Chemistry & Biology, Vol. 10, July, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S1074-5521(03)00154-6

Death, Taxes, and the Genetic Code?

The genetic code is an enduring feature of biology: only rare circumstances result in changes to translation of the code, at least in nature. Researchers are devising methods to engineer ribosome-synthesized polypeptides containing novel and potentially useful amino acids.

Ribosomal protein synthesis is a vital biological function that has been exploited as a valuable tool for research and for the production of medically and economically important proteins. Translation of the typical gene requires that the ribosome discriminate between a large number of similar aminoacyl-tRNA substrates with remarkable speed and processivity. However, while offering tremendous utility, proteins are normally limited to a standard set of 20 amino acids that encompass a narrow range of biochemical properties. The ability to artificially expand the variety of amino acids incorporated into growing peptide chains would be one way to increase the experimental and medical utility of proteins. For example, residues that serve as fluorescent tags or confer novel catalytic activities could provide breakthrough advances in protein technology. However, a major barrier to expanding the coding repertoire is the sheer complexity of the protein synthetic process. Changing the genetic code requires that a codon is reassigned to a corresponding tRNA and that the tRNA be acylated with a novel amino acid. Despite technical barriers to such alterations, recent progress by several groups lends hope that we may soon be able to engineer proteins with novel and useful amino acids.

The restrictive and defined chemistries of the canonical 20 amino acids' side chains have led nature to extensively supplement enzymes with metallic and organic cofactors, most of which perform critical catalytic functions that amino acid side chains alone simply cannot do. Nature has also dealt with the limited range of function by post-translational modification of amino acids. For example, the hydroxylation of proline and lysine is critical to the functions of many structural proteins such as collagen [1]. More exotically, certain redox enzymes contain the nonstandard amino acid selenocysteine incorporated at UGA codons [2]. This is achieved by an elaborate translational mechanism requiring a special tRNA and elongation factors as well as special message signals that recode UGA from stop to selenocysteine [3]. A similar mechanism is in place to incorporate pyrrolysine into proteins in methanogens [4]. The selenocysteine system can be considered parallel or orthogonal to the standard decoding system and can serve as a model for the biotechnologist. Several variations on this theme are under development in various labs.

One strategy is to create orthologous codon/tRNA/amino acid/ aminoacyl-tRNA synthetase (RS) combinations that operate in vivo. Technically, this is a challenging approach since not only does a codon and corresponding RNA have to be reassigned from the normal genetic coding functions, but the tRNA must also be efficiently acylated with the novel amino acid. This requires a specifically engineered RS, and, as if that were not enough, the cell must be able to tolerate the incorporation of the new amino acid into some fraction of its proteins. A potential advantage to an in vivo system, however, is relatively low cost, because specific components of the translational apparatus do not need to be purified for use in an in vitro translational system. Several groups have begun engineering RS enzymes to accommodate novel amino acids [5–7]. A preview article by Tamara Hendrickson published in last month's issue of *Chemistry & Biology* [8] discussed the Schultz group's recent progress toward the development of in vivo systems [e.g., 9, 10].

Another approach is to chemically attach unnatural amino acids to tRNAs in vitro. This avoids the requirement of engineering a specific RS, which may also mean that a wider variety of amino acids may be used. Two recently published papers use variations of this method to generate significant advancements in this field. Stephen Blacklow, Virginia Cornish, and collaborators [11] used purified components to construct an in vitro system for protein synthesis. Specific messages and corresponding tRNAs are synthesized, and the tRNAs are acylated with unnatural amino acids. These are then added to ribosomes and accessory factors to direct synthesis of the novel peptide, directed entirely by the engineered code. Thus far, they have achieved incorporation of four novel amino acids, all with yields in the tens of percent. This is remarkable considering that the tRNA substrates lacked nucleoside modifications, which are important for translational function [12]. Only short peptides were constructed, and this current system would not be economically competitive with chemical synthesis of defined peptides. It could be useful, however, for the synthesis of random libraries of peptides that contain unnatural amino acids. Moreover, this work is an intriguing proof of principle that may be developed for the production of longer polypeptides.

The in vitro synthesis of longer polypeptides faces several hurdles. One obstacle is increased efficiency. Unless yields can be increased, this system may be limited to either short peptides or to longer peptides that contain one of a few unnatural amino acids. The latter would still be extremely valuable because many applications will only require a single novel amino acid. Increased efficiency may also be achieved by optimizing tRNA sequence specificity and possibly specificity for the tRNA sequence to the amino acid [13]. Another tech-

nical complication that will need to be addressed for longer proteins is that all of the required aminoacyl-tRNAs must be included in the translational mix. Most simply, this could be achieved by adding cellular fractions containing the various translational components. In this case, however, the natural aminoacyl-tRNA for the reassigned codon would have to be prevented from acting, possibly with the use of a specific RS inhibitor.

In a report that was published in the June issue of *Chemistry & Biology*, Dougherty and coworkers [14] electroporated synthetic genes and chemically aminoacylated tRNAs directly into mammalian cells. Electroporation may allow higher transfection yields than other methods, such as biolistics, microinjection, and lipid-based carrier treatments, and it has the added advantage that it may also be applicable to a wide array of cell types. Other techniques, such as microinjection, may only work well with relatively large cells, like oocytes. Moreover, electroporation simultaneously transfects large populations of cells, whereas cells must be microinjected individually. Dougherty and coworkers electroporated both CHO cells and primary neurons. Two synthetic genes were used, one for green fluorescence protein and the other for the nicotinic acetylcholine receptor (nAChR). Each gene was engineered to contain the UAG codon at a single position and the aminoacyl-tRNA containing the appropriate anticodon. The electroporated transfectants expressed the encoded messages and made proteins containing the amino acid donated by the aminoacyl-tRNA. In separate experiments, tRNAs were acylated with either the natural serine or the unnatural 5,7-difluorotryptophan (F2trp), and both amino acids were incorporated.

Importantly, the F2Trp-engineered nAChR confers a characteristic effect on cell physiology. Using an oocyte microinjection system, Dougherty had previously shown that fluorinated tryptophans incorporated at residue 149 decrease the sensitivity of the receptor to acetylcholine [15]. In the current work, they use this phenomenon as an assay to demonstrate F2Trp incorporation in electroporated CHO cells. Thus, this work lends hope that reagent electroporation may greatly facilitate one of the most promising uses of the unnatural amino acid technology: the characterization of protein function in living cells.

I thank Dr. Rebecca Alexander for comments on a draft of this preview.

James F. Curran
Department of Biology
Wake Forest University
Winston-Salem, North Carolina 27109

Selected Reading

1. Towe, K.E. (2003). Science *300*, 1370–1371.
2. Böck, A. (2000). Biofactors *11*, 77–78.
3. Gesteland, R.F., Weiss, R.B., and Atkins, J.F. (1992). Science *257*, 1640–1641.
4. Srinivasan, G., James, C., and Krzycki, J.A. (2002). Science *296*, 1459–1462.
5. Kowal, A.K., Köhrer, C., and RajBhandary, U.L. (2001). Proc. Natl. Acad. Sci. USA *98*, 2268–2273.
6. Wang, L., Brock, A., Herberich, B., and Schultz, P.G. (2001). Science *292*, 498–500.
7. Sakamoto, K., Hayashi, A., Sakamoto, A., Kiga, D., Nakayama, H., Soma, A., Kobayashi, T., Kitabatake, M., Takio, K., Saito, K., et al. (2002). Nucleic Acids Res. *30*, 4692–4699.
8. Hendrickson, T.L. (2003). Chem. Biol. *10*, 475–476.
9. Mehl, R.A., Anderson, C., Santaro, S.W., Wang, L., Martin, A.B., King, D.S., Horn, D.M., and Schultz, P.G. (2003). J. Am. Chem. Soc. *125*, 935–939.
10. Chin, J.W., Cropp, T.A., Chu, S., Meggers, S., and Schultz, P.G. (2003). Chem. Biol. *10*, 511–519.
11. Forster, A.C., Tan, Z., Nalam, N.N.L., Lin, H., Qu, H., Cornish, V.W., and Blacklow, S.C. (2003). USA *100*, 6353–6357.
12. Curran, J.F. (1998). In Modification and Editing of RNA, H. Grosjean and R. Benne, eds. (Washington, D.C.: ASM Press), pp. 493-516.
13. Asahara, H., and Uhlenbeck, O.C. (2002). Proc. Natl. Acad. Sci. USA *99*, 3499–3504.
14. Monahan, S.L., Lester, H.A., and Dougherty, D.A. (2003). Chem. Biol. *10*, 563–571.
15. Zhong, W., Gallivan, J.P., Zhang, Y., Li, L., Lester, H.A., and Dougherty, D.A. Proc. Natl. Acad. Sci. USA *95*, 12088–12093.

Chemistry & Biology, Vol. 10, July, 2003, DOI 10.1016/S1074-5521(03)00151-0

Screening for Inducers of Kinase Degradation

Targeted small molecule-induced protein degradation is a promising approach to inhibit signaling within kinase cascades. In this issue, researchers describe a simple assay for the rapid, high-throughput identification of novel agents that promote degradation of the kinases Her2 and EGFR.

Her2 is a transmembrane receptor tyrosine kinase that heterodimerizes with other members of the Her family (e.g., epidermal growth factor receptor (EGFR)/Her1, Her3, and Her4) and promotes the transduction of proliferative and survival signals [1]. Her2 is overexpressed in a significant proportion of adenocarcinomas, and clin-