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SUPERSTOCK

ANTIMICROBIALS

Persisters come under fire

Bacterial persisters, which are slow-growing or non-growing phenotypic variants, complicate the treatment of chronic infections as they are recalcitrant to killing by antibiotics. Two studies now report novel approaches to eradicate persisters, and both of these strategies involve proteolysis by the ClpP protease.

The compound acyldepsipeptide (ADEP) from *Streptomyces hawaiiensis* kills growing cells by triggering the activation of ClpP, which leads to uncontrolled proteolysis of nascent peptides and eventual cell death. Conlon *et al.* reasoned that extended incubation with a more potent ADEP derivative, ADEP4, might activate ClpP in dormant persisters and lead to nonspecific degradation of mature proteins. Thus, the authors compared the proteomes of stationary phase *Staphylococcus aureus* populations with and without ADEP4 treatment and found that the addition of ADEP4 resulted in the degradation of more than 400 proteins. This result indicates that ClpP becomes a nonspecific protease in the presence of ADEP4 and causes uncontrolled proteolysis in non-replicating cells.

To determine whether ADEP4 is effective at killing persisters, Conlon *et al.* first tested its ability to eradicate *S. aureus* persisters that survive ciprofloxacin treatment. Unlike rifampicin, which had no killing effect, ADEP4 resulted in the eradication of persisters to the limit of detection. Similarly, ADEP4 showed potent activity against stationary phase *S. aureus* cultures, resulting in a 4-log reduction in cell count within 2 days. However, after 3 days, the population had regrown owing to the emergence of *clpP*-null mutants at a high frequency. The authors found that combining rifampicin with ADEP4 led to the eradication of the population to the limit of detection. This killing effect was even more pronounced when the cells were grown in minimal medium, which resulted in complete sterilization of the culture. The combination of ADEP4 and rifampicin was also tested on a range of *S. aureus* strains, including antibiotic-resistant clinical isolates, and was shown to be highly effective. Importantly, Conlon *et al.* also show that the combination of ADEP4 and rifampicin results in complete eradication of *S. aureus* biofilms *in vitro* and in a deep-seated chronic infection in a mouse model.

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In the second study, Kim *et al.* engineered a dual-control (DUC) inducible genetic switch that combines transcriptional repression of a target gene and regulated proteolysis of the encoded product and show that it can be used to identify and eliminate proteins that are required for *Mycobacterium tuberculosis* persistence. The DUC switch consists of two tetracycline repressors that suppress transcription at promoters containing *tet* operator sequences, in addition to the SspB adaptor protein, which targets proteins for degradation by ClpP. Thus, this switch enables silencing at the transcriptional and protein levels, which ensures that complete knockdown of the target protein occurs.

Nicotinamide adenine dinucleotide (NAD) is an essential cofactor for numerous biochemical reactions, but its requirement for persistence was unknown. Kim *et al.* used the DUC switch to block expression of *M. tuberculosis* NAD synthetase (NadE) in replicating cells and found that NadE was rapidly depleted, which resulted in growth arrest and bacterial death. The authors then assessed the requirement of NadE for persistence during starvation and under hypoxic conditions, and found that depletion of NadE led to the eradication of non-replicating *M. tuberculosis* persisters. Finally, depletion of NadE also resulted in a substantial reduction in *M. tuberculosis* colonization during both acute and chronic infection of mice, which confirms that NadE activity is essential for persistence *in vivo* and validates this protein as a potential target for antibiotics.

Considering the current antibiotic resistance crisis and the major threat that is posed by persisters, these two studies are an important advance for the development of novel antibacterial agents that have the ability to eliminate persisters.

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ORIGINAL RESEARCH PAPERS Conlon, B. P. *et al.* Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature* http://dx.doi.org/10.1038/nature12790 (2013) | Kim, J.- H. *et al.* A genetic strategy to identify targets for the development of drugs that prevent bacterial persistence. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1315860110 (2013)
FURTHER READING Balaban, N. Q. *et al.* A problem of persistence: still more questions than answers? *Nature Rev. Microbiol.* **11**, 587–591 (2013)