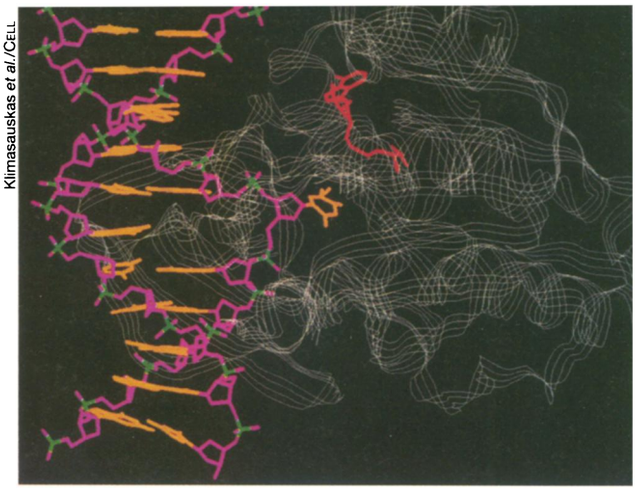
DNA Flips Out!

Enzymes repair and modify DNA in a surprising way

By JOHN TRAVIS



Klimasauskas *et al.*/CELL

In an act called base flipping, an enzyme (grey) binds to DNA's double helix and rotates a base (yellow) into a pocket within the enzyme.

Like cars, an organism's genes require frequent tuning and maintenance to function properly and avoid breakdowns. The mechanics responsible for this servicing are certain specialized proteins—enzymes that bind directly to DNA. Some of these enzymes tack atoms onto DNA, signaling whether a gene is turned on or off. Others scan the genome, searching for DNA in need of repair or removal.

Working on cars or genes can be awkward, however. To get at a broken part, auto mechanics may have to elevate the car on a lift or move undamaged parts out of the way. Similarly, to perform their biochemical maintenance, enzymes often must dramatically distort the normal helical shape of DNA.

The corkscrew structure of DNA consists of two linked strands, each a necklace of molecules called nucleotides, the fundamental building blocks of DNA. On each strand, a nucleotide bonds chemically to the nucleotide above and below it. In addition, creating ladderlike rungs between the strands, every nucleotide forms a weaker connection with a counterpart on the other DNA strand. This joining occurs between bases, a cluster of atoms each nucleotide possesses.

In DNA, bases come in four flavors—adenine, thymine, cytosine, and guanine. (RNA, a single-stranded molecule similar to DNA, substitutes the base uracil for thymine.) Since each base has a regular partner that it pairs with—adenine with thymine and cytosine with guanine—the sequence of nucleotide bases on one DNA strand determines the sequence on the other.

The attraction between bases on opposite strands and the bonds between the nucleotides that make up each strand confer a certain rigidity on DNA's double helix. That stiffness can make it difficult for an enzyme to position itself properly against a nucleotide or its base. As a result, when enzymes bind to DNA, they sometimes bend the DNA or throw a kink into its double helix—temporary distortions that provide greater access to specific parts of a strand. In some cases, an enzyme completely unzips the double helix, splitting it into two distinct strands.

According to recent research, however, enzymes have another way of tackling DNA. Some apparently pry apart a base pair, then rotate one of the freed nucleotides, bringing its base out of the confines of the double helix and into the enzyme's active site, a pocket within the protein's structure. The enzyme can then remove this pocketed base from its nucleotide or modify the base and sling it back into its proper position.

Until last year, scientists had never caught an enzyme performing this kind of remarkable maneuver, which they call base flipping. It's as if an automobile mechanic lifted the engine out of car, conducted repairs, and then casually dropped the engine back into place.

"It came as a total surprise to us. In retrospect, of course, it looks like the obvious thing to do. It's a simple and elegant way to do chemistry on a base," says Nobel laureate Richard J. Roberts of New England Biolabs, a biotech firm in Beverly, Mass. Roberts, along with three investigators from Cold Spring Harbor Laboratory in New York, published the first description of a base-flipping enzyme in the January 28, 1994 CELL.

Since that initial report, researchers have confirmed a second case of base flipping and have interpreted the shapes of other enzymes as suggesting that this unusual mechanism occurs in many DNA-protein encounters, including the essential ones by which enzymes repair damaged DNA. Enzymes used by humans, viruses, and bacteria all appear to employ this base-flipping ability.

Indeed, investigators believe they have belatedly discovered one of life's more basic genetic tricks. "Anything that's preserved so completely between [the bacterium] *Escherichia coli* and humans is very fundamental. We think this is a very ancient paradigm for DNA-protein interaction," says John Tainer of the Scripps Research Institute in La Jolla, Calif.

Base flipping first came to light in a study of methylation, the process by which enzymes join a small assembly of atoms, called a methyl group, to a base. Though researchers know several reasons why bacteria add methyl tags to their DNA, methylation's role in higher organisms is murkier.

Some suggest that methylation permits a process called gene imprinting, a poorly understood phenomenon in which offspring turn on genes inherited from one parent, but not the seemingly identical genes provided by the other. "There are a lot of biological phenomena that have been associated with methylation . . . but just what its primary function is in vertebrates is unknown," says Roberts.

Until last year, the mystery of methylation included not only its purpose but also how enzymes accomplished the task. Consider, for example, the methyltransferases that Roberts and his colleagues study. This family of enzymes affixes a methyl group onto a particular atom of a cytosine base.

The transfer, however, can only happen if the enzyme interacts with its target cytosine in a certain orientation—an angle almost perpendicular to the cytosine's bond with its guanine partner on the opposite strand. However, the nucleotides stacked above and below cytosine in the double helix normally render such a configuration impossible.

This "attack trajectory problem," as Gregory Verdine of Harvard University labels it, was just one of the issues that confounded investigators trying to resolve methyltransferase's mode of action. "This reaction can't normally take place, so the enzyme must do something interesting," explains Verdine.

The bewilderment evaporated when Roberts and his colleagues caught one methyltransferase red-handed. They crystallized this enzyme bound to a short strand of DNA that included a cytosine base. Shining X rays through the crystal

and monitoring how the atoms reflected or bent the light, these investigators deduced the shape of the DNA and the bound enzyme.

These X rays showed clearly that methyltransferase had, like an old-fashioned jukebox plucking a selected record out of a large stack and positioning it under a needle for playing, gracefully flipped a cytosine out of the stacked base pairs of the DNA and guided it into the enzyme's active site. Presumably, the enzyme then fastens a methyl group onto the base and releases it back into the double helix.

In the July 14 CELL, Verdine and his coworkers at Harvard describe a second instance of this unusual interaction. Once again, a cytosine had been flipped out of the helix so that a methyltransferase could latch onto it.

Enzymes that remove methyl groups may also flip bases. On occasion, methyltransferases fasten methyl groups to the wrong base or to an inappropriate region of the correct base. Bruce Demple of Harvard School of Public Health in Boston points to the Ada repair enzyme, made by *E. coli*, as one biochemical mechanic capable of fixing this type of error. When it spots guanine with a methyl group incorrectly tied to the base's oxygen atom, the enzyme binds to DNA and transfers the methyl group onto itself.

The Ada enzyme's active site is buried deep within the protein, which would make it difficult for the enzyme to interact with a guanine locked into a double helix. As a result, Roberts believes Ada must flip the guanine out. Though no one has enough data yet to confirm that, Demple agrees it's a reasonable assumption. Still, Ada would also have to modify its own shape, he argues, noting that the tunnel leading into the enzyme's active site is normally too small for guanine to move through.

Repairing damaged DNA may represent another essential task in which base flipping is routine.

"As soon as nature discovers how to do something, she tends to do it everywhere," says Roberts. "We think that repair enzymes that are recognizing mismatches might well use this same mechanism."

The mismatches he speaks of happen when a DNA base couples to the wrong companion on the opposite strand. For example, the base uracil mistakenly shows up in DNA every few hundred thousand bases or so, creating mismatches.

Consequently, almost every organism has developed a family of enzymes, commonly called UDGases, to remove uracil from DNA.

Earlier this year, the first depictions of the three-dimensional structure of UDGases appeared—one by Tainer and his coworkers in the March 24 CELL and another in the Feb. 9 NATURE by Laurence Pearl of the University of Edinburgh and his colleagues. Both groups resolved their enzyme's structure while it was bound to a single uracil.

This structural information suggests, they say, that UDGases flip the base out without dramatically distorting the rest of the double helix. But researchers can prove such enzymes flip bases only by examining a UDGase affixed to a complete DNA molecule, says Tainer.

Once an enzyme like UDGase separates a base from its nucleotide, other repair enzymes must heal the lesion by removing the remainder of the nucleotide and replacing it with another nucleotide containing the appropriate base. Without this replacement nucleotide, there would be gaps in the DNA strands. The enzymes that copy DNA during cell division would then grind to a halt when they encountered these genetic potholes.

"The repair of these sites is essential," explains Demple, because the loss of even one base "represents a total loss of genetic information in that strand."

Accordingly, enzymes called exonucleases pick up where UDGases and other base cutters leave off, excising damaged nucleotides. Even though the nucleotides the enzymes target no longer carry bases, Tainer and his colleagues suggest that exonucleases may still flip bases. From the structure of one such enzyme, which they describe in the March 23 NATURE, these researchers conclude that an exonuclease may recognize a nucleotide that needs pruning by flipping out the "orphan" base—the one opposite the damaged site—and binding to that.

The most audacious example of base flipping that has been proposed involves the vital photolyase enzymes. Unlike many DNA repair enzymes, these do not simply help excise damaged genetic material—they directly repair wounds. Photolyases tackle a common type of damage, a bond that forms between thymines and cytosines next to each other on the same strand. The sun's ultraviolet light creates these so-called pyrimidine dimers, which prevent proper DNA replication and can lead to skin cancer and other malignancies.

In a complicated reaction driven by blue light, photolyases repair the altered strand by severing the unnatural link forming the dimer. The presumed site of this dimer-breaking chemistry lies buried within the repair enzyme, according to a description of the enzyme's structure reported in the June 30 SCIENCE by Aziz Sancar of the University of North Carolina at Chapel Hill School of Medicine and his colleagues (SN: 7/8/95, p.20). The researchers propose that this enzyme flips both bases into its active site, breaks the dimer bond, and then thrusts the detached bases back into their proper positions.

Sancar believes that juggling two bases may not be the limit of this flipping mechanism. He studies a repair enzyme found in *E. coli* that removes a dozen nucleotides at a time from DNA. Sancar admits he has no proof yet but says "it is conceivable you can flip out 12 nucleotides."

The biggest question in base flipping may be how enzymes accomplish it. "How does the base get out? The issue of the pathway is going to be an exciting one for the future," says Verdine.

DNA may naturally "breathe," flipping bases in and out all the time. An enzyme might then simply catch a base when it was outside the helix.

But Roberts and other investigators believe it more likely that enzymes actively flip bases. For the repair enzymes, Demple suggests picturing a molecular vacuum cleaner that surveys DNA, constantly trying to "suck" out a base. Since mismatched or orphan bases are less tightly bound in the double helix, an enzyme might flip them more easily than it could a normally paired base.

Tainer and his colleagues have a different analogy: a dentist's probe. When they model DNA fastened to their UDGase structure, it appears that one of the enzyme's amino acids, a leucine, would protrude into the DNA. Tainer speculates that this leucine probes the double helix, popping out most mismatched bases. But only uracil fits snugly into the enzyme's active site, offering an explanation for what Tainer calls the needle in the haystack problem: How does a UDGase distinguish between uracil and the thousands of surrounding bases—especially cytosine, which differs from uracil by only a single atom?

Less than 2 years old, the concept of base flipping has certainly inspired a frenzy of speculation. Reviewing the topic in the July 14 CELL, Roberts theorizes that the mechanism evolved billions of years ago as a way of simply recognizing mismatched bases. One primitive enzyme would flip out a base, enabling a second one to chemically tinker with it.

Over time, he says, more complicated enzymes capable of both tasks, such as UDGases and methyltransferases, would have evolved.

Investigators believe base flipping will turn up in other forms of DNA-protein interactions. Among the most fundamental, notes Roberts, is the replication of DNA that is needed when cells divide.

To make a copy of DNA, enzymes called polymerases must first unzip the double helix, creating two single strands of nucleotides. Then, by pairing the appropriate bases, enzymes construct a second strand of nucleotides for each strand from the original DNA. This creates two copies of the parent double helix. Base flipping, muses Roberts, "looks as though it is step one of unzipping a helix." □