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Doing nature one better: Expanding the genetic code in living mammalian cells

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Researchers at the Salk Institute for Biological Studies have developed a novel strategy to expand the natural repertoire of 20 amino acids in mammalian cells, including neurons, and successfully inserted tailor-made amino acids into proteins in these cells. In a powerful demonstration of the method's versatility, they then used unnatural amino acids to determine the operating mechanism of the "molecular gates" that regulate the movement of potassium ions in and out of nerve cells.

"In the past, this type of engineering has been mainly restricted to bacteria or in yeast, and it was very challenging to efficiently incorporate unnatural amino acids in mammalian cells. But most biomedical questions have to be studied in the cells of higher organisms and animal models to arrive at meaningful answers," explains Lei Wang, Ph.D., an assistant professor in the Chemical Biology and Proteomics Laboratory, who led the current study published in the July issue of *Nature Neuroscience*.

The genetic code, which is shared by plants, animals and bacteria, includes 64 codons encoding 20 different amino acids and three stop signals. Being able to expand the code and insert non-natural amino not only greatly enhances researchers' ability and precision, but also provides novel tools for addressing challenging questions insurmountable with conventional means.

"We had tried using conventional mutagenesis to introduce mutations

into the potassium channel but it didn't give us any answers," says Paul A. Slesinger, Ph.D., an associate professor in the Peptide Biology Laboratory, who collaborated with Wang on the current study. "Being able to incorporate bulky unnatural amino acids into living mammalian cells really made all the difference," he adds.

During his graduate studies, Wang pioneered a method to accommodate additional amino acids in bacteria. His approach mimicked the strategy every cell relies on to incorporate conventional amino acids into proteins: During protein synthesis, amino acids are brought out one by one by molecules known as transfer RNAs (tRNA) and added to the growing protein chain according to the instructions spelled out in the genetic code till a stop codon — for which no corresponding tRNA/amino acid pair exists — lets everybody know that this particular job is done.

From a large pool of mutated aminoacyl-tRNA synthetases — the enzyme that loads tRNAs with their corresponding amino acids — Wang selected the one that would attach a desired artificial amino acid to a tRNA that recognizes one of the stop codons. Every time the stop codon appeared in the genetic code, the new tRNA would insert the artificial amino acid.

But doing the same trick in mammalian cells becomes way more complicated. Simply transferring the bacterial genes into mammalian cells doesn't work since they flat out refuse to produce bacterial tRNAs. While it is easy to screen large numbers of mutated aminoacyl-tRNA synthetases in bacteria and yeast, it can't be done in mammalian cells in the same way. But Wang and his team got around both obstacles.

"We found that we could coerce mammalian cells to express bacterial tRNAs by using the H1 promoter," says first author Wenyuan Wang, Ph.D., a postdoctoral researcher in Wang's laboratory. Relying on yeast

to do the dirty job of finding a synthetase that recognizes tRNA and attaches the right unnatural amino acid helped them to overcome the second challenge. “Using yeast for the selection process and then transferring the enzyme for use in mammalian cells may sound like a naïve idea, but members from the same kingdom behave very similarly in terms of tRNA synthetases and it worked,” he adds.

After a green fluorescence protein-based functional assay in various mammalian cells and neurons literally gave them the green light, Wang teamed up with Slesinger, who studies ion channels in the brain, to illustrate that this technology can solve otherwise intractable biological questions.

When a signal travels along a nerve cell, the potassium channel Kv1.4, which belongs to a class of so-called fast-inactivating ion channels, opens briefly and then quickly shuts down. Structural studies had suggested that in a process similar to threading a needle the channel’s flexible head feeds through a small portal and blocks the central pore of the channel. Wang and Slesinger used the new unnatural technology as a molecular ruler to answer the question whether increasing the size of the thread had an effect on the speed of inactivation"

“We introduced mutations into the thread, so it would be too big to fit through the hole,” says Wang, “but we couldn’t see a difference with natural amino acids.” Adding even bulkier, artificial amino acids provided the answer. “Now the process of inactivation was really slow, supporting the hypothesis that the diameter of the flexible head plays a crucial role in the fast inactivation of this channel,” adds Slesinger.

Source: Salk Institute