AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology®

COMMENTARY

Cues from the Membrane: Bacterial Glycerophospholipids

Zachary D. Dalebroux

Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

ABSTRACT In this issue of the *Journal of Bacteriology*, V. W. Rowlett et al. unveil new *Escherichia coli* circuitry linking membrane glycerophospholipid (GPL) homeostasis to bacterial stress response and adaptation mechanisms (J Bacteriol 199:e00849-16, 2017, https://doi.org/10.1128/JB.00849-16). Glycerophospholipids comprise critical components of the dual-membrane envelope of Gram-negative bacteria and participate in many processes. The new evidence suggests that, in some instances, distinct *E. coli* GPL molecules function for distinct biochemistry and bacteria sense perturbations in membrane GPL concentrations to coordinate survival strategies. Understanding GPL sensing and remodeling mechanisms will be important moving forward, given the breadth of function for these molecules in bacteriology.

KEYWORDS *Enterobacteriaceae*, *Escherichia coli*, barrier function, global regulatory networks, membrane structure, phospholipid-mediated signaling, phospholipids, stress response

Accepted manuscript posted online 24 April 2017

Citation Dalebroux ZD. 2017. Cues from the membrane: bacterial glycerophospholipids. J Bacteriol 199:e00136-17. https://doi.org/10.1128/JB.00136-17.

Editor Victor J. DiRita, Michigan State University

Address correspondence to zdalebro@ouhsc.edu.

For the article discussed, see https://doi.org/10.1128/JB.00849-16.

Evolution provided nature with lipid amphiphiles to build barriers known as membrane bilayers. As a boundary and scaffold, the lipid bilayer comprises the principal surface of most cellular life. Humans have exploited the amphipathic properties of lipids for millennia and studied their biochemistry for nearly a century. Nevertheless, sparse knowledge exists of how particular lipids impact cell biology, especially in prokaryotes. In this issue of the *Journal of Bacteriology*, Rowlett et al. provide a biochemical and physiologic *tour de force* and analysis of *Escherichia coli* mutants lacking either the phosphatidylethanolamine (PE) or cardiolipin (CL) family of glycerophospholipids (GPL) (1).

Eugene P. Kennedy (1919 to 2011) and colleagues elucidated the GPL biosynthesis pathway between the mid-1950s and early 1970s by assaying rat liver mitochondria and *E. coli* extracts for catalysis (2–4). During the late 1970s and early 1980s, bacterial-mutant screens and gene-mapping approaches made it possible to identify the loci for these activities. Kennedy's pupils embraced the new approaches, especially Christian R. H. Raetz (1946 to 2011) and William Dowhan, an author of the work discussed here (1). Raetz thrust the field forward by designing groundbreaking mutant screens to identify lesions in genes like *cdsA*, *pss*, *psd*, and *pgsA* by colony autoradiography and *in vivo* activity screening (5–9). The approach worked elegantly, and by the 1990s, many *E. coli* open reading frames for Kennedy pathway enzymes had been mapped and mutated. Several genes for GPL biosynthesis were conditionally essential for bacterial viability, making their study complex. Rowlett et al. (1) revisit historic mutants, including a Pss-null mutant that lacks PE lipids and is viable only in the presence of nonphysiologic concentrations of $MgCl_2$ (20 to 50 mM) (10). The PE-deficient strain is compared to a more recently engineered CL-deficient *E. coli clsABC* triple mutant, in which what we now understand to be three distinct CL synthase enzymes are deleted (11).

Rowlett et al. identify strong phenotypes for PE- and CL-deficient *E. coli* strains, including dramatic alterations in morphology, envelope structure, O antigen chain

length, carbon flux into surface molecules, outer membrane (OM) protein folding, energy production, membrane potential, metabolism, and biofilm formation (1). Removing PE has pleiotropic effects on *E. coli* physiology, and titrating *pss* expression in the mutant rescues phenotypes in a dose-dependent manner. Cardiolipin deficiency attenuates many of the same functions as PE depletion in the bacteria. Cardiolipin depletion also causes varied effects on bacterial synthesis and assembly of surface carbohydrates and biofilm formation. For example, the CL-deficient bacteria produce a longer O antigen structure than the wild type, while PE-depleted cells generate a more truncated molecule. For the latter, the O antigen chain length is restored to the wild type upon *pss* induction. Furthermore, the CL-deficient *E. coli* cells do not form biofilms, while bacteria in which PE is deleted are biofilm hyperproducers. Finally, bacteria in which CL is deleted that are exposed to hydrogen peroxide and basic pH grow better than the unexposed cells, a response not observed for the wild type. The data support the idea that *E. coli* cells employ CL molecules for specific functions.

Many of the enzymes and activities for GPL biosynthesis are conserved between *E. coli* and mammals. Cardiolipin is a major exception. The primary bacterial CL synthase for *E. coli*, ClsA, catalyzes a reaction whose substrates and mechanisms are distinct from those of the mammalian CL synthase in mitochondria (12). In fact, both ClsA and ClsB catalyze a condensation reaction between two phosphatidylglycerol (PG) substrates to form the CL product (11, 13). The third *E. coli* enzyme, ClsC, catalyzes a reaction involving PG and PE substrates (11). Therefore, multiple mechanisms and enzymes for CL synthesis exist, supporting the idea that the pathway is under strong positive selection for variation and redundancy. In further support of specialized roles for CL in Gram-negative bacteriology, the genes encoding *pss*, *psd*, and *pgsA* are each conditionally essential for bacterial viability, but *clsA*, encoding the major CL synthase, is not (10, 14–17). In fact, even the *clsABC* triple mutant assessed by Rowlett et al. shows no growth rate attenuation in rich broth medium compared to the growth of the wild type (1). Therefore, unlike most other major GPL families, CL is biochemically and genetically redundant for *E. coli* and performs specialized nonessential functions.

To understand the stress responses elicited by PE and CL deficiency, the authors developed gene-reporter assays, Western blots, and second-messenger measurements to detect the abundance and activity of key regulators. Many of the promoters chosen for the reporter constructs are regulated by more than one transcription factor, making the results challenging to interpret quantitatively. Fortunately, the authors probe further and directly measure the protein and nucleotide abundance. The analysis provides a snapshot of the stress response elicited by the loss of either PE or CL. They blot for the housekeeping sigma factor, RpoD, the alternative sigma factors, RpoE, RpoH, and RpoS, a periplasmic protease, DegP, a chaperone, DnaK, and a heat shock-related stress protein, GroL. The data suggest that PE- and CL-deficient cells activate a variety of cytoplasmic and envelope-related stress response mechanisms. Both PE- and CL-deficient bacteria activate the stringent response pathway, since the levels of the nucleotide alarmones guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), collectively referred to as ppGpp, increase in the mutants compared to their levels in the wild-type bacteria. Subtle differences in the levels of key regulators exist between the PE and CL deletion mutant bacteria. In general, PE depletion caused dramatic increases in all the stress-related regulatory molecules analyzed and a strong decrease in the housekeeping molecule RpoD. In contrast, CL deficiency caused a less dramatic increase in the regulators, especially RpoE and RpoS, while the levels of RpoD were not affected. Thus, *E. coli* might harbor distinct sensory mechanisms to detect perturbations in either PE or CL abundance. Cardiolipin deletion increased the level of ppGpp but did not cause a concomitant decrease in RpoD, suggesting that the response to CL perturbation is complex. A principal role for the ppGpp second messenger is to decrease *rpoD* promoter activity, such that RNA polymerase is made

available to the alternative sigma factors. Therefore, bacterial adaptation to CL depletion might involve the simultaneous expression of homeostatic and stress response systems. The results are consistent with *E. coli* sensing membrane lipid perturbation by regulatory mechanisms.

Evidence in the literature supports the idea that bacterial stress sensors might respond to GPL concentrations directly. For example, the CpxA inner membrane (IM) sensor kinase responds to a variety of signals originating within the *E. coli* envelope, and PE and PG deficiencies induce CpxA activity (18–21). Phosphatidylethanolamine is synthesized from serine, and the Pss enzyme associates with the ribosome (22). The primary ppGpp synthetase in *E. coli*, RelA, also interacts with the ribosome, where it detects uncharged tRNAs that accumulate in response to amino acid starvation (23). Perhaps signaling occurs between Pss and RelA at the ribosome to control ppGpp increases during fluctuations in amino acid substrate availability. Finally, the OM sensor lipoprotein, RcsF, detects OM stress, including alterations in lipid content (24). It is now recognized that RcsF can be threaded through the lumen of OM beta-barrel proteins to access the OM outer leaflet and detect lipid perturbations (25, 26). Therefore, *E. coli* regulators harbor mechanisms to sense and respond to alterations in membrane lipid content.

From the late 1970s to the early 1980s, Raetz and others continued to probe the mutants that were defective for GPL biosynthesis. A particular temperature-sensitive mutant has a mutation in *pgsA*, the enzyme that catalyzes the first committed step in the synthesis of PG and CL, which caused the accumulation of two unknown glycolipid species within the *E. coli* membrane, lipids X and Y (27). These molecules were identified as synthetic precursors to the lipid A disaccharolipid, which comprises the majority of the OM outer leaflet and forms the hydrophobic base of the LPS superstructure. The data strongly indicated a critical intersection between acidic GPL and lipid A biosynthesis. However, the field was propelled toward identifying the genes encoding the lipid A biosynthesis enzymes. Soon it was determined that lipid A could be regulated by Gram-negative bacteria to increase resistance to antibiotics and decrease pathogen recognition by innate immune receptors. Until recently, there was minimal evidence to suggest that Gram-negative bacteria regulate the GPL composition of the envelope in response to changes in their environment. The exception again was CL, whose content increases upon entry into stationary phase (28, 29). Recent studies in *Salmonella enterica* serovar Typhimurium demonstrate that the PhoPQ virulence regulators control OM acidic-GPL content to increase OM barrier function and intracellular survival during pathogenesis in mice (30). The chemical modifications regulated include increased CL trafficking to the OM and increased activity of the OM palmitoyltransferase/phospholipase A1 enzyme, PagP (30, 31). In fact, PhoPQ activation promotes PagP palmitoylation of both lipid A and PG substrates within the OM outer leaflet (31).

Gram-negative bacteria likely harbor a variety of mechanisms to alter membrane GPL content. However, it remains challenging to study GPL molecules, in part owing to the biochemical complexity of the cell envelope, which consists of several distinct layers of varied chemical composition.

Within the dual-membrane cell envelope of *E. coli*, GPL structures interact with other lipid amphiphiles, such as neutral glycerolipids, lipopolysaccharides, membrane-derived glycolipids, and capsular exopolysaccharides. Unfortunately for experimentalists, bacteria rely upon GPL to synthesize, bind, and assemble most of the other envelope constituents. This has made gene knockout studies difficult to interpret. Consequently, progress in understanding the signals and regulatory conditions that cue bacteria to alter membrane GPL composition has been slow, and sparse knowledge exists for how alterations in GPL composition and structure affect physiology and adaptation. Only recently have we begun to understand the mechanisms by which GPL perturbance might cue bacterial sensors of stress. The work by Rowlett et al. (1) and others continues to probe this biology, and many questions remain.

ACKNOWLEDGMENT

Work in my laboratory was funded by National Institutes of Health award number P20GM103447.

REFERENCES

1. Rowlett VW, Mallampalli VKPS, Karlstaedt A, Dowhan W, Taegtmeyer H, Margolin W, Vitrac H. 2017. Impact of membrane phospholipid alterations in *Escherichia coli* on cellular function and bacterial stress adaptation. J Bacteriol 199:e00849-16. https://doi.org/10.1128/JB.00849-16.
2. Bublitz C, Kennedy EP. 1954. Synthesis of phosphatides in isolated mitochondria. III. The enzymatic phosphorylation of glycerol. J Biol Chem 211:951–961.
3. Kennedy EP, Weiss SB. 1956. The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 222:193–214.
4. Kanfer JN, Kennedy EP. 1962. Synthesis of phosphatidylserine by *Escherichia coli*. J Biol Chem 237:PC270–PC271.
5. Ganong BR, Leonard JM, Raetz CR. 1980. Phosphatidic acid accumulation in the membranes of *Escherichia coli* mutants defective in CDP-diglyceride synthetase. J Biol Chem 255:1623–1629.
6. Nishijima M, Raetz CR. 1979. Membrane lipid biogenesis in *Escherichia coli*: identification of genetic loci for phosphatidylglycerophosphate synthetase and construction of mutants lacking phosphatidylglycerol. J Biol Chem 254:7837–7844.
7. Raetz CR. 1976. Phosphatidylserine synthetase mutants of *Escherichia coli*. Genetic mapping and membrane phospholipid composition. J Biol Chem 251:3242–3249.
8. Raetz CR. 1975. Isolation of *Escherichia coli* mutants defective in enzymes of membrane lipid synthesis. Proc Natl Acad Sci U S A 72:2274–2278. https://doi.org/10.1073/pnas.72.6.2274.
9. Raetz CR, Larson TJ, Dowhan W. 1977. Gene cloning for the isolation of enzymes of membrane lipid synthesis: phosphatidylserine synthase overproduction in *Escherichia coli*. Proc Natl Acad Sci U S A 74:1412–1416. https://doi.org/10.1073/pnas.74.4.1412.
10. DeChavigny A, Heacock PN, Dowhan W. 1991. Sequence and inactivation of the *pss* gene of *Escherichia coli*. Phosphatidylethanolamine may not be essential for cell viability. J Biol Chem 266:5323–5332.
11. Tan BK, Bogdanov M, Zhao J, Dowhan W, Raetz CR, Guan Z. 2012. Discovery of a cardiolipin synthase utilizing phosphatidylethanolamine and phosphatidylglycerol as substrates. Proc Natl Acad Sci U S A 109:16504–16509. https://doi.org/10.1073/pnas.1212797109.
12. Schlame M. 2008. Cardiolipin synthesis for the assembly of bacterial and mitochondrial membranes. J Lipid Res 49:1607–1620. https://doi.org/10.1194/jlr.R700018-JLR200.
13. Guo D, Tropp BE. 2000. A second *Escherichia coli* protein with CL synthase activity. Biochim Biophys Acta 1483:263–274. https://doi.org/10.1016/S1388-1981(99)00193-6.
14. Pluschke G, Hirota Y, Overath P. 1978. Function of phospholipids in *Escherichia coli*. Characterization of a mutant deficient in cardiolipin synthesis. J Biol Chem 253:5048–5055.
15. Kikuchi S, Shibuya I, Matsumoto K. 2000. Viability of an *Escherichia coli pgsA* null mutant lacking detectable phosphatidylglycerol and cardiolipin. J Bacteriol 182:371–376. https://doi.org/10.1128/JB.182.2.371-376.2000.
16. Shiba Y, Yokoyama Y, Aono Y, Kiuchi T, Kusaka J, Matsumoto K, Hara H. 2004. Activation of the Rcs signal transduction system is responsible for the thermosensitive growth defect of an *Escherichia coli* mutant lacking phosphatidylglycerol and cardiolipin. J Bacteriol 186:6526–6535. https://doi.org/10.1128/JB.186.19.6526-6535.2004.
17. Suzuki M, Hara H, Matsumoto K. 2002. Envelope disorder of *Escherichia coli* cells lacking phosphatidylglycerol. J Bacteriol 184:5418–5425. https://doi.org/10.1128/JB.184.19.5418-5425.2002.
18. Grabowicz M, Silhavy TJ. 2017. Envelope stress responses: an interconnected safety net. Trends Biochem Sci 42:232–242. https://doi.org/10.1016/j.tibs.2016.10.002.
19. Keller R, Ariöz C, Hansmeier N, Stenberg-Bruzell F, Burstedt M, Vikström D, Kelly A, Wieslander Å, Daley DO, Hunke S. 2015. The *Escherichia coli* envelope stress sensor CpxA responds to changes in lipid bilayer properties. Biochemistry 54:3670–3676. https://doi.org/10.1021/acs.biochem.5b00242.
20. Mileykovskaya E, Dowhan W. 1997. The Cpx two-component signal transduction pathway is activated in *Escherichia coli* mutant strains lacking phosphatidylethanolamine. J Bacteriol 179:1029–1034. https://doi.org/10.1128/jb.179.4.1029-1034.1997.
21. Itou A, Matsumoto K, Hara H. 2012. Activation of the Cpx phosphorelay signal transduction system in acidic phospholipid-deficient *pgsA* mutant cells of *Escherichia coli*. Biochem Biophys Res Commun 421:296–300. https://doi.org/10.1016/j.bbrc.2012.04.003.
22. Raetz CR, Kennedy EP. 1972. The association of phosphatidylserine synthetase with ribosomes in extracts of *Escherichia coli*. J Biol Chem 247:2008–2014.
23. Dalebroux ZD, Swanson MS. 2012. ppGpp: magic beyond RNA polymerase. Nat Rev Microbiol 10:203–212. https://doi.org/10.1038/nrmicro2720.
24. Konovalova A, Mitchell AM, Silhavy TJ. 2016. A lipoprotein/β-barrel complex monitors lipopolysaccharide integrity transducing information across the outer membrane. eLife 5:e15276. https://doi.org/10.7554/eLife.15276.
25. Konovalova A, Perlman DH, Cowles CE, Silhavy TJ. 2014. Transmembrane domain of surface-exposed outer membrane lipoprotein RcsF is threaded through the lumen of beta-barrel proteins. Proc Natl Acad Sci U S A 111:E4350–E4358. https://doi.org/10.1073/pnas.1417138111.
26. Cho SH, Szewczyk J, Pesavento C, Zietek M, Banzhaf M, Roszczenko P, Asmar A, Laloux G, Hov AK, Leverrier P, Van der Henst C, Vertommen D, Typas A, Collet JF. 2014. Detecting envelope stress by monitoring beta-barrel assembly. Cell 159:1652–1664. https://doi.org/10.1016/j.cell.2014.11.045.
27. Nishijima M, Raetz CR. 1981. Characterization of two membrane-associated glycolipids from an *Escherichia coli* mutant deficient in phosphatidylglycerol. J Biol Chem 256:10690–10696.
28. Cronan JE, Jr. 1968. Phospholipid alterations during growth of *Escherichia coli*. J Bacteriol 95:2054–2061.
29. Hiraoka S, Matsuzaki H, Shibuya I. 1993. Active increase in cardiolipin synthesis in the stationary growth phase and its physiological significance in *Escherichia coli*. FEBS Lett 336:221–224. https://doi.org/10.1016/0014-5793(93)80807-7.
30. Dalebroux ZD, Edrozo MB, Pfuetzner RA, Ressl S, Kulasekara BR, Blanc MP, Miller SI. 2015. Delivery of cardiolipins to the *Salmonella* outer membrane is necessary for survival within host tissues and virulence. Cell Host Microbe 17:441–451. https://doi.org/10.1016/j.chom.2015.03.003.
31. Dalebroux ZD, Matamouros S, Whittington D, Bishop RE, Miller SI. 2014. PhoPQ regulates acidic glycerophospholipid content of the *Salmonella* Typhimurium outer membrane. Proc Natl Acad Sci U S A 111:1963–1968. https://doi.org/10.1073/pnas.1316901111.