijing University. He was later selected through an examination to come to the United States for graduate study in molecular biology. But like any good betting man, Han tries to play his cards to advantage; his lab studies not humans, but the much simpler system of the transparent, 959-cell, 1-millimeter-long roundworm, *Caenorhabditis elegans*—their chosen lab model for the last 12 years.

“Fatty acid profiles are pretty much the same for all worms,” he says. “We’ll use the worms to study how these enzymes are organized to maintain precise levels of fatty acids.”

HOW WORMS RESPOND

To understand how worms respond to raising and lowering different fatty acids, Kniazeva and Han came up with an approach that

blends classic genetics and biochemistry with cutting-edge genomic technology.

In the first round of their approach, the team knocked out eight of the predicted elongase enzyme genes in worms using RNA interference (RNAi). The technique causes worm cells to destroy the RNA message that would normally direct a cell to make a specific elongase. The RNAi effectively shuts down production of the enzyme, thereby dramatically reducing the level of the fatty acids it generates. Having this kind of control allowed Han’s lab to begin to characterize the elongases and their corresponding products.

Results of the first round of studies, published in the January 2003 issue of *Genetics*, showed that the ELO-2 enzyme acts to elongate palmitate, a 16-carbon fatty acid, into longer chains with 18 and 20 carbons. If the enzyme is knocked out using RNAi, then palmitate piles up and the longer-chain fatty acids decrease. The upset in the fatty acid profile causes multiple problems for the worms, which grow slowly, lay fewer eggs, and change their rhythmic digestive pattern.

Such diverse and serious disruptions hint at the importance of having just the right balance of fatty acids. Two other elongase knockouts, ELO-5 and ELO-6, led to lower levels of branched fatty acids whose functions in animal cells are not clearly understood. These worms stopped growing abruptly at the first larval stage, indicating that these fatty acids must be crucial to worm growth and development. (This work has recently been submitted for publication.)

The second round of the approach involved running a DNA microarray analysis on two elongase knockout worm strains. By surveying the whole worm genome, the microarray tells Kniazeva which genes’ expression levels go up or down in response to the missing enzyme and fatty acid. From those results, she picked a number of candidates that seemed most likely to be involved in regulating fatty acid production. Now, she has made RNAi knockouts for 25 genes and used gas chromatography to get the fatty acid readout from each one. From the analysis, they have identified genes that are likely to be involved in branched fatty acid production. If need be, the team will go back and do a microarray analysis for knockouts of these regulatory genes as well, repeating the process until each gene and its corresponding enzyme or protein is placed in the correct spot of the fatty acid synthesis pathway. The group has learned a great deal from the previous work of other lipid researchers, including Michael S. Brown, HHMI Trustee Joseph L. Goldstein, and HHMI investigator David J. Mangelsdorf, all at the University of Texas Southwestern Medical Center at Dallas.

These days, Han says, you have to look at the whole genome to get the complete picture. “And as a worm geneticist,” he says, “it makes it easy to go after functions.” Observing the RNAi-treated worms should quickly reveal any problems in muscle, nerve, feeding, or reproductive systems that the disrupted fatty acid metabolism may cause.

Han explains why he thought the problem of fatty acid regulation was worth taking the lab down a new path: “Since not many people are working in this area, our contribution to science as a whole can be bigger.” And besides, he says with a gambler’s grin, “As a scientist, I *have* to have the attitude that I have nothing to lose.”

—KENDALL POWELL