CHEMICAL BIOLOGY

Engineering life

A selection strategy to produce ribosome•mRNA pairs that operate independently of the natural cellular machinery in *Escherichia coli* could be used for the creation of complex artificial networks in cells.

When people talk about intelligent engineering of life forms by adding desirable characteristics, they are almost invariably talking about genetic engineering which involves the alteration of endogenous genes or the addition of new genes. Much genetic engineering involves doing things that could be done through simple managed breeding as has been done for hundreds of years. There are other mechanisms of performing such engineering, however, which are potentially more powerful. This work is taking place in the arena of synthetic biology.

Synthetic biology aims to do things that would be effectively impossible using selective breeding. This involves the creation of new cellular components that operate independently of the normal cellular machinery except for where they are purposely designed to interact with endogenous cellular components. This would seem to be a daunting task, and indeed it is, but there has been considerable progress in this nascent field.

Jason Chin was involved in some of the early work in synthetic biology when he was in Peter Schultz's lab. There he worked on expanding the genetic code with novel codon and aminoacyl tRNA synthetase–tRNA combinations that allow one to engineer an organism capable of incorporating unnatural amino acids into proteins (Chin *et al.*, 2003). As an independent investigator, he is now working on a different form of synthetic biology that involves creating novel ribosome•mRNA pairs that function independently of endogenous ribosomes and mRNA in *E. coli*. Such pairs can be used to create synthetic networks and functions in cells.

Chin's first work in this new area has now been published in *Nature Chemical Biology* where he describes a new tunable selection strategy that allowed them to evolve ribosome•mRNA pairs that function as orthogonal translational machinery (Rackham & Chin, 2005). "The term orthogonal basically means the new ribosome doesn't translate the cellular mRNA and the old ribosome doesn't translate the new mRNA," explains Chin.

Their selection strategy exploits the fact that in bacteria the binding of the ribosome to mRNAs is dependent on a seven-nucleotide binding domain near the AUG initiation codon. They first designed a library of mRNAs containing all possible sequence combinations in this region, and subjected it to negative selection. This allowed them to derive a population of ribosome binding sequences that would not function with the endogenous ribosome. Next they used a library of mutated 16S rRNA sequences to select for artificial ribosomes that could specifically translate these mRNAs. "So now we have mRNAs that are not translated by the endogenous ribosomes, thus orthogonal, and we have ribosomes that now translate that orthogonal mRNA," says Chin.

They went on to demonstrate that expression of these mutant ribosomes had no noticeable effect on the health or growth of bacteria. Expression of multiple orthogonal ribosome•mRNA pairs expressing different proteins allowed them to create a Boolean logic AND function driven by the programmed synthesis of three different components , each of which was required to obtain a positive output.

The selection method described here promises to allow the creation of many more orthogonal pieces of cellular machinery. This could include not only the kind of ribosome•mRNA pairs described here but also ribosomes capable of decoding extended codons similar to those already described by Chin. Use of these new tools of synthetic biology could allow the realization of currently unimaginable applications.

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RESEARCH PAPERS

Chin, J.W. *et al.* An expanded eukaryotic genetic code. *Science* **301**, 964–967 (2003).

Rackham, O. & Chin, J.W. A network of orthogonal ribosome•mRNA pairs. *Nat. Chem. Biol.* **1**, 159–166 (2005).

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