Nature Reviews Microbiology | AOP, published online 9 February 2015; doi:10.1038/nrmicro3443

SYNTHETIC BIOLOGY

GMOs in lockdown

Genetically modified organisms (GMOs) are becoming widely used in agriculture and bioremediation, and for the production of pharmaceuticals and biofuels. However, alongside their increased use, there are growing concerns about the potential risk that these organisms could escape into the environment. Now, two studies report the design of novel GMOs that are dependent on synthetic amino acids (sAAs) and therefore cannot grow in natural ecosystems.

> "novel GMOs that are dependent on synthetic amino acids … cannot grow in natural ecosystems"

To generate GMOs that require sAAs for growth, Mandell, Lajoie *et al.* and Rovner *et al.* took advantage of a recoded *Escherichia coli* strain in which all UAG stop codons are replaced with synonymous UAA stop codons and that lacks release factor 1 (RF1), which terminates translation at UAG sites. Therefore, by inserting specific aminoacyl-tRNA synthetase (aaRS)–tRNA pairs into this strain, the authors were able to genetically reassign UAG to encode sAAs.

Mandell, Lajoie *et al.* engineered this strain so that UAG recodes for L-4,4′-biphenylalanine (bipA), and then created multiple mutants with redesigned essential proteins that incorporate bipA into their functional regions. By comparing bacterial growth in the presence and absence of bipA, the authors identified several mutants that were metabolically dependent on bipA and characterized their escape frequencies (that is, their ability to grow in the absence of bipA). Notably, two of these synthetic auxotrophs displayed very low escape rates: a mutant in which bipA is incorporated into adenylate kinase (adk); and a mutant in which bipA is part of tyrosyl-tRNA synthetase (tyrS). Combining the adk and tyrS mutations lowered the escape rate of the double-mutant strain even further. Interestingly, whole genome sequencing revealed that double-mutant colonies that were able to grow in the absence of bipA contained mutations in the Lon protease, which degrades misfolded proteins. This led the authors to hypothesize that disruption of Lon prevents the degradation of poorly folded adk and tyrS, which occurs when standard amino acids are incorporated in place of bipA. To overcome this resistance mechanism, they re-engineered the bipA aaRS (BipARS) so that it requires bipA for folding, which abrogated its residual ability to use standard amino acids instead of bipA. Indeed, the combination of the three mutations (adk, tyrS and BipARS) resulted in a strain with undetectable escape mutants when grown for up to 14 days.

Using a similar strategy, Rovner *et al.* engineered *E. coli* to recode UAG with one of three different sAAs: p-acetyl-L-phenylalanine (pAcF); p-iodo-L-phenylalanine (pIF); or p-azido-L-phenyalanine (pAzF). The authors isolated several synthetic auxotrophs and also found that the escape frequency was reduced when multiple mutations were combined in a single strain. In particular, a triple-mutant strain that incorporated pAzF in the functional sites of MurG, DnaA and seryl-tRNA synthetase (SerS) displayed very low escape rates. Sequencing of the few escape mutants revealed mutations at one of the three *E. coli* genes encoding tyrosine tRNAs (*tyrT*, *tyrV* or *tyrU*), so the authors proposed that deletion of *tyrT* and *tyrV* would prevent the acquisition of mutations in the remaining tRNA (*tyrU*) and reduce the escape rate of the resulting mutant. Indeed, the simultaneous deletion of *tyrT* and *tyrV* in the triple mutant resulted in a strain with undetectable escape frequencies following culture for more than 20 days.

Finally, both groups assessed the ability of these novel GMOs to be rescued by metabolic cross-feeding or by horizontal gene transfer (HGT). In contrast to normal auxotrophs, which can sometimes grow by scavenging for metabolites from neighbouring cells, the new *E. coli* strains were unable to grow in media supplemented with bacterial lysates or in proximity to wild-type bacteria. Similarly, both strains were resistant to HGT-mediated escape because the different sAA-dependent enzymes are distributed throughout the genome.

Taken together, these studies delineate a strategy for the generation of safer GMOs that are dependent on synthetic metabolites and are therefore unable to grow in the natural environment.

Cláudio Nunes-Alves

BRAND X PICTURES

ORIGINAL RESEARCH PAPERS Mandell, D. J., Lajoie, M. J. *et al.* Biocontainment of genetically modified organisms by synthetic protein design. *Nature* http://dx.doi.org/10.1038/nature14121 (2015) | Rovner, A. J. *et al.* Recoded organisms engineered to depend on synthetic amino acids. *Nature* http://dx.doi.org/10.1038/nature14095 (2015)