Reprogramming the Genetic Code

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less than 20 μm across, calcified cyanobacterial sheaths have a good, but highly episodic, microfossil record (*10*), reflecting the response of sheath calcification to environmental effects. Well-calcified cyanobacterial sheaths first became conspicuous in the marine geologic record about 1200 million years ago (*11*), remained relatively common until about 100 million years ago, and today are scarce in seawater but locally abundant in fresh water (*6*). Their scarcity in rocks older than about 1200 million years has attracted particular attention. It seems that sheath calcification rarely occurred in marine cyanobacteria during the first half of their history. A possible explanation for this "Precambrian Enigma" (*6*) is that the high carbon dioxide levels during this period meant that cyanobacteria did not need to use bicarbonate (*6*).

It is not yet known whether the controlled intracellular calcification shown by Gloeomargarita has a geologic record. If it does, it might be much less erratic than the one for calcified sheaths. Couradeau *et al*. suggest that ancient relatives of Gloeomargarita may indeed have carried out intracellular calcification. They reason that Gloeomargarita has ancestral features and that the alkalinity export involved in sheath calcification might require cellular mechanisms that did not evolve until later.

However, it may be difficult to find geologic evidence. Tiny benstonite inclusions like those in Gloeomargarita would not be easy to identify with confidence in Precambrian rocks. Recognizing this difficulty, Couradeau *et al*. suggest that carbonate deposits containing barium and strontium, present in the benstonite, might provide a better geologic indicator for this style of biomineralization than the granules themselves.

Couradeau *et al*.'s discovery raises some intriguing possibilities, but assessing their importance for the fossil record will be challenging. In addition to searching for scarce calcified sheaths, we now need to be on the lookout for subtle traces of intracellular calcification. This is likely to cause some head scratching. At least we know why our skulls are so well calcified. Or do we?

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MOLECULAR BIOLOGY

Reprogramming the Genetic Code

Incorporating unnatural amino acids into proteins presents challenges in expanding the genetic code.

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The genetic code provides rules by which a genome is decoded to produce proteins of defined amino acid composition and sequence. These rules, which specify 61 codons (triplets of nucleotides) that code for the 20 common amino acids, and 3 codons that signal the termination of protein synthesis, are near-universally conserved in living organisms. Despite conservation of this code and the translational machinery that enforces it, a growing body of work addresses the challenges in reprogramming the genetic code. Designer amino acids, created by synthetic chemistry, can now be incorporated into specific sites in proteins of interest in vitro, in cells, and most recently in a whole animal (see the figure). These routes to unnatural polymer synthesis and evolution are already facilitating the study of cellular processes including protein interactions, protein conformational changes, posttranslational modification biology, and the kinetics of protein transport and cell signaling with a new level of molecular precision (*1*). Emerging developments may allow the synthesis and evolution of new materials and therapeutics.

The fidelity of natural protein synthesis is maintained by specific aminoacylation of a transfer RNA (tRNA) with an amino acid, and the ribosomal decoding of each tRNA in response to a cognate codon on a messenger RNA (mRNA) (*2*, *3*). Incorporating unnatural amino acids (with different chemical and physical properties that differ from those of the common amino acids) into proteins requires methods for loading them onto tRNAs and unique codons that can be specifically decoded by "orthogonal" tRNAs, which have been engineered not to be recognized as substrates for endogenous aminoacyl–tRNA synthetases. Chemical methods to aminoacylate orthogonal tRNAs designed to recognize a stop codon (the amber codon), and the use of these tRNAs in translation reactions in vitro, provided the first general route to incorporating unnatural amino acids into proteins (*4*). Recent advances have expanded the scope of in vitro protein synthesis. For example, quadruplet codons (four nucleotides) that can be read, albeit poorly, on the natural ribosome have been used to incorporate several unnatural amino acids into a single protein (*5*). In vitro translation can also be reconstituted from purified factors, allowing particular aminoacyl–tRNA synthetases and tRNAs to be omitted from the translation reaction. The resulting "blank" codons can then be reassigned to new amino acids by introducing orthogonal tRNAs bearing anticodons complementary to the blank codons (*6*, *7*). Ribozymes that catalyze the aminoacylation of tRNAs with unnatural amino acids and other monomers including alpha hydroxy acids (*8*) have provided an accessible alternative to chemical aminoacylation of tRNAs. This method has allowed the synthesis and directed evolution of unnatural peptides and cyclic peptides bearing a range of unnatural amino acids and monomers (*8*). Thus far, in vitro approaches have been used to synthesize and evolve antibiotic-like molecules and to label proteins for fluorescence resonance energy transfer experiments (*5*, *8*).

Extending unnatural protein synthesis to cells has also seen progress. The injection of a chemically aminoacylated orthogonal tRNA, engineered to recognize an amber codon, into *Xenopus laevis* oocytes has facilitated new insight into the structure and function of membrane proteins, including

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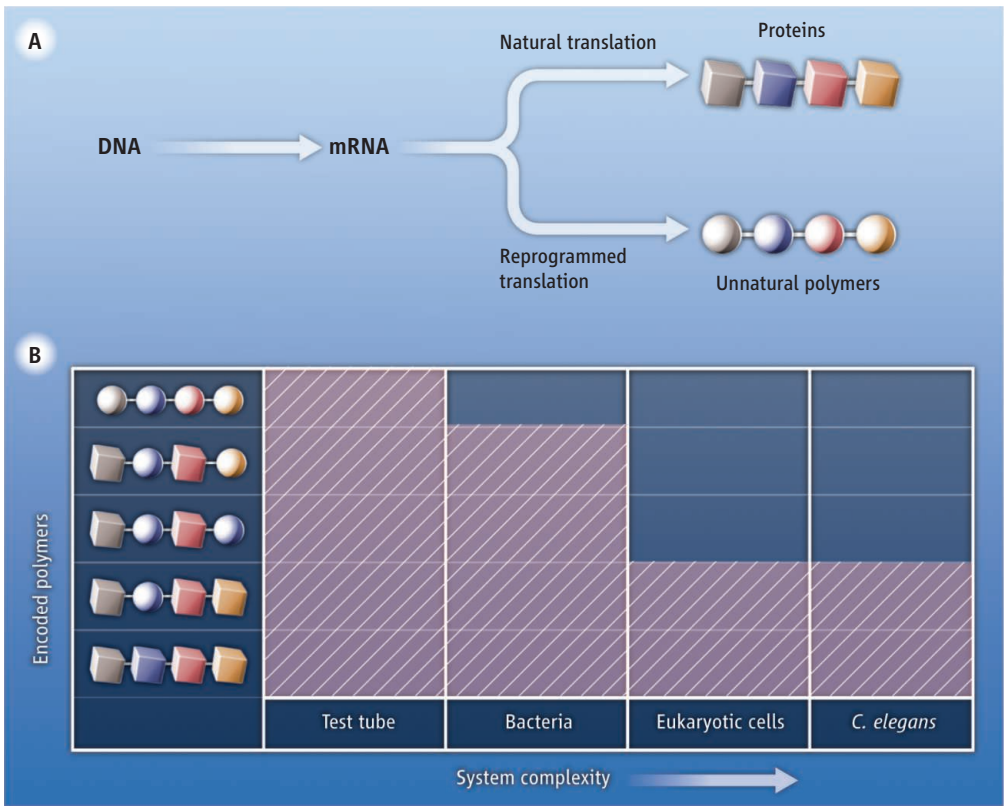

Unnatural translation. (**A**) DNA is transcribed to mRNA, and the natural translational machinery decodes the mRNA to produce polymers of the natural 20 amino acids (cubes). Reprogrammed translation allows the introduction of unnatural amino acids (spheres) into proteins. (**B**) Reprogramming the genetic code in systems of increasing complexity.

ion channels, through the incorporation of unnatural amino acids (*9*). Orthogonal aminoacyl–tRNA synthetase–tRNA pairs that do not cross-react with endogenous synthetases and tRNAs can specifically recognize a particular unnatural amino acid and efficiently direct its incorporation in response to a unique (usually the amber) codon. Such pairs have now been described in particular host cells (*1*, *10*). The pyrrolysyl–tRNA synthetase–tRNA pairs from *Methanosarcina* species can be used to incorporate unnatural amino acids into protein in bacteria, yeast, mammalian cells, and even a whole animal, the worm *Caenorhabditis elegans* (*1*). Indeed, the extension of unnatural amino acid–based tools—developed in cells—to plants and animals (*11*) may ultimately provide approaches for investigating more complex phenomena, such as neural processing and development, with new levels of molecular, spatial, and temporal precision.

Because all the codons are used in the genome for protein synthesis, the incorporation of multiple unnatural amino acids into proteins in cells requires additional blank codons. To address this issue, a parallel and independent or "orthogonal" translation pathway has been created in the model bacterium *Escherichia coli* (*12*, *13*). An orthogonal ribosome, which translates orthogonal mRNAs that are not substrates for the natural ribosome, has been created. Altered, by directed evolution in the laboratory, it can read quadruplet codons with extended anticodon tRNAs that are poorly decoded on the natural ribosome. This provides a set of blank quadruplet codons that have been assigned to new amino acids by means of orthogonal synthetases and tRNAs, creating a parallel translation pathway in the cell (*12*, *13*). This approach has been used to genetically direct the formation of a nanoscale redox-insensitive cross-link that may be useful in trapping discrete, functional conformations of proteins and creating stabilized protein therapeutics.

Emerging advances in genome engineering (*14*, *15*) may generate complementary approaches to providing additional blank codons. By replacing the codons in the genome with a reduced set of codons that encode all 20 amino acids and deleting the endogenous tRNAs that decode the replaced codons, it may be possible to convert some sense codons into blank codons. The number of blank sense codons that can be independently decoded may be constrained by the extensive wobble pairing rules by which many tRNAs decode several codons. Experimental efforts at codon replacement may reveal the extent to which protein folding and homeostasis, and cellular robustness are evolutionarily embedded in natural codon usage (*16*).

Another challenge to increasing the number of distinct amino acids that can be incorporated into proteins in cells is the creation of additional orthogonal aminoacyl–tRNA synthetase–tRNA pairs. The recent de novo generation of such pairs by directed evolution in *E. coli* offers one solution (*17*).

It has been more than 40 years since in vitro polyester synthesis was demonstrated (through the chemical modification of tyrosine on a tRNA) (*18*). However, many challenges remain in reprogramming the translational machinery of cells for the encoded synthesis of diverse polymers. Future progress will provide new insight into fundamental questions in biology and promises routes to new materials and therapeutics.

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