Early evolution maximized the 'spellchecking' of protein sequences

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As letters of the alphabet spell out words, when amino acids are linked to one another in a particular order they "spell out" proteins. But sometimes the cell machinery for building proteins in our bodies makes a mistake and the wrong amino acid is inserted. The consequences can be devastating, resulting in a garbled protein that no longer has the correct function, possibly leading to cancers and other diseases.

Now scientists at The Scripps Research Institute have examined how an enzyme responsible for adding one amino acid, alanine, to proteins has come to have its own spellchecker. In their paper published in the August 7, 2009, issue of *Science*, Scripps Research Professor Paul Schimmel and colleagues show that two separate functions—alanine adding and editing—were joined together in a single enzyme during early evolution, in a way that greatly enhances these activities. The findings provide a glimpse into how enzyme functions have evolved.

"The work is also an example of the power of a multi-disciplinary approach to one specific study," says Schimmel, who is Ernest and Jean Hahn Professor and Chair in Molecular Biology and Chemistry and a member of the Skaggs Institute for Chemical Biology at Scripps Research. "Often one study is based on computer-based informatics, or x-ray crystallography, or enzyme function analysis. But here all of these methods and more were brought to bear on an issue."

Building Proteins...

Proteins are made of chains of amino acids linked to one another by ribosomes, the protein factories of cells. Small RNA molecules, called transfer RNAs (tRNAs), transport specific amino acids to the ribosomes so that they can be added to a growing chain.

Each type of tRNA is attached to, or charged with, only one type of amino acid by one enzyme, with 20 enzymes for 20 amino acids. But sometimes a tRNA gets stuck with the wrong amino acid, or is mischarged. In 2006 Schimmel's group, in collaboration with the Ackerman group at Jackson Laboratories, showed that when the enzyme that adds alanine to tRNAs—called alanyl-tRNA synthetase (AlaRS)—mischarges its tRNA (tRNA Ala), the error leads to the accumulation of misfolded proteins and causes mice to develop a devastating neurological disease.

Because of such consequences, a cell has a vested interest in not misspelling its proteins, especially when it comes to adding alanine. How does it ensure nothing goes wrong?

...And Editing Them

Proteins, including enzymes, are often divided into domains—parts of a protein that can evolve, function, and exist independently of the rest. One domain of AlaRS is responsible for aminoacylation, the chemical reaction that adds alanine to tRNA Ala. A second domain, the editing domain, removes amino acids other than alanine added to tRNA Ala by mistake.

Many cells also contain "free-standing" alanine editing enzymes, called AlaXps, that are separate from AlaRS. "The idea is that if the editing domain of AlaRS does not catch the error in aminoacylation then there is another chance," explains Schimmel. "The AlaXp can remove the wrong amino acid from mischarged tRNA Ala."

In AlaRS, the two domain are joined together. But there is third domain, C-Ala, attached to the end of this enzyme, present in AlaRS of every living species. C-Ala acts as a functional bridge between the aminoacylation and editing functions. This C-Ala domain is also present in some, but not all, free-standing AlaXps.

"We think that the earliest AlaXps did not have C-Ala," says Min Guo, a postdoctoral fellow in Schimmel's lab. "Then AlaXps got bigger during evolution by the addition of C-Ala. They then came together with the aminoacylation function through gene fusion."

Guo and Schimmel decided to figure out the function of C-Ala.

A Bridge Between Two Functions

As a first step, they determined the structure of C-Ala by x-ray crystallography, a technique used for obtaining the three-dimensional arrangement of proteins and other molecules by bombarding them with x-rays. They discovered that the structure of C-Ala looks a lot like another type of protein that binds DNA. They therefore tested whether C-Ala binds tRNA Ala.

All tRNAs have three-dimensional shapes that look like a capital letter "L ." Through a series of experiments, Guo and Schimmel determined that C-Ala binds to the "elbow" of tRNA Ala, the region that joins the two arms of the L-shaped tRNA.

The researchers next asked, "What is the significance of this binding?"

To find out, they took the AlaRS protein apart—separating the aminoacylation domain from the editing domain with or without C-Ala—and then put different combinations of these protein regions back together again.

The researchers discovered that the aminoacylation domain on its own adds alanine to tRNA Ala's, but not very well. Adding the editing domain with C-Ala makes aminoacylation more efficient. "When you add these two halves of the protein they can speak to one another, through the binding of C-Ala to tRNA," says Guo.

But when the scientists added the editing domain without C-Ala, there was no difference in aminoacylation activity.

"The C-Ala domain strongly enhances the collaboration between the aminoacylation and editing domains. It makes them work together synergistically," explains Schimmel.

Guo and Schimmel's analysis further indicates that the earliest organisms probably had the aminoacylation and editing functions as separate enzymes, but because of the importance of correctly adding alanine to proteins, these two activities were soon brought together by C-Ala sometime during primordial life.

The results were entirely unexpected. "We always imagined that domains are joined together in evolution by linkers," says Schimmel. "But here the linker C-Ala acts in a way that greatly enhances their functions, and enables them to work as true partners."

In addition to Schimmel and Guo, co-authors of the paper "The C-Ala Domain Brings Together Editing and Aminoacylation Functions on One tRNA" include Yeeting E. Chong, Kirk Beebe, Ryan Shapiro, and Xiang-Lei Yang from Scripps Research.

Source: The Scripps Research Institute (news : web)