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LOW-FIDELITY DNA POLYMERASES

Enzymes that make lots of 'mistakes'may have functional and evolutionary significance

STU BORMAN, C&EN WASHINGTON

A viral dna polymerase that catalyzes the synthesis of DNA with a high rate of base pair mismatches is the latest example of a series of error-prone polymerases that have amazed and intrigued the biomedical community.

These low-fidelity DNA polymerases flout the conventional rules of DNA base pairing. They also may have specialized functions and evolutionary implications that are as yet almost completely unknown. And they may provide a new type of research tool for synthesizing novel forms of DNA to order.

Polymerase X (Pol X) from African swine fever virus catalyzes the formation of traditional Watson-Crick base pairs--guanine with cytosine and adenine with thymine--but also catalyzes guanine-guanine mismatches with high frequency. The enzyme's unique behavior was identified and characterized by graduate student Alexander K. Showalter and chemistry professor Ming-Daw Tsai of Ohio State University [*J. Am.*

ENZYME BEHAVIOR **Tsai (left) and Showalter's discovery that polymerase X catalyzes base pair mismatches adds to body of evidence that challenges the traditional philosophy of**

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Chem. Soc., **123,** 1776 (2001)]. The discovery of Pol X and other low-fidelity polymerases "suggests that the traditional philosophy of DNA replication may need to be rethought," Tsai says.

Peter Strazewski of the Institute of Organic Chemistry at the University of Basel, Switzerland, whose research interests include base pairing and mutagenesis, notes that the ability of the swine fever virus genome "to encode and control its own mutagenicity, possibly site- or maybe base-specifically, is a fascinating and potentially very important finding, both for evolutionary and medicinal research."

NOBEL LAUREATES James Watson and Francis Crick first proposed in 1953 that the faithful replication of DNA was based on the formation of selective hydrogen bonds between the specific nucleotides that join to form base pairs. "That idea has been firmly rooted in the dogma of DNA polymerase fidelity for almost 50 years," Tsai says.

The principle was shaken somewhat by chemistry professor Eric T. Kool of Stanford University and coworkers when they synthesized shape mimics of conventional DNA bases that form Watson-Crick base pairs but can't hydrogen bond. Their work suggested that geometry rather than hydrogen bonding is the critical factor in base pair formation. But the central theme that one base demands its Watson-Crick partner has remained more or less intact.

The advent of mismatch polymerases is now challenging that theme once again. "There are now many DNA polymerases known that are highly error-prone, and some even insert a wrong base opposite a template nucleotide better than a correct base," says biological chemistry and genetics professor Louise Prakash of the University of Texas Medical Branch, Galveston, whose group has discovered and characterized some of the key low-fidelity DNA polymerases. For example, Pol-h catalyzes the formation of guanine-thymine mismatches, albeit less frequently than it makes correct adenine-thymine base pairs [*J. Biol. Chem.,* **274,** 36835 (1999)]. Pol-i, on the other hand, inserts a guanine 10 times more often or a thymine five times more often than it incorporates the correct nucleotide adenine opposite thymine [*Nature,* **406,** 1015 (2000)].

Asked to comment on the Showalter-Tsai study, Kool says, "Pol X is what I would consider to be a very nice third example, after Pol-h and Pol-i--and certainly one of the more extreme examples--of a very low-fidelity DNA polymerase. And it's the first that's a viral polymerase

instead of a human polymerase, so that makes it new and interesting as well. It has remarkably low fidelity, and it is also remarkable that it makes guanine-guanine mismatches so frequently." Pol-h and Pol-i make primarily guanine-thymine mismatches.

ANOTHER DISTINCTIVE feature of Pol X is that it's the first polymerase to catalyze five base pairings--the four Watson-Crick permutations plus the guanine-guanine mismatch, all with comparable activities. Pol-i, for example, "has very low fidelity for one particular base pair in part due to a greatly diminished catalytic activity for the corresponding correct pair," Tsai says.

Research geneticist Thomas A. Kunkel of the National Institute of Environmental Health Sciences, Research Triangle Park, N.C., who specializes in DNA replication fidelity, notes that most polymerases make "a particular mismatch, say a guanine coming in opposite a thymine," about one time for every 10,000 adenines they put in correctly. "So they're considered accurate."

But for the guanine-thymine pair, Pol-h has an error rate as high as one in 16. And Pol-i "is now the most extreme example that anybody knows about," Kunkel says, "because instead of an error rate of one in 10,000 or even one in 16, it has an error rate of three in four. So it does guanine-thymine mismatches more efficiently than it puts together correct adenine-thymine pairs. That's totally stunning. It must have a highly specialized function because it violates the standard Watson-Crick pairing dogma, but nobody knows what that function is."

Showalter and Tsai have now found that "the error rate of Pol X is one in two for the guanine-guanine mismatch," Kunkel says. "In other words, it will do the mismatch about as well as it will do a correct guanine-cytosine base pair. The really unusual feature is the relative efficiency of formation of the guanine-guanine pair, which is uniquely high for that type of mismatch. That's what's really striking about the study."

So the Ohio State group's study "is amazing, with one caveat," Kunkel says. "The caveat is that polymerases have the opportunity to perform repair or replication using a wide variety of different sequences, but Showalter and Tsai analyzed the enzyme's incorporation properties at only one specific template nucleotide position. It's hard to know whether that's really going to be a
general feature of this polymerase or whether there's something highly unusual about the site they investigated." Nevertheless, he adds, "that doesn't dampen my

enthusiasm for the work because it's very exciting that the viral polymerase makes errors at an efficiency close to that of correct base pair formation."

PRAKASH

Researchers would now like to know how the polymerases misincorporate bases "and why they do it, why they evolved that way," Kool says. "It's totally unexplained activity. You would expect most polymerases that replicate DNA to have high fidelity. So why do some have very low fidelity"

From a structural standpoint, he speculates that "very loose active sites" may account for the mismatch behavior. The active sites of low-fidelity polymerases are most likely "not tightly constrained around the DNA," Kool believes. "At least that's my working hypothesis."

Furthermore, researchers working in this field would like to know why some polymerases have evolved into mismatch catalysts. From an evolutionary perspective, it's possible that a guanine located opposite a thymine "might not always be an error," Kunkel says. "Under some circumstances, it might be the right thing to do to stabilize a genome. These specialized polymerases may actually confer a selective advantage on a cell by virtue of their ability to generate mismatches." Showalter and Tsai propose in their paper that Pol X catalyzes mismatches and thus promotes mutation because viruses need to mutate reasonably rapidly.

Studies of mismatch polymerases are important from a basic research standpoint in that they will potentially provide a deeper understanding of how low-fidelity enzymes work and the evolutionary biology of why they exist. Ironically, such studies could also lead to a better understanding of the accurate replication of genetic information.

Possible practical applications of the work include developing new ways of synthesizing DNA containing modified nucleotides. "At a conference several years ago, I said I could imagine a day when one could make designer polymerases with almost any kind of specificity, once we knew enough about polymerases," Kunkel says. "There's a lot of flexibility in the active sites of low-fidelity polymerases, and if we understood this better it would potentially allow us to design enzymes to do exactly what we want them to do, with a range of biotech applications."

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