Researchers uncover chemical basis for extra 'quality control' in protein production

December 9 2009

December 9, 2009 -Even small errors made by cells during protein production can have profound disease effects, and nature has developed ways to uncover these mistakes and correct them. Though in the case of one essential protein building block—the amino acid alanine—nature has been extra careful, developing not one, but two checkpoints in her effort to make sure that this component is used correctly.

Now, scientists at The Scripps Research Institute have discovered the chemical basis for why these extraordinary efforts are necessary. The work was published in the December 10, 2009 issue of the prestigious journal *Nature*.

"What is shown here with the 'serine paradox' is just the tip of the iceberg," said senior author Paul Schimmel, who is the Ernest and Jean Hahn Professor and Chair of Molecular Biology and Chemistry and a member of The Skaggs Institute for Chemical Biology at Scripps Research. "In the coming years, there will be an increasing awareness of the role of mistranslation in human diseases and of how nature has struggled to find solutions to attenuate mistranslation and its consequences."

Spelling with Amino Acids and Proofreading for Errors

As letters of the alphabet spell out words, when amino acids are linked to

one another in a particular order they "spell out" proteins. When amino acids are put in the wrong order, "spelling errors" (mistranslations) occur, often with devastating consequences for the health of the organism.

Normally, small RNA molecules, called transfer RNAs (tRNAs), transport specific amino acids to the ribosomes, the protein factories of cells, so that amino acids can be added to their correct place in a growing chain. At the beginning of this process, 20 tRNA enzymes, one for each of the 20 common amino acids, select the proper amino acid to be transported by a tRNA and join them together.

However, as Senior Research Associate Min Guo of the Schimmel-Yang lab, who is first author of the new paper, noted, "Sometimes there are mistakes. Where there is supposed to be alanine, there is a serine or glycine instead."

In 2006, the Schimmel-Yang lab contributed to research led by the Ackerman group at Jackson Laboratories that showed the consequences of this particular mistake in protein building for a strain of mutant mice. When the enzyme that adds the amino acid alanine to tRNAs—called alanyl-tRNA synthetase (AlaRS)—mischarges its tRNA (tRNA Ala), the error leads to the accumulation of misfolded proteins and the mice display severe neurological and other defects. Another study by the Schimmel-Yang lab showed that E. coli bacteria with a similar mutation become very sensitive to serine and glycine, dying when these amino acids (but not others) are added to the culture.

Clearly, nature has a vested interest in avoiding such costly errors.

In fact, the Schimmel-Yang group showed in a *Nature* and a Science paper published in January 2008 and August 2009, respectively, that nature plays it extra safe with the quality control of alanine in protein

production. Alanine's tRNA synthetase, AlaRS, not only loads the tRNA with an amino acid, but also checks to make sure it attached the right one. In addition, many organisms, from bacteria to humans, have an extra freestanding "spellchecker" molecule—in the form of a protein called AlaXp—to ensure that alanine is not confused with other amino acids.

"The editing function is redundant," said Guo. "This leads to several questions: Why are the cells so sensitive to alanine mistakes in particular? Why did AlaXps evolve so early? And why are redundant proteins still present that you supposedly don't need?"

A Case of Mistaken Identity

In the new *Nature* paper, the scientists used a variety of techniques, including x-ray cryatallography and kinetic and mutational analysis, to answer these questions.

The results showed that one reason for the difficulty AlaRS has in distinguishing alanine from serine and glycine is that the active site on the AlaRS molecule is a large, flexible pocket. Instead of acting as a rigid lock for a single key, the cavity flexes to hold not only its target alanine, but also similar-size molecules serine and glycine.

But serine and glycine are not exactly the same size as alanine. That's where the "serine paradox" comes in. Glycine is smaller and serine is larger than alanine. Current theory would not predict that molecules that are both larger and smaller than alanine would be a problem for AlaRS.

"The reason we call it a paradox is because none of the other tRNA synthesases have a problem mis-activating both a smaller and a bigger amino acid," said Guo. "Theoretically, the tRNA synthesases should have the most problem recognizing a smaller amino acid, because a

smaller one can also go into the binding pocket. The smaller one is easy to understand. Now we explain the bigger one."

Unexpectedly, the new study's results also revealed that within AlaRS's binding pocket the acidic group of Asp235 creates an extra hydrogen bond with the larger serine molecule. This additional bond turns out to be the major force that helps to secure the misplaced serine in the pocket, despite its larger size. However, x-ray analysis showed that Asp235 is also critical for holding the amino group of alanine. Attempts by the Schimmel-Yang lab (and also most likely by eons of evolution) to replace Asp235 with another residue failed. In fact, the scientists found that to make a change that would eliminate the interaction with serine would also impact negatively on the interaction with the correct amino acid, alanine. So, nature developed another solution by creating AlaXp, which is specifically designed to provide a second check and eliminate any serine that is attached to tRNA (Ala).

Together, AlaRS's large, flexible pocket and the additional hydrogen bond with serine explain the chemical basis for frequent confusion of glycine and serine for alanine and the need for additional checks to make sure that alanine, not one of its look-alikes, is incorporated into a protein when called for.

"Now we can understand why nature takes so much effort to invent two editing checkpoints," said Guo. "That's what is necessary to have the correct sequence of amino acids in a protein. To me, it's amazing that a single chemical feature can determine the fate of almost every organism on earth. Organisms from bacteria to humans share the same biological solution that nature found to this critical chemical problem."

Source: The Scripps Research Institute (news : web)