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Common Enzyme is a Key Player in DNA Repair

A quarter century after they discovered it, researchers have identified the job of one of the most common DNA-damage response proteins. The enzyme has puzzled scientists because it is present in nearly every organism, which suggests that it is crucial to life, and yet, in laboratory experiments, its function has remained a mystery.

The discovery suggests that the enigmatic enzyme known as DinB DNA polymerase is specialized for proficient and accurate replication of a particular kind of damaged DNA, report Graham Walker, an HHMI professor at the Massachusetts Institute of Technology, and his colleagues in the January 12, 2006, issue of the journal *Nature*. HHMI professors are leading research scientists who received \$1 million grants from the Institute to bring innovative teaching to the undergraduate classroom.

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- Graham C. Walker

DNA is assaulted daily by toxic chemicals, metabolic byproducts, sunlight, and other forms of radiation. Most of the nicks and dings are quickly fixed by the cell's fleet of precision DNA repair processes, which can surgically excise and replace a faulty section. But sometimes a damaged bit of DNA slips through unrepaired. A faulty nucleotide—the basic constituent of DNA—can stall the temperamental DNA replication machinery as it unwinds and copies the genome in dividing cells. In humans, uncorrected DNA errors passed along to the new daughter cells can lead to cancer.

When DNA integrity is at stake, a family of proteins known as translesion DNA polymerases comes to the rescue. They cannot actually repair a damaged strand of DNA, but they can smooth over the problem by inserting a

nucleotide opposite the damaged partner, so the DNA replication machinery can finish duplicating the chromosome despite the damaged DNA. This trick is usually referred to as a DNA damage tolerance mechanism. "It enables the replication to keep going and tolerate the damage, even though it doesn't get rid of it," Walker explained.

The molecular building blocks of DNA are adenine, cytosine, guanine, thymine - A, C, G, and T in the shorthand of molecular biology. In different combinations, they make up the genes that code for the proteins that carry out the routine and complex tasks necessary for life.

Now, researchers in Walker's lab have discovered that the enzyme DinB is exceptionally skilled at copying over a particular kind of damage to the G nucleotide. When the main DNA replication machinery stalls at the damaged G, the DinB translesion polymerase recognizes the G and adds its chemical partner, C, to form the correct base pair in the new chromosome.

"It was a pretty striking result," Walker said. "Not only did DinB copy over the damaged G nucleotide, it was 10 to 15 times better at copying the damaged G than copying the undamaged G. Up until now, it didn't look like that useful a polymerase."

Experiments led by MIT graduate student Daniel Jarosz and former postdoctoral fellow Veronica Godoy revealed that when bacteria that are missing DinB are exposed to a chemical that causes DNA damage, these bacteria are 1,000 times more likely to die than normal bacteria. Godoy is now an assistant professor of biology at Northeastern University in Boston. She and Jarosz also found that DinB can only repair G damage of a certain size. For example, they said, DinB cannot efficiently copy over a particularly bulky type of damage caused by a chemical found in charcoal-broiled steaks.

The researchers also discovered that a single change in the amino acids that make up DinB will strip it of its unique talent and change it into an ordinary polymerase. When a protein is that easy to modify, scientists assume its function must be important to have survived several billion years of evolution.

"In general, we think that nature evolved this function to help us survive DNA damage that would otherwise kill us," said Walker. "It's probably a kind of damage that all cells encounter frequently in life."

Walker and colleagues found that DinB's unique ability to repair DNA damage extends to mice and archaea, an ancient and diverse group of single-celled organisms. In nearly every organism, they believe, the same specialized enzyme is poised to patch over a certain kind of blemish so that it does not permanently damage the genome.

The DinB gene and its protein product were first discovered in Walker's lab in 1980, long before scientists knew anything about translesion polymerases. Then graduate student Cynthia Kenyon, now a biologist at University of California, San Francisco who has done groundbreaking work on the genetic basis of aging in worms, systematically identified the genes turned on in bacteria exposed to DNA damaging agents, the so-called SOS response. Kenyon named the damaged-inducible (Din) genes in order of discovery, and DinB was the second one she found.

"Knocking it out didn't seem to have an obvious effect," Walker said. "We couldn't find anything interesting in the mutant bacteria." Since then, the known DinB family has grown to include five related proteins in bacteria, 10 in yeast, and four in people.

"We found it 25 years ago," said Walker about DinB, "and we're just now finding out what it does."