CHEMICAL BIOLOGY

Addicting bugs to nonstandard amino acids

Scientists can maintain orthogonal translation systems over many bacterial generations by making an essential gene depend on a nonstandard amino acid.

Life on Earth may be based on 20 canonical amino acids, but clever scientists can add novel functions to creatures by reassigning the UAG amber stop codon to code for a 21st, nonstandard amino acid. Although useful in many biological applications, these orthogonal translational systems tend to be quite inefficient. One problem is that organisms typically find an alternative pathway to fitness that does not require the new amino acid. Researchers have not yet been able to coerce a bacterium to utilize a nonstandard amino acid across its whole proteome.

New work from Andrew Ellington's lab at the University of Texas at Austin addresses this challenge with a strategy to make *Escherichia coli* survival dependent on a nonstandard amino acid. The work follows

in the footsteps of recently reported biological containment strategies for genetically modified organisms, which oblige them to depend on a nonstandard amino acid. To generalize such an approach, the team engineered TEM-1 β -lactamase, an antibiotic-resistance protein found in Gram-negative bacteria, to incorporate a tyrosine analog at a site required for its enzymatic activity. In the presence of a β -lactam antibiotic, the survival of the engineered bugs hinges on incorporation of the tyrosine analog into the engineered TEM-1, which prompts them to maintain an active orthogonal translation system.

To make sure that wily bugs could not readily find an alternative pathway to survival, the group tested whether any canonical amino acids could replace the tyrosine analog and thus restore activity of the engineered TEM-1. Although phenylalanine could substitute for the tyrosine analog,

because a single point mutation to the reassigned codon would not code for phenylalanine, they reasoned that the orthogonal translation system could be maintained for the long term. This was borne out in successful serial culture experiments comprising hundreds of generations, with no 'escape' events.

Ellington's team's approach was successful in several *E. coli* strains, *Shigella flexneri*, *Salmonella enterica*, *Yersinia ruckeri* and even the evolutionarily distant *Acinetobacter baylyi*. They propose that the 'addiction' strategy could also be applied to other enzymes to introduce nonstandard amino acids into the genetic code of many other organisms.

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RESEARCH PAPERS

Tack, D.S. *et al.* Addicting diverse bacteria to a noncanonical amino acid. *Nat. Chem. Biol.* doi:10.1038/nchembio.2002 (18 January 2016).