QnAs with John E. Cronan

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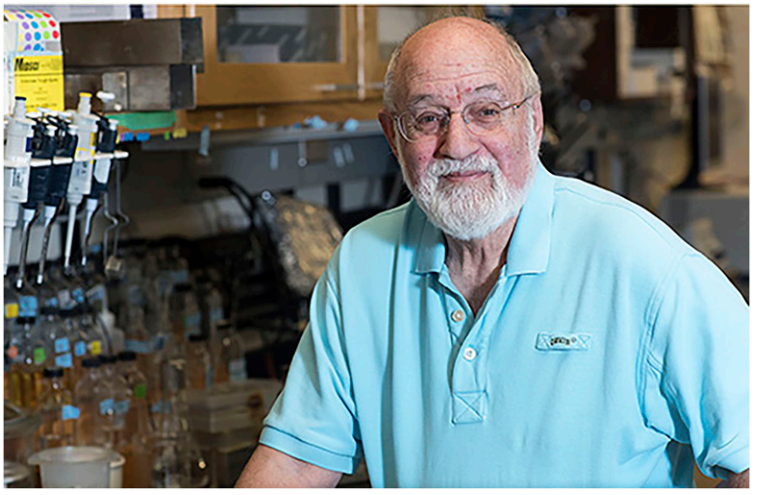
John E. Cronan has spent a long and distinguished research career studying the various pathways by which lipids and fatty acid-derived cofactors, such as biotin and lipoic acid, are synthesized. Cronan and his colleagues have uncovered several important biosynthetic pathways, including one for the cofactor lipoic acid. These biomolecules play a wide variety of roles in ensuring normal cellular function. A professor of microbiology and biochemistry at the University of Illinois at Urbana–Champaign, Cronan was elected to the National Academy of Sciences in 2017. Cronan tells PNAS about his latest research on lipoic acid synthesis, the focus of his Inaugural Article (1).

PNAS: How did you become interested in studying lipids?

Cronan: It actually goes way back to when I was an undergraduate, when I did a summer internship at [the University of California, Los Angeles] and started working on fatty acids. As a result, I knew something about lipids when I went to graduate school with Daniel Wulff at [the University of California,] Irvine. Way back then, we didn't know the lipid composition of [*Escherichia*] *coli* in a physiological context, and I [was] working on lipids in bacteria for my thesis. I then went to Washington University St. Louis to postdoc with Roy Vagelos, who had done seminal biochemical work on fatty acids. His laboratory didn't have anybody [who] was doing genetics. So I studied the genetics of some of these fatty acid pathways, learned some enzymology, and had two productive years there. Then I went to Yale as a faculty member and stayed there for 8 years, and then moved to the University of Illinois. I've always worked on bacterial lipids, although along the way I've also worked a little bit on yeast and plant lipids.



John Cronan. Image courtesy of Steph M. Adams (University of Illinois at Urbana–Champaign, Urbana, IL).

PNAS: What led to your Inaugural Article (1) on lipoic acid biosynthetic pathways in bacteria?

Cronan: At some point I became interested in vitamins that are made off the fatty acid biosynthetic pathway, one of which is lipoic acid. Lipoic acid had been discovered about 60 years ago, but nobody knew how it was made. We got started on that, and found that there are two pathways for this, a simple *E. coli* pathway, and a more complicated pathway in *Bacillus subtilis*. A protein called GcvH [glycine cleavage H protein] acts as sort of a hub in the *B. subtilis* pathway. It accepts octanoic acid, which is converted to lipoic acid, and shuttles it on to these other proteins. What piqued my curiosity was whether the GcvH proteins of bacteria that don't do it that way, such as *E. coli*, are different from the *B. subtilis* one.

PNAS: What did you find?

Cronan: Eric Smith, at the Santa Fe Institute, had proposed that the GcvH glycine cleavage system is actually the primordial pathway (2). I had liked that idea and I thought he had made a strong argument, and so I fully expected that if we substituted the *E. coli* GcvH for the *B. subtilis* GcvH it wouldn't work. But it does, and a variety of others also have hub function. We repeated the experiment a few times, including with GcvHs from diverse sources, and we kept getting the same answer. It turns out some other bacteria cheerfully make the same stuff using different pathways and different enzymes. Eric had done his analysis

This is a QnAs with a member of the National Academy of Sciences to accompany the member's Inaugural Article on page 647 in issue 4 of volume 115.

Published online June 11, 2018.

on *Aquifex*, which has five of these GcvH proteins, and thus we thought it was only reasonable to look at *Aquifex* and see whether these putative GcvH proteins had this capacity to substitute for the *B. subtilis* GcvH protein. We had some of them that do, and some that don't, some that look like GcvHs but aren't, some that can do the hub function, and some that can't.

PNAS: What is the significance of these findings?

Cronan: It's an example of protein "moonlighting" done by a very small protein, a protein that has a highly conserved structure but is still is able to do two jobs. This whole idea of protein moonlighting is an interesting one that has come up in recent years. You have a protein that does reaction *A*, and it can learn how to do reaction *B* sometimes without losing reaction *A*. The idea of one gene/one protein/one enzyme has been overturned at several levels now, and this is another example of that. We knew for a long time that one protein can have more than one activity, but something as small as the GcvH proteins having two jobs is unexpected.

PNAS: Are there any hypotheses for how GcvH's primordial function is conserved in *E. coli* despite the influence of genetic drift?

Cronan: That's an interesting question, and we've seen some other examples of this. Maybe this thing is conserved because it plays a structural role or could have functions in other pathways we don't know about. The hand-wavy argument is simply that evolution isn't over yet.

PNAS: What follow-up experiments are you planning to do?

Cronan: Right now we're working on the human lipoic acid biosynthetic pathway. We've actually shown that the human pathway is essentially the *B. subtilis* pathway and there are some neonatal disorders caused by lack of pathway enzymes. We're also looking at some other bacteria that are interesting. One thing we found in collaboration with a German group is a new function for lipoic acid in bacteria that oxidize sulfur compounds. In the sulfur-oxidizing bacteria, again the GcvH–look-alike proteins don't do the GcvH reaction. We will also study the *Aquifex* GcvH proteins, to see if we can figure out the differences among them. We would have to take a genetic approach, because eye-balling these things doesn't give you any clear idea of what's going on, they're fairly diverse. It's a challenge, but we've built up a fairly complete toolbox over the years.

1 Cao X, Hong Y, Zhu L, Hu Y, Cronan JE (2018) Development and retention of a primordial moonlighting pathway of protein modification in the absence of selection presents a puzzle. *Proc Natl Acad Sci USA* 115:647–655.

2 Braakman R, Smith E (2014) Metabolic evolution of a deep-branching hyperthermophilic chemoautotrophic bacterium. *PLoS One* 9:e87950.