cell division that is much like the blebbing of PMN. Goldfarb notes that, although homologous genes have not been identified, 'it doesn't mean an autologous process does not occur' in mammalian cells. A similar blebbing event has been observed in Bloom's disease – a genetic disease that results from a mutation in a DNA helicase gene – in which 'nuclear microvesicles' are released into the cytoplasm [6]. Regardless, Klionsky finds the study of biodegradation 'fascinating overall'. Although most research has focused on biogenesis, he says 'there is always this homeostasis. You have to have both [processes], you can't have just one'.

Although the research undertaken here might not lead to the development of novel therapeutic agents, it brings us a step closer to understanding the ways in which eukaryotic cells recycle materials in specific and refined ways.

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Scientists expand the genetic code

Vida Foubister, freelance writer

Ever wonder whether mankind would be better off with a genetic code that uses 21 amino acids, one more than the standard 20, to make proteins? It soon might be possible to find out. Scientists at The Scripps Research Institute (http://www.scripps.edu/) have created a bacterium that synthesizes an unnatural amino acid, *p*-aminophenylalanine (*p*AF), and incorporates it into proteins with a fidelity and efficiency rivaling that of the 20 natural amino acids [1].

'They've really redesigned *Escherichia coli*,' said Michael Ibba, Assistant Professor of Microbiology at The Ohio State University (http://www.osu.edu/). 'They chose what amino acid to add to the genetic code of this organism and they gave the organism the machinery to make the amino acid and use it.'

Although there is nothing special about *p*AF, a known synthetic amino acid with a structure similar to tyrosine, this study demonstrates the potential to make changes that are more interesting, both scientifically and therapeutically. 'The nice thing about their approach is that it's modular,' said Virginia Cornish, Assistant Professor of Chemistry at Columbia University (http://www.columbia.edu/). 'While they can only have 21 total amino acids, what that 21st amino acid is they can vary fairly easily.'

A complete system

The challenge facing the Scripps scientists was to put all the pieces necessary for the biosynthesis of *p*AF and its incorporation into protein in *E. coli* and ensure they worked together. 'In order to go from 20 to 21 amino acids, we had to add a biosynthetic pathway, add a synthetase, add a tRNA and add a codon for that new amino acid,' said Ryan A. Mehl, now an Assistant Professor of Chemistry at Franklin & Marshall College (http://www.fandm.edu/). 'We stole the machinery from other organisms and modified it.'

First, a biosynthetic pathway that enabled *E. coli* to make *p*AF from simple carbon sources was needed. Three genes from *Streptomyces venezuelae*, which lead to the production of *p*-aminophenylpyruvic acid as an intermediate metabolite in the conversion of chorismate to chloramphenicol, were introduced into *E. coli* (Fig. 1). A native *E. coli* enzyme, aromatic aminotransferase, completes the biosynthesis to *p*AF.

To incorporate the resulting 21st amino acid into protein, a tRNA–synthetase pair from *Methanococcus jannaschii* was introduced into the same bacterium. The tRNA was chosen because of its specificity for TAG, a nonsense codon that normally tells the cell machinery to stop making protein. However, in this system, the synthetase was altered to recognize *p*AF and load it onto the tRNA. Because of that modification, the unnatural amino acid is incorporated into protein in response to the TAG stop codon.

1359-6446/03/$ – see front matter PII: S1359-6446(03)02635-7

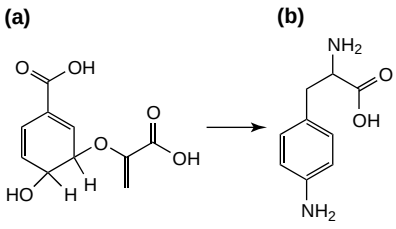

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Figure 1. A novel bacterium with a 21 amino acid genetic code converts **(a)** chorismate, an intermediate in the synthesis of aromatic amino acids, into **(b)** *p*-aminophenylalanine (*p*AF) and incorporates this unnatural amino acid into proteins in response to the amber nonsense codon UAG. Figure reproduced, with permission, from Ref. [1].

To determine whether *p*AF was being generated, amino acids were extracted from cells grown in minimal media, separated by HPLC and then measured by MS. Finally, the fourth codon of the sperm whale myoglobin gene was converted to a TAG stop codon, and the insertion of *p*AF into the protein at this site was confirmed by N-terminal sequencing.

Exploiting this new chemistry

The ability to site-specifically modify proteins holds tremendous potential both for basic scientific research and drug design. 'Historically, the way we've tried to understand biological systems is by isolating components in test tube and analyzing them there,' Cornish said. 'But what you really want to know is how they function in the cell.'

The possibilities include using a biophysical probe, such as fluorophores, to directly tag a protein within living cells. 'This approach would allow you to follow the protein in real time with a microscope and watch various biological processes,' said Christopher Anderson, Graduate Student at Scripps. Another probe called a photocrosslinker, which can be activated by ultraviolet light to crosslink proteins, could be used to stabilize transient protein–protein interactions within cells [2,3].

There are also several potential therapeutic protein applications. The addition of a ketone [4], a functional group that is not present in any of the natural 20 amino acids, would give scientists a 'reactive handle,' Anderson said. 'You create a site that is totally unique in that molecule and can be selectively modified.' Scientists could also use this approach to site specifically attach polyethylene glycol, a long polymer that improves bioavailability, to therapeutic proteins. Today, the PEGylation of commercially available proteins, such as insulin and erythropoietin, is nonspecific.

It might also be possible to use the insertion of an unnatural 21st amino acid to enable *E. coli* to make post-translational modifications that are essential for the function of some therapeutic proteins. Erythropoietin, for example, requires glycosylation.

Future work

The Scripps lab, lead by Peter G. Schultz, Professor of Chemistry, plans to pursue many of those applications. It is also is working to develop new systems that enable *E. coli* to incorporate unnatural amino acids with chemical structures that differ from *p*AF. As well as modifying tRNA–synthetase pairs, the group will work to add amino acid biosynthetic pathways because of their potential to reduce costs by eliminating the need for unnatural amino acids in the growth media. In the future, these biosynthetic pathways might also enable applications in more complex organisms where feeding the animal large amounts of an unnatural amino acid could be toxic.

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