299a Breaking the Degeneracy of the Genetic Code

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We have previously demonstrated that a wobble phenylalanine (Phe) codon can be re-assigned to a Phe analog in appropriately engineered strains of *E. coli*. We hypothesized that the concept of breaking the degeneracy of the genetic code can be generalized to other wobble codons. In this work, multiple-site-specific incorporation of an unnatural amino acid in response to a wobble leucine (Leu) codon was realized by use of an *E. coli* strain outfitted with a yeast transfer RNA ($ytRNA^{Phe}_{CAA}$) capable of Watson-Crick base-pairing with the UUG Leu codon. $ytRNA^{Phe}_{CAA}$ was charged with L-3-(2-naphthyl)alanine (2-Nal) by a co-expressed mutant yeast phenylalanine tRNA synthetase. The UUG codon was thereby partially re-assigned to 2-Nal, whereas the other five Leu codons remained assigned to Leu. Addition of a competitive inhibitor of *E. coli* leucyl-tRNA synthetase, 4-aza-DL-Leu, into the culture medium enhanced incorporation of 2Nal at the UUG codon by lowering the levels of competing *E. coli* leucyl-tRNAs charged with Leu. Furthermore, in order to obtain more active yPheRS mutants, screening methods based on multiple specific incorporation of nonnatural amino acids are being developed. The method presented here is applicable to diverse protein engineering objectives including glycosylation and pegylation.