PNAS LETTER

A conditional nature for the synthetical lethality between defects in lipid and peptidoglycan biosynthesis

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We write to provide an alternative view on the synthetic lethality between lipid and peptidoglycan synthesis (*fabH* and *nlpI*) reported in a recent PNAS article, "Cross-talk between phospholipid synthesis and peptidoglycan expansion by a cell wall hydrolase" by Som and Reddy (1). Our viewpoint centers on the conditional nature of this lethality, driven by the weakened cell envelope's inability to withstand compressive turgor pressure.

Som and Reddy (1) attributed the synthetic lethality of *Escherichia coli* K-12 MG1655 Δ*fabH* Δ*nlpI* to peptidoglycan hydrolase activity being very detrimental under a limited fatty acid supply. However, studies in multiple Δ*fabH* strains showed clear evidence of a defective outer membrane (OM) (2, 3). An intact OM preserves cell shape and size under a hyperosmotic stress (4). Further, strengthening the OM could sufficiently suppress the morphological defect induced by a less active elongasome (5). The OM and peptidoglycan provide an integral mechanical role in sustaining the cell envelope, and the survival of mutants with severe defects in both required an optimised osmotic condition to alleviate shape deformation stress. However, Som and Reddy (1) used a high salt lysogeny broth (LB) medium, and we suspect that this may have exerted substantial turgor pressure on a Δ*fabH* Δ*nlpI* mutant to trigger conditional lethality.

We constructed an MG1655 Δ*fabH* Δ*nlpI* mutant with palmitate and oleate supplementation under adjusted osmotic conditions (whole genome sequencing confirmed no suppressor mutations). As anticipated, a reduced viability of the Δ*fabH* Δ*nlpI* mutant correlating with NaCl concentrations was observed (Fig. 1). In contrast, the double mutant showed full spectrum viabilities in the LB medium without NaCl (Fig. 1). This observation underscores the mechanical coupling of peptidoglycan and OM in surviving compressive loads, i.e., dysregulated peptidoglycan hydrolase activity reduced cell viability under hyperosmotic stress in an OM-compromised background. Remarkably, higher cell loads (10^0 to 10^{-2} dilutions) for Δ*fabH* Δ*nlpI* were nonviable in salt-containing LB mediums, while further diluted spots were viable (Fig. 1), suggesting that vulnerabilities to turgor pressure correlated with the number of inoculated cells. We attributed this to

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The authors declare no competing interest.

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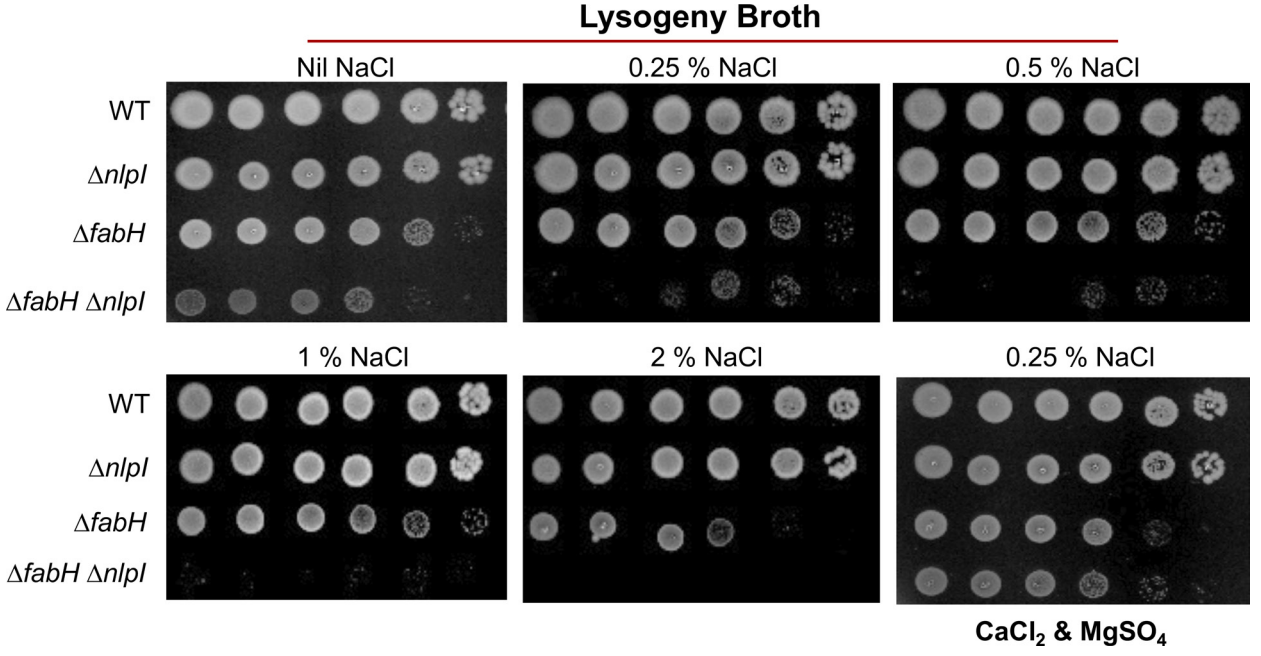

Fig. 1. Conditional lethality of *E. coli* K-12 MG1655 mutants grown in LB agar with various concentrations of NaCl at 37 °C. Serially diluted overnight cultures (OD_{600} adjusted) grown with oleate and palmitate were spotted and plated. The *Right Bottom* panel inoculums were grown with the supplementations of 2.5 mM $CaCl_2$ and 2.5 mM $MgSO_4$.

competition for OM stabilising divalent cations deficient in the LB medium (6). Indeed, the high cell load lethality was rescued by supplementation with calcium and magnesium (Fig. 1).

The conditional lethality of the Δ*fabH* Δ*nlpI* mutant underscores the importance of having a regulatory network that coordinates lipid and peptidoglycan synthesis, as proposed by Som and Reddy (1). The mutant's inability to survive emphasizes the need for such a network, as it cannot support any processes that might further harm the cell envelope. This also draws attention to the considerations necessary when creating genetic constructs like the Δ*fabH* Δ*nlpI* mutant. We also hope that this letter presents sufficient evidence for the cautious use of LB medium when studying the bacterial cell envelope.

1. N. Som, M. Reddy, Cross-talk between phospholipid synthesis and peptidoglycan expansion by a cell wall hydrolase. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2300784120 (2023).
2. Z. Yao, R. M. Davis, R. Kishony, D. Kahne, N. Ruiz, Regulation of cell size in response to nutrient availability by fatty acid biosynthesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E2561–E2568 (2012).
3. Y. Hong *et al.*, Loss of β-ketoacyl acyl carrier protein synthase III activity restores multidrug-resistant *Escherichia coli* sensitivity to previously ineffective antibiotics. *mSphere* **7**, e0011722 (2022).
4. E. R. Rojas *et al.*, The outer membrane is an essential load-bearing element in Gram-negative bacteria. *Nature* **559**, 617–621 (2018).
5. E. M. Fivenson *et al.*, A role for the Gram-negative outer membrane in bacterial shape determination. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2301987120 (2023).
6. S. Wee, B. J. Wilkinson, Increased outer membrane ornithine-containing lipid and lysozyme penetrability of Paracoccus denitrificans grown in a complex medium deficient in divalent cations. *J. Bacteriol.* **170**, 3283–3286 (1988).