NEW AMINO ACID DISCOVERED; FUNDAMENTAL BUILDING BLOCK OF LIFE (May 23, 2002)

COLUMBUS, Ohio - Two teams of researchers from Ohio State University reported today that they had identified the 22nd genetically encoded amino acid, a discovery that is the biological equivalent of physicists finding a new fundamental particle or chemists discovering a new element.

Two papers describing the discovery appear in the current issue of the journal *Science*. Prior to this, scientists had believed that there were only 21 natural amino acids -- the key building blocks of proteins.

For 30 years after the discovery of the structure of DNA and the unraveling of the genetic code, scientists believed that there were only 20 natural amino acids. Then in 1986, researchers broke that numerical barrier announcing that the 21st had been discovered.

Finding a 22nd suggests that even more of these basic biological building blocks may be found using modern genome sequencing techniques.

The discovery grew out of some very basic biochemistry examining how a particular type of microbe - methanogens - can convert methyl-containing compounds into methane. While researchers have long understood the biochemical mechanisms for how acetate and carbon dioxide are converted to methane, they didn't understand how a common class of compounds - the methylamines - is transformed into this gas.

One research group, led by Joseph A. Krzycki, an associate professor of microbiology, had been working for several years with a particular strain of microbe, *Methanosarcina barkeri*. This organism, a member of the recently identified domain Archaea, is able to convert monomethylamine, dimethylamine and trimethylamine into this greenhouse gas.

Krzycki's research group had isolated specific proteins related to the process in 1995 and, two years later; they had isolated and sequenced one of the genes responsible. Then in 1998, they published a paper showing that the gene had a component called an in-frame amber codon that behaved unusually.

Codons are three-letter "words" identifying the bases DNA uses to specify particular amino acids as building blocks of proteins. Normally, codons signal the start of a protein, its end or a particular amino acid used to construct it. Surprisingly, the codon Krzycki's team identified should have signaled a stop to protein building but it did not.

"Joe and his colleagues found this happening in genes important for all three of the methylamine compounds - something that wasn't supposed to happen," explained Michael Chan, an associate professor of biochemistry and chemistry at Ohio State. Chan led the second research team that identified and determined the structure of the amino acid.

The realization of the codon's odd behavior suggested the possibility of a new amino acid, but the researchers knew there might be other explanations as well. Krzycki and his colleagues sliced the protein into smaller bits called peptides, and began sequencing them, a process which usually ultimately reveal the amino acid responsible for the protein.

"That all seemed to point to this being just lysine, one of the normal amino acids," Chan said. Regardless, Krzycki asked Chan and Ph.D. student Bing Hao to start working on deducing the crystalline structure of the protein containing the amino acid. At the end of the two-year process, Hao and Chan had determined the structure of the protein, part of which revealed a new amino acid.

At the same time, Krzycki was looking for other evidence. He, along with doctoral students Gayathri Srinivasan and Carey James, was eventually able to identify the specific transfer-RNA (tRNA) needed to insert the new amino acid into protein, as well as another important enzyme essential to the process. These two discoveries, along with the detailed crystalline structure, convinced the teams that they had found a new genetically encoded amino acid -- pyrrolysine - the 22nd known to science.

"We realized that we had to know which tRNA would decode that amber codon," Krzycki said. "Finding it was an essential part of the puzzle."

He believes this will be a very rare amino acid, given the fact that it has taken so long to identify it. However, Krzycki believes it is likely to be found in other situations - in other organisms - aside from methanogens. He's philosophic about the importance of the discovery: "This shows us that the genetic code, and therefore, evolution is much more plastic than people might have thought."
Chan agrees, pointing to the strong possibility that finding a 22nd genetically encoded amino acid should stimulate the search for a 23rd or a 24th. "With so many researchers dissecting so many genomes now, it's reasonable to suggest that there might be more waiting to be found.

"I think this work will cause researchers to start looking at genetic sequences that they might have thought at first were simply aberrations," he said. "Instead, they might signal discoveries like ours."