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Previews

Yielding at Stop Codons: Expanding the Genetic Code

Codon-specific incorporation of noncoded amino acids into proteins can diversify the genetic code. Now, in both *E. coli* and *S. cerevisiae*, iterative rounds of selection can be used to isolate aminoacyl-tRNA synthetases that aminoacylate suppressor tRNAs with noncoded amino acids.

The invariance of the genetic code is taken for granted. With the unusual exceptions of selenocysteine [1] and pyrrolysine [2, 3], biosynthetic proteins are restricted to the 20 encoded amino acids, and further diversity is only introduced by posttranslational modification or with cofactors. In most cases, the relationships between the coded amino acids and their respective codons are also invariant. Stops are stops and coding is coding—get the picture? Sadly, for scientists interested in deconstructing complex biological questions in vivo, nature didn't select the most useful amino acids. In fact, heretical as it may seem, as biochemical and biophysical tools, some of the encoded amino acids could even be considered boring and useless—at least to those interested in mapping the inner workings of the cell.

Thus, expanding the genetic code by controlled introduction of a variety of different and useful amino acids into the proteome offers great biotechnological promise. Imagine the possibilities if one could design or choose any noncoded amino acid and incorporate it into any protein, at any position, in vivo, in any cell line. For example, nonhydrolyzable phosphonates could be used to characterize signal transduction pathways [4]. Fluorescent amino acids could illuminate protein trafficking patterns or protein-protein interactions. One can also envision applications for the incorporation of heavy metal derivatives, crosslinkers, and enzyme affinity labels, among others [5]. Recent advances in noncoded amino acid incorporation in response to suppressor stop codons in vivo (Figure 1; and as reported by Schultz and coworkers in this issue of *Chemistry & Biology* [6] and in a recent issue of the Journal of the American Chemical Society [7]) may soon make these applications a reality.

Early efforts to infiltrate the genetic code focused on conservative substitutions, like trifluoroleucine for Leu [8] and selenomethionine for Met [9]; these noncoded amino acids were incorporated throughout the proteome. The first major breakthrough in the field came with the discovery of suppressor tRNAs, combined with the development of methods to chemically aminoacylate these tRNAs with noncognate amino acids [10–12]. These aminoacylated suppressor tRNAs participate in protein translation by readthrough of specific stop codons, leading to incorporation of the unnatural amino acid into emerging peptide chains (Figure 1). Thus, the ability to site specifically incorporate any chemically accessible amino acid into proteins, at least in vitro, was achieved. More than 100 structurally diverse amino acids have now been incorporated into proteins using this method, demonstrating that once a tRNA is aminoacylated, the translation apparatus is reasonably promiscuous for different amino acids [13]. Different approaches have also been undertaken in order to introduce the usefulness of aminoacylated suppressor tRNAs into living cells. For example, aminoacylated suppressor tRNAs can be microinjected into *Xenopus* oocytes [14], and direct uptake of aminoacylated tRNAs into mammalian cell cultures has been demonstrated [15].

Efforts have also been directed toward manipulating one or more aminoacyl-tRNA synthetase (AARS). The AARSs are the enzymes that are responsible for aminoacylating tRNAs in vivo, with each AARS being highly specific only for its cognate amino acid [16]. Thus, if an AARS could be mutagenized to recognize only a suppressor tRNA and only a new noncoded amino acid, then this mutant AARS/tRNA pair would be ideal for the incorporation of unnatural amino acids in vivo. The one caveat is that the new AARS must not aminoacylate any wild-type tRNAs, and the suppressor tRNA must not be a substrate for any wild-type AARSs. Several examples of AARS/tRNA pairs that fit these "orthogonality" requirements have now been reported [17–20].

Recently, Schultz and coworkers have taken these efforts a step further by designing methods to select novel orthogonal AARSs in vivo in both prokaryotic [7] and eukaryotic systems [6]. The elegance of these selection strategies is that they are independent of the nature of the desired noncoded amino acid. Of course, a new AARS needs to be selected for each new amino acid; however, the selection schemes are based only on the presence or absence of the amino acid in the selection media, not on the nature of its side chain. In each case, positive selection is used to identify mutated AARSs that can aminoacylate a suppressor tRNA at a level sufficient to enable readthrough of a critical stop codon (conferring antibiotic resistance in *E. coli* [7] or enabling Gal4-mediated transcriptional activation in *S. cerevisiae* [6]). This selection is conducted in the presence of the unnatural amino acid of choice. Next, negative selection is applied to weed out AARS variants that are not specific for the unnatural amino acid by removing this amino acid from the media and selecting only those variants that can no longer read through stop codons. In *E. coli*, cells were sorted based on a fluorescent signal [7], whereas in *S. cerevisiae* [6] cells were sorted based on sensitivity to 5-fluoroorotic acid (5-FOA). Both of these reports used tyrosyl-tRNA synthetase (TyrRS) as a starting point for the selection of a specific orthogonal AARS. For this reason, the current selection schemes have been weighted toward hydrophobic amino acids. Importantly, however, these strategies will surely prove applicable to other AARSs, opening the door for a wider array of unnatural amino acid targets. In *E. coli*, the prokaryotic selection scheme was used to select a new AARS specific for *p*-aminophenylalanine: a *p*-aminophenylalanyl-tRNA synthetase, or *p*AFRS [7].

In a clever twist to their *E. coli* selection scheme, Schultz and coworkers have generated a fully indepen-

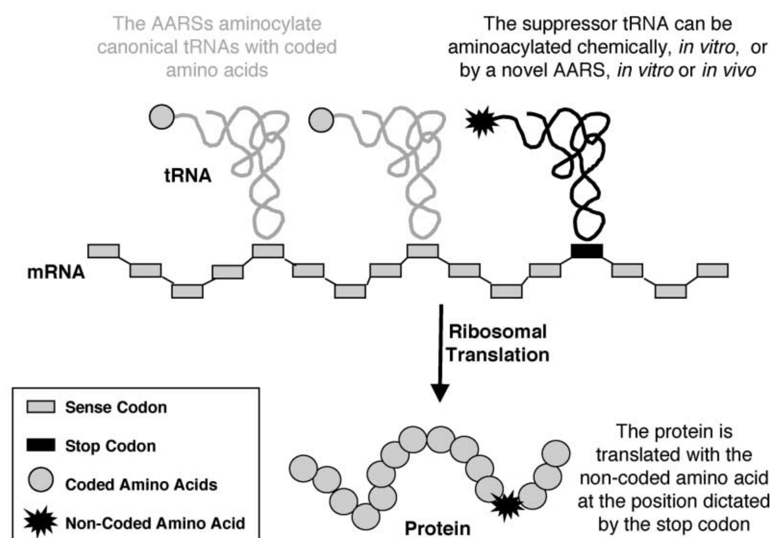

Figure 1. Codon Suppression as a General Method for the Incorporation of Noncoded Amino Acids into Biosynthetic Proteins

dent strain of *E. coli* with a 21 amino acid genetic code and the ability to biosynthesize its own 21st amino acid, *p*AF [7]. Three *Streptomyces venezuelae* genes critical to the biosynthesis of *p*AF were introduced into *E. coli* (*papA, papB*, and *papC*). When combined with an *E. coli* aminotransferase, the enzymes encoded by these genes were shown to biosynthesize *p*AF in vivo from chorismate. Finally, the introduction of the gene for pAFRS and its orthogonal tRNA (mutRNA$_{CUA}^{Tyr}$) generated a strain that was fully capable of making and incorporating *p*AF into proteins via suppression of the amber stop codon (TAG) [7].

Because of the efforts of these researchers, in the not-so-distant future it may be possible for scientists to order kits for the selective misincorporation of a wide variety of biotechnologically interesting amino acids. All that will be required is the construction of a plasmid with an engineered stop codon at the position targeted for unnatural mutagenesis. Thus, stop codons don't necessarily mean stop anymore.

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