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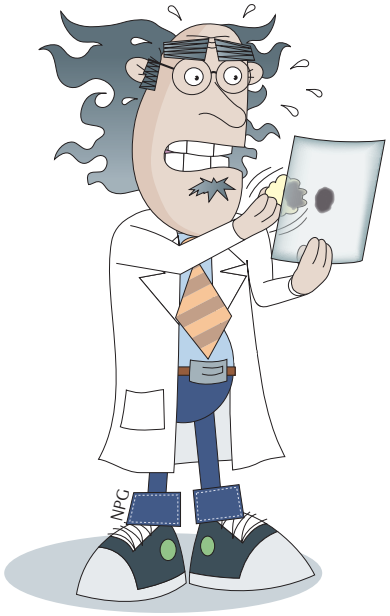
BACTERIAL PHYSIOLOGY

A persistent magic spot

Bacterial persisters are rare phenotypic variants that enter a slow-growing or non-growing state and are transiently tolerant to antibiotics. However, the molecular basis of persistence has proved difficult to elucidate. Gerdes and colleagues now provide direct evidence that the alarmone guanosine tetra- or pentaphosphate ((p)ppGpp) triggers the formation of *Escherichia coli* persisters by activating toxin–antitoxin (TA) loci in a regulatory cascade involving inorganic polyphosphate (polyP) and the Lon protease.

TA loci encode a stable toxin that inhibits bacterial growth and a labile antitoxin that counteracts toxin activity, thereby reversing the non-growing state. Previous work by Gerdes and colleagues showed that successive deletion of ten *E. coli* type II TA loci leads to a cumulative reduction in persistence and that deletion of Lon (which degrades all known *E. coli* type II antitoxins) produces a sharp decline in persister formation. These data support a model in which persistence depends on Lon-mediated degradation of antitoxins.

To investigate this further, the authors examined the potential role of the only known activator of Lon, polyP, which is synthesized by polyP kinase (PPK) and degraded by exopolyphosphatase (PPX). Interestingly, in cells lacking PPK and a double mutant lacking both PPK and PPX, a substantial reduction in persister formation was observed. By contrast, persistence increased in cells that accumulated polyP owing to *ppx* deletion and in cells overexpressing PPK. However, increasing the level of polyP had only a minor effect on persistence in the strain lacking Lon and in the strain lacking ten TA loci, indicating that polyP-mediated persistence requires both Lon and TA activity.



The stringent response regulator (p)ppGpp controls polyP concentration by competitively inhibiting PPX. In a strain lacking the (p)ppGpp synthetases RelA and SpoT, persistence was reduced dramatically, suggesting that this nucleotide positively controls persistence by promoting polyP accumulation. Furthermore, controlled overexpression of a constitutively active form of RelA in a wild-type strain led to a 35-fold increase in persistence, whereas only modest increases were observed in the strains lacking Lon or the ten TA loci and in the double mutant lacking PPK and PPX. Collectively, these findings show that (p)ppGpp-induced persistence requires polyP, Lon and TA activity.

The model predicts that activation of TA loci correlates with reduced growth. Indeed, using a microfluidic device to monitor the growth of single cells, the authors found that TA transcription was stochastically switched on in a small fraction of cells and that high TA expression correlated with growth arrest. Importantly, these cells were resistant to high doses of ampicillin and could resume growth after removal of the antibiotic, consistent with the phenotype of persister cells. The authors then went on to directly test whether (p)ppGpp controls the stochastic switch to slow growth and antibiotic tolerance. Cells with high levels of (p)ppGpp emerged at a comparable frequency to persister cells and were unable to divide, insensitive to ampicillin and resumed growth after ampicillin removal, indicating that (p)ppGpp triggers the persistent state.

Together, these findings provide the first direct evidence for a molecular mechanism underlying the persistence phenotype. The regulatory cascade begins with an increase in (p)ppGpp levels in a subpopulation of cells, which promotes polyP accumulation and consequent antitoxin degradation, resulting in toxin-mediated slow growth and antibiotic tolerance. The mechanistic basis for the stochasticity in (p)ppGpp levels is unknown; however, the authors propose that some rare cells in the population might encounter starvation conditions, which is consistent with the induction of (p)ppGpp synthesis during starvation and the strong growth-phase dependence of persistence.

Christina Tobin Kåhrström

> “(p)ppGpp-induced persistence requires polyP, Lon and TA activity”

ORIGINAL RESEARCH PAPER Maisonneuve, E., Castro-Camargo, M. & Gerdes, K. (p)ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. *Cell* **154**, 1140–1150 (2013)

FURTHER READING Balaban, N. Q. *et al.* A problem of persistence: still more questions than answers? *Nature Rev. Microbiol.* **11**, 587–591 (2013)