NON-NATURAL AMINO ACIDS

A synthetase swap

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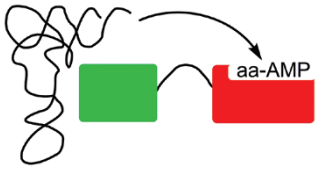

The enzymatic incorporation of non-natural amino acids into proteins currently relies on self-acylating 'flexizymes' and engineered tRNA synthetases with altered substrate specificity. However, engineering synthetases to accept new substrates of interest is not always straightforward, and flexizymes are limited to cell-free translation. As an alternative approach, Giessen *et al.* envisioned that the analogous chemistry performed by adenylation (A) domains found within nonribosomal peptide synthetases could be coopted for use as surrogate tRNA synthetases, allowing facile access to hundreds of amino acid substrates. To test their hypothesis, the authors designed a fusion protein with the tRNA-recruiting multisynthetase complex accessory protein Arc1p to direct PheA as a representative A domain to a non-native tRNA substrate. Modeling based on an existing PheA structure and a newly acquired crystal structure of Arc1p suggested that a short linker connecting Arc1p to the C terminus of PheA would facilitate catalysis. Adenylation assays of constructs containing one of four linkers confirmed that PheA's activity was not disrupted by the Arc1p fusion, whereas aminoacylation assays using $tRNA^{Phe}$ further demonstrated that the fusion proteins were able to catalyze tRNA loading. The best construct, containing an 8-amino-acid linker, displayed ~10% efficiency as compared to a native phenylalanine synthetase, PheRS; though this activity leaves room for improvement, it is likely sufficient for initial applications. Importantly, the reactions were equally efficient with L- or D-phenylalanine in the PheA constructs, whereas D-phenylalanine was only minimally processed by PheRS. As Arc1p is known to bind multiple tRNAs, the authors confirmed five other tRNAs could also be loaded with L- or D-phenylalanine. Analytical methods and a functional assay using a cyclodipeptide synthase, which uses amino acid–loaded tRNAs as substrates, further confirmed amino acid attachment at the expected terminal adenosine. Though future work will be needed to engender specificity in tRNA interactions if this method is to be used in cells, the approach opens up new opportunities for amino acid incorporation based on A domain diversity. *CG*

QUORUM SENSING

mRNA tug of war

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Bacteria such as *Vibrio harveyi* respond to changes in population density by regulating the expression of genes such as *luxR* that promote collective behaviors, including bioluminescence and biofilm formation. At low cell density, phosphorylation of the response regulator LuxO activates the transcription of the Qrr family of small regulatory RNAs (sRNAs). These sRNAs directly base pair with and thereby block the expression of mRNAs, including the quorum sensing regulators *luxM*, *luxO* and *luxR*. However, it was not known whether repression of the different mRNA targets occurs through identical or distinct regulatory mechanisms. To determine whether Qrr3 exhibits preference for particular target mRNAs, Feng *et al.* used an *Escherichia coli* competition assay expressing different combinations of target mRNAs encoding fluorescent tags in the presence of Qrr3 sRNA. They found that Qrr3 uses three distinct mechanisms to repress target gene expression. Base pairing of *luxM* mRNA with Qrr3 resulted in the coupled degradation of both mRNA and sRNA molecules, whereas base pairing of *luxR* mRNA to Qrr3 caused *luxR* but not the Qrr3 sRNA to be degraded. Finally, *luxO* binding to Qrr3 blocked LuxO protein production through Qrr3-mediated sequestration. Considering that *luxM* was known to bind the first and second stem loops of Qrr3 (SL1 and SL2) while *luxR* and *luxO* bind SL2, the specific base pairing patterns to Qrr3 might dictate the particular repression mechanism. Indeed, the authors found that modifying *luxM* mRNA to base pair only to SL2 blocked Qrr3 degradation, whereas modulating the binding strength of *luxR* or *luxO* mRNA to SL2 drove whether an mRNA was catalytically degraded or sequestered. Finally, mathematical modeling coupled with experiments showed that degradation of an mRNA target produced the highest level of immediate repression while sequestration provided the weakest and slowest rate of responsiveness and that these different mechanisms serve to control the dynamics and potency of quorum sensing. *GM*

PROTEIN AGGREGATION

Curling damage

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Many neurodegenerative diseases are associated with protein misfolding, which leads to the formation of ordered aggregates of proteins called amyloid fibers. Amyloid fibers can also be formed as a normal, functional process unrelated to misfolding. For instance, the *Escherichia coli* CsgA protein is secreted from cells in an unpolymerized form and assembles into extracellular amyloid called curli, which are important in surface attachment and biofilm formation. Although the factors required for curli fiber formation have been identified, it has been unclear how curli formation is prevented inside the cell, where it can be cytotoxic. To address this question, Evans *et al.* tested components of the *csg* operons for inhibitory activity *in vitro* by generating periplasmic extracts (PEs) from *csg* mutants. PEs from cells lacking CsgE, a chaperone required to direct CsgA to CsgG for secretion, could inhibit CsgA amyloid assembly *in vitro*, whereas PEs from cells lacking CsgC could not. Fractionation of the PE from *ΔcsgG* cells—which could inhibit assembly—as well as an intact-cell curli assay implicated CsgC in inhibiting CsgA amyloid formation. When CsgC was deleted, CsgA aggregates were intracellular, cell viability was compromised, and cellular stress associated with the abnormal accumulation of misfolded proteins was induced. CsgC could inhibit CsgA amyloid formation *in vitro*, as detected by far-UV CD spectroscopy and NMR, presumably by stabilizing a preamyloid intermediate of CsgA, as detected by an amyloid conformation-specific antibody. Although the authors could not detect a stable interaction between CsgA and CsgC by native gel electrophoresis, they were able to map the regions of CsgA through which CsgC acts using the *in vitro* assay. Finally, they found that *E. coli* CsgC could inhibit amyloid formation of CsgA from *Salmonella enterica* and *Citrobacter koseri* as well as human α-synuclein, which shares an 8-amino-acid motif with CsgA. Having a dedicated amyloid inhibitor may be what distinguishes functional amyloid from disease-associated amyloid. *MB*

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