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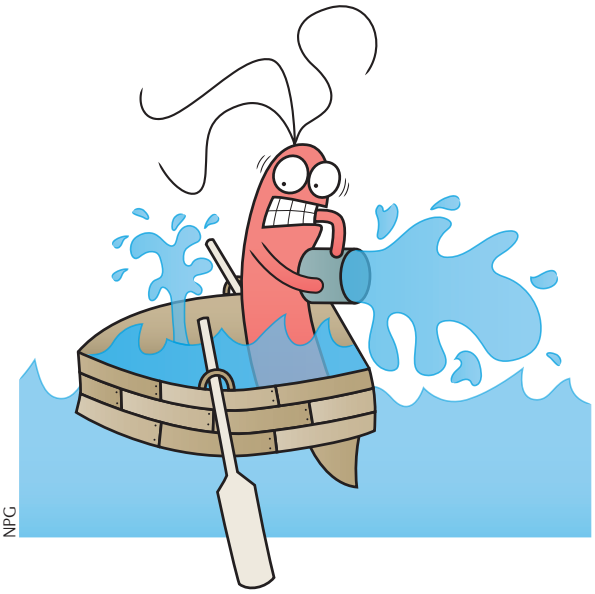
BACTERIAL PHYSIOLOGY

Persisters are under the pump

> persister cells are latent but active

Persisters are a small group within a bacterial population that exhibit tolerance to drugs and survive treatment with antimicrobials. They are phenotypically distinct from antibiotic-resistant bacterial cells, and drug tolerance in persisters seems to involve the induction of a dormant, metabolically inactive state. However, although previous studies have identified several mechanisms that promote persistence, it is not known whether antibiotics effectively enter and accumulate in persister cells. In a recent study, Pu, Zhao, Li *et al.* show that in addition to dormancy, enhanced efflux activity contributes to the formation of persister cells, and suggest that persister cells are latent but active.

The authors treated *Escherichia coli* cells with a β-lactam antibiotic, isolated drug-tolerant persister cells and subsequently used a fluorescently labelled antibiotic to measure intracellular concentrations of the drug in those cells. They found that the fluorescent antibiotic accumulated in persister cells, which suggests that they are permeable to the drug; however, the accumulated concentration of antibiotic in persisters was lower than that in the total cell population. To test how persister cells decrease the intracellular levels of the antibiotic, the authors generated mutants that lack the porin channel proteins OmpF and OmpC, which facilitate the entry of β-lactam antibiotics through the outer membrane, or TolC, which is a component of several efflux systems that export cellular β-lactams. Although, the intracellular drug concentration was decreased in porin-knockout mutants in the total cell population, the levels of antibiotic were still lower in mutant persisters, which suggests that additional mechanisms exist that prevent the accumulation of the antibiotic in persister cells. Persister cells that had defective TolC efflux pumps had increased concentrations of the antibiotic compared with wild-type persister cells and, furthermore, the intracellular levels of the antibiotic were similar to those in the total Δ*tolC* mutant population. This led the authors to suggest that a higher efflux rate, rather than lower membrane permeability, is responsible for the reduced accumulation of antibiotic in persister cells. In agreement with this, RNA-sequencing analysis revealed that persisters had increased expression levels of multi-drug efflux-associated genes compared with the total cell population, whereas the genes that encode the porins OmpF and OmpC only had slightly higher levels of transcription in persisters. The authors went on to show that the expression of *tolC* negatively correlated with the accumulation of antibiotic but positively correlated with the frequency of persister formation. Moreover, the treatment of cells with antibiotics and specific efflux pump inhibitors decreased the formation of persisters and cell survival, which highlights the crucial role of efflux for drug tolerance in persisters.



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Finally, the transcriptome sequencing data also identified other genes that were expressed at higher levels in persisters than in the total cell population, including toxin genes, stress response genes and genes that are associated with a slow metabolism, all of which have been implicated in promoting the dormant state of a cell. To determine the contribution of those differentially expressed genes to persistence, the authors generated a fluorescent protein fusion library and a knockout library. Interestingly, the authors found that following treatment with antibiotics, increased expression levels of *tolC* led to a greater enrichment of bacterial persisters than increased expression levels of other persistence-related genes, whereas the deletion of *tolC* resulted in a more substantial reduction in the formation of persisters.

In summary, the results of this study suggest that persisters combine two seemingly contradictory mechanisms to survive attack by antibiotics: in addition to passive dormancy, bacterial persisters use active efflux to pump antibiotics out of the cell to survive. These findings could lead to novel therapeutic strategies that combine antibiotics with efflux pump inhibitors to target drug tolerance.

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ORIGINAL ARTICLE Pu, Y., Zhao, Z., Li, X. *et al.* Enhanced efflux activity facilitates drug tolerance in dormant bacterial cells. *Mol. Cell* **62**, 284–294 (2016)