CANCER RESEARCH

Slow DNA Repair Implicated In Mutations Found in Tumors

Researchers studying cancer have long assumed that the areas of DNA most susceptible to damage from radiation and reactive chemicals are the same ones mutated in cancer cells. Problem is, the relationship doesn't always hold: Some "hotspots" for genetic damage are rarely mutated in cancer cells, while some infrequently damaged sites are frequently seen mutated in tumors. In recent years, biologists have begun to suspect that the missing link is a set of enzymes capable of repairing genetic damage. If the damage is repaired quickly, their thinking goes, there will be no mutation to pass along to other cells, including those that might become cancerous. But where the repair process is slow—expect problems.

Proving this idea, however, depends on being able to measure—with great sensitivity—the rates at which enzymes repair damaged DNA. Over much of the last decade, researchers have been refining their ability to track these rates. Now a pair of studies from a team of California researchers brings this process into its sharpest focus yet. On page 1438, Shuwei Gao and his colleagues Régen Drouin and Gerald Holmquist at the Beckman Research Institute at the City of Hope hospital in Duarte, California, show that the rate of DNA repair can vary as much as 15-fold within a single human gene. And on page 1436, their teammates Silvia Tornaletti and Gerd Pfeifer tie slow rates of repair in regions of the *p53* tumor supressor gene—which may be implicated in up to 50% of human cancers—with hotspots for genetic mutations that have been associated with cancer.

If a similar relationship is shown in other genes, this "could be a significant change in the way mutagenecists look at the causes of cancer," says Aziz Sancar, a professor of biochemistry and biophysics at the University of North Carolina, Chapel Hill. Such a revision, agrees Stanford biologist Phil Hanawalt, may not only flesh out theories of how cancer develops, but spark new therapies as well, as researchers aim to speed laggardly DNA repair in normal cells and sabotage repair in tumor cells in hopes of killing the cells before they can reproduce.

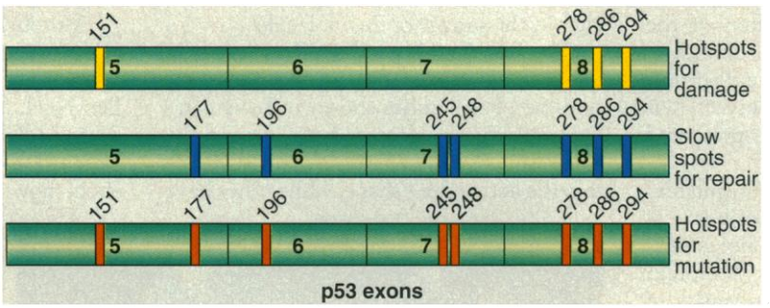
Researchers have known for some time that rates of DNA repair can vary. In 1992, for example, Douglas Brash of Yale University revealed that individual stretches of the same gene in *E. coli* bacteria have diferent repair rates. To prove the same holds true for human genes, the California team improved on a technique known as ligation-mediated polymerase chain reaction, or LMPCR. The method, invented by Barbara Wold of Caltech, is a way to determine the position of breaks in a DNA strand. "My idea," says Holmquist, who switched to this line of research from his studies in chromosome evolution just 4 years ago, "was to convert DNA damage into DNA breaks and then map it with this technique to determine where the damage occurs."

It wasn't as easy as it sounds. DNA can be damaged in hundreds of ways, which meant the group had to find enzymes that break DNA at only one type of damage site, allowing them to track and measure damage from a specific source. They settled on two enzymes—one from a bacteria, the other from a virus that infects bacteria—which together break DNA at a specific type of damage caused by ultraviolet light. UV-B in sunlight is the chief culprit in skin cancer. But the main damage it inflicts on DNA, the formation of an aberrant chemical structure known as cyclobutane pyrimidine dimers (CPDs), is identical to the damage caused by UV-C, the light source the group used for convenience reasons. Since the enzyme pair break the DNA strand at each CPD they encounter, that gave the team the ability to find exactly where UV-C—and by implication UV-B—do their damage.

Enzymes in hand, the group set about irradiating cultures of human skin cells with UV-C light and mapping the locations of genetic damage. Other groups of cell cultures were irradiated, then given half an hour to 64 hours to repair any damage from UV-C. After that, the amount of damage in the groups was compared at each damage site. In this way, the group built up a picture of how fast UV damage is repaired at each CPD site in the *p53* gene and in PGK1, a well-studied "housekeeping" gene that codes for a protein found in all cells.

Much to their surprise, Gao and his colleagues found that along the PGK1 gene, regions separated by only a few nucleotide base pairs had repair rates differing by as much as 1000%. The reasons why aren't clear yet, but one possibility, says Pfeifer, is that DNA's tightly folded structure might prevent repair proteins from reaching some sites as quickly as others.

If they don't yet know what causes the repair rates to vary widely, the Beckman team does have a handle on the relationship between slow DNA repair and genetic mutations found in skin cancer. In the study of *p53*, Tornaletti and Pfeifer found that seven of eight hotspots for mutation along the gene suffered from slow DNA repair (see diagram). Meanwhile, only four of these sites were among those most frequently damaged by UV radiation—implying that slow repair makes a large contribution to subsequent mutations. "It's not how easily DNA is damaged or how fast it is repaired, but a combination of these two biological mechanisms" that produces the mutations seen most often in skin cancer, says Holmquist.



Faulty repair. Seven of eight hotspots for mutation in the *p53* gene match up with "slow spots," where DNA repair lags.

ILLUSTRATION: E. CARROLL

Among researchers, this new understanding has already triggered a rush to influence the rate of DNA repair as a new weapon against cancer. "The race is on," says David Mitchell, a photobiologist at the University of Texas M.D. Anderson Cancer Center in Smithville. Perhaps the most promising potential strategy, suggest Hanawalt and Holmquist, is disrupting DNA repair in tumor cells. "That way you can cause cells that don't know how to repair themselves to self destruct," says Holmquist. Another approach might be to help speed up repair in the areas that are known to be mutagenic hotspots, he adds.

On the basic research front, says Pfeifer, the first push will be to find enzymes that break DNA at damage sites other than those caused by ultraviolet light. That will allow investigators to expand the use of LMPCR to mutagens such as benzo(a)pyrene, found in cigarette smoke. The new technique is also likely to create a race to explain why neighboring base pairs along a gene receive vastly different repair service. Eventually, these studies might provide the tools to improve upon the body's own genetic repairs.

–Robert Service