SYNTHETIC BIOLOGY

Tailor-made genetic codes

Expanding the range of amino acids polymerizable by ribosomes could enable new functionalities to be added to polypeptides. Now, the genetic code has been reprogrammed using a reconstituted *in vitro* translation system to enable synthesis of unnatural peptides with unmatched flexibility.

Michael C. Jewett and Vincent Noireaux

The translation apparatus is the cell's factory for protein synthesis, stitching together amino acid substrates into sequence-controlled polypeptides (proteins) from a defined template. With protein elongation rates of up to 20 amino acids per second and an accuracy of 99.99% (ref. 1), prokaryotic translation is faster and more accurate than many enzymatic conversions that require only a single biocatalyst and a single reactant. This extraordinary synthetic capability has driven extensive efforts to harness the translation apparatus for novel functions.

Pioneering efforts have demonstrated that it is possible to genetically encode more than the 20 naturally occurring amino acids[2–5]. This powerful tool can be used to expand the functionality of proteins and peptides. As a result, reprogramming of the genetic code has emerged as one of the defining opportunities in synthetic biology[2]. Some of the potential goals include providing platforms for the synthesis of polymers containing unnatural amino acids that can be selected from a large catalogue; enabling new biocontainment strategies; expanding the chemical reactions conducted by and within living systems; and developing new materials and therapeutics. So far, the major route to these goals has consisted of engineering the translation machinery of living organisms, such as *Escherichia coli*, to fabricate cells that can synthesize unnatural polymers. The current approaches, however, usually allow the site-specific incorporation of just a few unnatural amino acids into proteins[6,7] — for instance by reassigning the UAG stop codon. This limits the potential of genetic code reprogramming. Creating tailor-made genetic codes with 'blank' codons that can be re-assigned to multiple distinct unnatural monomers would require substantial re-engineering of the entire apparatus to keep cells alive. Freed from the need to sustain life, cell-free systems are more amenable to engineering design.

About half a century ago, Nirenberg and co-workers developed one of the first cell-free

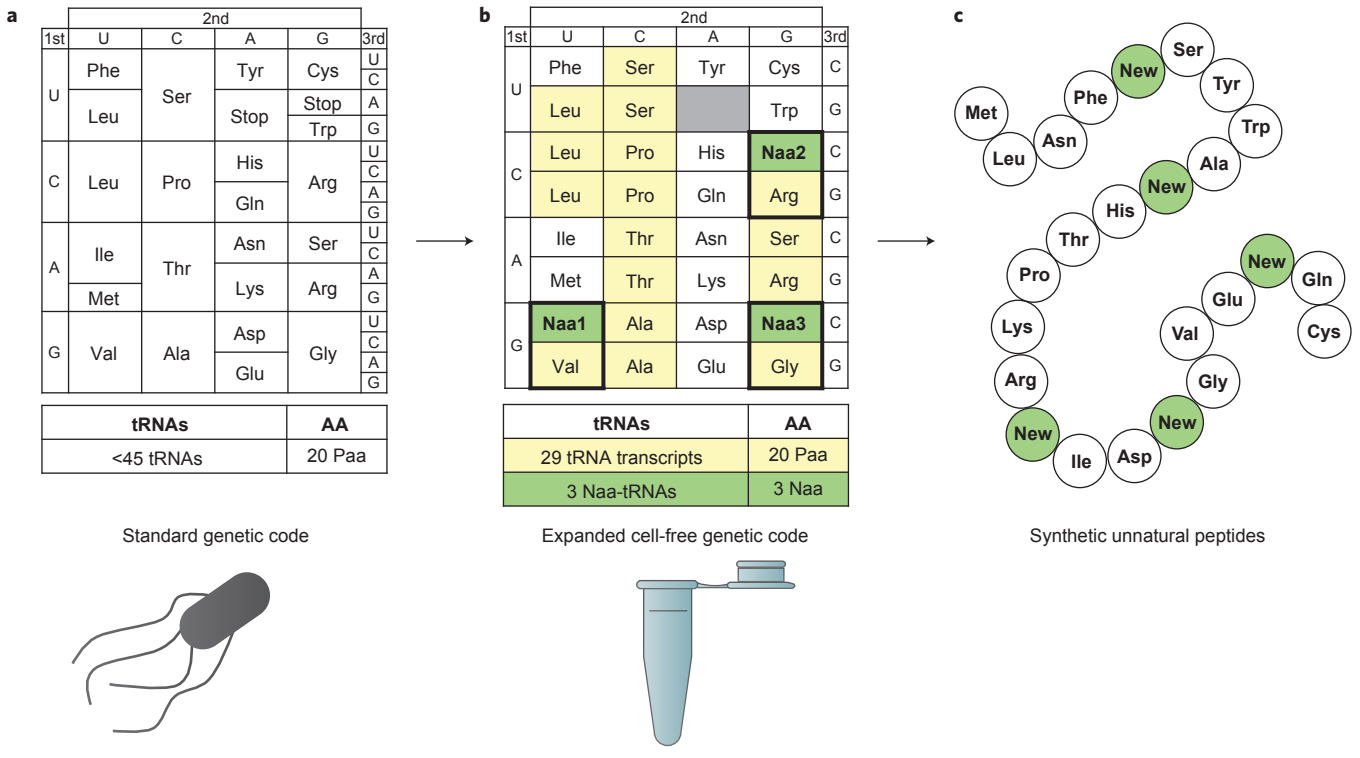

Figure 1 | Reprogramming the genetic code. **a**, The standard genetic code table as found in living organisms such as *E. coli*. **b**, A customized genetic code is created by only using either G or C in the third position. Eleven boxes (2 Ser, 2 Leu, 2 Arg, 1 Pro, 1 Thr, 1 Val, 1 Ala, 1 Gly) are coded for by more than 1 codon, the redundant codons can therefore be reallocated to new amino acids without removing any of the twenty naturals ones. As an example, three boxes (Val, Gly, Arg) of this expanded genetic code are reassigned to three new unnatural amino acids (Naa1, 2, 3). **c**, The PURE cell-free expression system can then be used to synthesize synthetic peptides containing the three unnatural amino acids, at any position and in unlimited number. Figure adapted from ref. 10, NPG.

translation systems to decipher the genetic code and establish the standard RNA-codons table[8] (Fig. 1a). In the past fifteen years, a new generation of cell-free expression systems, more versatile and high yielding, has emerged to understand, harness, and expand the capabilities of natural systems. In one example, the PURE technology[9] (protein synthesis using recombinant elements) has been advanced to produce proteins in test tube reactions. This *E. coli*-based reconstituted cell-free protein synthesis system, made of purified molecular components, allows the user to define all of the elements and set their concentrations. Writing in this issue of *Nature Chemistry*[10] Hiroaki Suga and co-workers elegantly re-design the standard genetic code and use the PURE technology to effectively expand the number of different unnatural amino acids that can be directly polymerized into peptides during translation. The redundancy found in the standard genetic code is decreased without removing any of the 20 canonical amino acids. Ultimately this approach could create a dozen 'free' codon boxes that can be reassigned to non-proteinogenic amino acids, although Suga and co-workers have only re-assigned three new codons so far.

To create a customized genetic code, Suga and co-workers first realized that all of the standard amino acids are coded when the third codon base is limited to either C or G (Fig. 1), reducing the number of boxes from 64 to 32. This reduction leaves one stop codon and eleven redundant codons that can be used for new amino acids. As a demonstration of their conceptual framework, three of the eleven free boxes are reallocated to unnatural residues, each replacing one of the redundant valine, arginine and glycine codons (Fig. 1b).

To achieve specific incorporation during translation, the number of required tRNAs, 45 out of 86 *in vivo*, is reduced to 29, enough to cover the twenty natural amino acids, while the three other tRNAs are synthesized with non-proteinogenic amino acids. Using a technique previously developed by the team, the 32 tRNAs are produced using *in vitro* transcription reactions, and then each one is loaded with its specific amino acid using a ribozyme called flexizyme. A messenger RNA that codes for the desired polypeptide sequence is then transcribed *in vitro* and added to cell-free translation machinery containing only the tRNAs necessary to incorporate twenty natural amino acids and three unnatural ones from a catalogue of eight different flavours. Translation of the mRNA then produces the peptide containing the unnatural amino acids at any desired position along the peptide chain (Fig. 1c). The number of unnatural amino acids incorporated into the polypeptide is potentially unlimited — although this requires very efficient rates of incorporation.

A step-by-step demonstration of this approach is provided, supported by biochemical characterizations and validated by mass spectrometry. Transcripts of increasing lengths, incorporating up to four codons for three different unnatural amino acids, are successfully translated *in vitro*, with negligible crosstalk and contamination. Synthetic peptides of up to 32 amino acids are polymerized. A natural anti-cancer macrocyclic peptide, containing five unnatural amino acids, is synthesized by using a modified start codon.

The platform presented by Suga and co-workers represents a tour de force in the construction of tailor-made genetic codes, holding promise to make small peptides with novel functionalities. Indeed, the possibility to produce synthetic peptides incorporating multiple, distinct unnatural amino acids at any position and in unlimited number may allow ribosome repurposing in fundamentally novel ways. This could have profound implications for production, screening, and selection of peptidomimetic, or non-standard peptides as novel drugs. While the system has not yet supported the synthesis of proteins, and the approach would not be easily feasible in cells because one would need to additionally evolve multiple mutually independent aminoacyl tRNA synthetase systems as well as radically recode genomes, the new technology will shed light on which sense codons might be most easily recoded. By testing sense suppression in ways not yet possible in cells, this work will contribute understanding to tRNA decoding, as well as open new coding channels for genetic code expansion. ❐

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OXIDE INTERFACES

Mismatched lattices patched up

Controlling interfaces between transition-metal oxides and dissimilar structures is crucial for practical applications, yet has remained a quandary. Now, a coherent interface that bridges a perovskite and a fluorite structure has been formed using judiciously chosen metal cations.

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Semiconductor and oxide heterostructures are omnipresent in modern society, as components of a wide variety of devices. A growing challenge for manufacturing layered devices, such as nanocapacitors, light emitting diodes and batteries, is ensuring that heterointerfaces are stable and do not hinder performances — interfacial phenomena are especially important as devices are increasingly being scaled down.

Well-behaved interfaces are achieved by interleaving isostructural components — that is, sharing structurally identical cation and anion sublattices. This maintains lattice coherency without large stresses that may otherwise produce misfit dislocations or form unintended secondary phases. Nonetheless, successful technological materials exhibit a wide range of structure types, inevitably leading to interfaces