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Classics

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Exploring the World of Phospholipids and Their Interactions with Proteins: The Work of William Dowhan

The Gene Encoding the Phosphatidylinositol Transfer Protein Is Essential for Cell Growth
(Aitken, J. F., van Heusden, G. P., Temkin, M., and Dowhan, W. (1990) *J. Biol. Chem.* 265, 4711–4717)

A Phospholipid Acts as a Chaperone in Assembly of a Membrane Transport Protein
(Bogdanov, M., Sun, J., Kaback, H. R., and Dowhan, W. (1996) *J. Biol. Chem.* 271, 11615–11618)

William Dowhan's curiosity about the connections between phospholipids and proteins associated with them goes back as far as his days as a graduate student with Esmond Snell at the University of California, Berkeley. In these two JBC Classics, his group's ability to manipulate biochemical and molecular genetics tools to answer fundamental questions about lipid biology shines through. "William Dowhan and his research group have made many contributions to the biochemistry of phospholipid metabolism and membrane biogenesis," says Robert Simoni at Stanford University.

Bill Dowhan (*right*) is shown here with the late Chris Raetz (*left*), who was a longtime collaborator and friend, and his former postdoctoral advisor, the late Gene Kennedy, on the occasion of Kennedy's 90th birthday in 2009 (photo courtesy of William Dowhan).

The first paper, published in 1990, documented the importance of phosphatidylinositol/phosphatidylcholine transfer proteins *in vivo*. Dowhan's group, which has been based at the University of Texas Medical School since 1972, used a combination of biochemistry and genetics to clone the protein's gene. Dowhan had first heard of phospholipid transfer proteins in 1969, when he began his postdoctoral training with Eugene (Gene) Kennedy at Harvard Medical School. At his very first Kennedy lab meeting, the discussion centered around a publication that had just come out (1). The paper described "one of the first observations of proteins in the soluble phase that transferred lipids between bilayers," recalls Dowhan. "No one could figure out what these proteins really did *in vivo*, but they knew the proteins had this function" of transferring lipids between membranes.

As he moved through his career, Dowhan focused on cloning and characterizing genes and purifying enzymes responsible for phospholipid metabolism in *Escherichia coli*. Then came a sabbatical in 1983 with Gottfried (Jeff) Schatz at the Biozentrum of the University of Basel in Switzerland, that expanded Dowhan's research directions into yeast genetics. He says the opportunity to work with Schatz "got me into the possibility of looking for this phosphatidylinositol/phosphatidylcholine transfer protein (PI-TP) in yeast, which I probably would have never done if I hadn't taken this sabbatical."

Fresh from his sabbatical, Dowhan started tracking down the protein and its gene *in vivo*. "I submitted a grant at that time with some preliminary data that we had begun to purify to

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homogeneity the PI-TP from yeast, which had never been done before. Fortunately, we got the grant," he says.

The Dowhan group managed to purify PI-TP from yeast. "The most important part was using basic biochemistry and understanding how to purify proteins before the advent of genetically tagging proteins for affinity chromatography," explains Dowhan.

For the next step in the process of finding the gene for the protein, Dowhan and colleagues had to apply reverse genetics because the yeast genome was not available in the late 1980s. They sequenced the amino terminus of the protein, made the corresponding oligonucleotide probes, tested yeast cDNA libraries with those probes, and pulled out the gene. "We still didn't know the role PI-TP played in cell function. But now we had the sequence of the gene and the knock-out mutant was not viable," notes Dowhan. "So we published" the findings.

At the same time, Vytas Bankaitis, now at the University of North Carolina, had been working on cloning the *SEC14* gene in yeast, which is necessary for vesicular transport. "It turns out we had missed the DNA sequence," Dowhan says. From Bankaitis' work, it was obvious that "PI-TP was the product of the *SEC14* gene. It all came together in a joint report in *Nature*. Now we had a function associated with the *SEC14* gene, which we didn't have before," Dowhan explains (2). "We had a phenotype of a mutant lacking this phospholipid transfer protein, which then stopped vesicular transport."

This initial link between phospholipid metabolism and vesicular transport opened up the field to characterization of the Sec14 protein superfamily in a broad range of biological systems. These proteins contain lipid-binding domains, which sense membrane lipid composition and integrate lipid metabolism and lipid-mediated signaling with an array of cellular processes.

The second JBC Classic focused on a different feature of phospholipids: their role in protein folding. Dowhan was fascinated by membrane proteins ever since he was a graduate student and had gone to the Kennedy laboratory as a postdoctoral fellow, intending to purify the membrane component expressed by the *lac* operon for lactose transport in *E. coli*. He was unsuccessful because, at that time, the necessary detergents were not available. Once the lactose permease was purified (3), Dowhan noticed in the literature that other researchers mentioned that when the protein was reconstituted in liposomes missing phosphatidylethanolamine, the protein was defective in energy-dependent uphill transport. Dowhan recalls that he wondered, "Was that an artifact of the liposome system or was that also true *in vivo*?"

To get to the bottom of this observation, Dowhan's group used *E. coli* to generate null mutants of what were considered to be absolutely essential genes for phospholipid synthesis and cell viability. They created a null mutant of the *pssA* gene, which encodes the committed step to the synthesis of the major phospholipid, phosphatidylethanolamine. By establishing conditions in which bacterial cells lacking phosphatidylethanolamine remained viable, the investigators were able to identify and characterize different cell phenotypes caused by the missing phospholipid both *in vivo* and *in vitro*. In collaboration with Ronald Kaback at UCLA, Dowhan's group showed that phosphatidylethanolamine was essential for the proper folding of an epitope of lactose permease that was also necessary to support the energy-dependent uphill transport of lactose. "Studies by others have since shown a similar chaperone role for lipids in other bacteria, plants and mammalian cells," notes Simoni.

To obtain their data, the investigators developed a new technique, the Eastern-Western blot. In this method, membrane proteins were delipidated and partially denatured by SDS. The proteins underwent gel electrophoresis and then were transferred to a solid support by Western blotting. A series of individual lipids were then laid over the proteins at a 90° angle so that the investigators could see, after incubating with conformation-specific antibodies, which lipids helped which membrane proteins regain proper conformation.

This technique was used to establish that phosphatidylethanolamine was necessary in a late step of folding of lactose permease, but was not necessary to maintain the final folded state. This observation suggested that lipids act as molecular chaperones in helping protein maturation. "This paper set the stage for understanding how lipids affect the topological organization of wild-type proteins in the membrane," notes Dowhan.

Dowhan and his collaborator Mikhail Bogdanov have continued using bacterial mutants in phospholipid metabolism to systematically manipulate the native membrane lipid compositions during the cell cycle. They have analyzed the transmembrane domain orientation of

membrane proteins to establish the molecular basis for lipid-dependent organization of lactose permease and other secondary transporters (4).

Dowhan says his work has two take-home messages. One is that "Lipids aren't just glorified biological detergents," he says. "They have specific roles" in the cell. The other message is in the power of numbers. Dowhan says the more techniques applied to solve a biological mystery, the more likely the mystery will be successfully solved.

Robert Simoni at Stanford University (JBC Associate Editor) nominated the papers as Classics and Rajendrani Mukhopadhyay (ASBMB's Senior Science Writer) wrote the introduction.

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