

DNA's Mr. Fix-it

When our DNA is damaged—as it is every day—an elegant repair network steps in, sensing and repairing genetic errors billions of times over the course of our lives.

Like begets like. Dogs never give birth to cats, and apple trees won't sprout from acorns. That is life's central dogma, arising from the dependable passage of DNA intact and in the same order from one cellular generation to the next. However, tens of thousands of times a day, the DNA in virtually every cell in every living organism, including humans, suffers genetic damage, chemically induced mutations, and simple mix-ups of one form or another.

Some of these genetic mishaps come about during the course of natural cell division; others arise from external sources such as pollution or medication. Too many mutations or alterations and the cell simply dies.

That's good because it helps to ensure that only healthy cells divide. If a cell with damaged DNA survives, it may convey permanent mutations, perhaps resulting in life-threatening illnesses. Many common cancers, including lymphomas and leukemias, can develop from damaged DNA. Some researchers suspect that Alzheimer's disease and even aging itself may

be the cumulative result of damaged DNA.

Despite the abuse our DNA endures, our individual genomes usually stay basically intact because DNA has a remarkable capacity for repair. Our cells have built-in, highly efficient machinery that finds and fixes "genetic typos."

THE FIELD HAS EXPLODED

Scientists used to think there wasn't much reason to worry about the integrity of DNA—they believed that innately it was shielded from harm and fundamentally sta-

GRAHAM JOHNSON

Mechanisms of DNA Repair

Base excision repair

- ↓ Base mutates spontaneously or suffers damage
- ↓ Damage specific DNA glycosylase recognizes and flips-out malformed base
- ↓ Glycosylase catalyzes hydrolytic excision of incorrect base
- ↓ AP endonuclease and phosphodiesterase remove sugar phosphate
- ↓ Replication machinery replaces proper base using complementary strand as template

Nucleotide excision repair

- ↓ Base suffers damage
- ↓ Specific DNA repair proteins bind damage and kink DNA
- ↓ Endonuclease binds kink complex and nicks strand
- ↓ Helicase unwinds and removes bad section
- ↓ Replication machinery replaces proper bases using complementary strand as template

Single-strand annealing

- ↓ Strands broken by radiation or chemical means
- ↓ Exposed ends degraded and genetic information lost
- or
- ↓ Proteins immediately bind ends and join strands without degradation
- ↓ Specific repair proteins protect exposed ends and seek one another
- ↓ Proteins unwind DNA until random homologous regions bind
- ↓ Unpaired single-strand ends cut and ligated

ble. This immunity, it was reasoned, allowed genetic information to pass reliably from generation to generation.

The damaging effect of UV radiation and X-rays on genetic materials was already recognized in the early part of the 20th century, and the realization that cells sometimes could correct genetic mutations after damage emerged in the 1930s. Nonetheless, says Stanford University geneticist Philip C. Hanawalt, one of the pioneers in the field of DNA repair, “For

many years after the discovery of the double-helical DNA structure, we thought the genetic material must be incredibly well-protected and not subject to chemical alteration.” But now, Hanawalt says, “We know that damage to DNA occurs all the time and that DNA repair is essential to maintain the genome.”

In recent years, genetic investigators have discovered more and more about the many ways DNA can be damaged. Researchers have learned much about the complex genetic

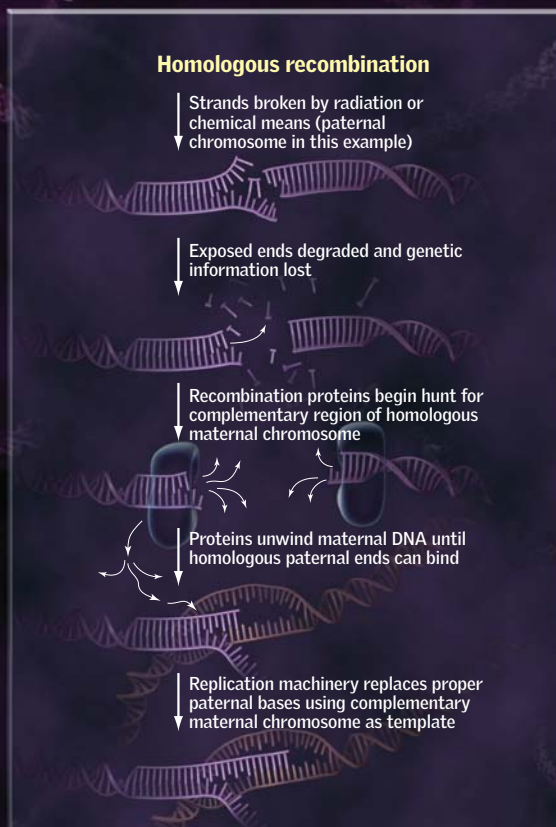
machinery that cells deploy to fix broken, cut, mutated, and misplaced genetic materials. Out of that evolving understanding has emerged a deeper awareness that DNA is truly dynamic and that responses to genetic damage are nearly as fundamental to life—and health—as is the genetic code itself.

Errol C. Friedberg of the University of Texas Southwestern Medical Center at Dallas wrote the major textbook on DNA damage and repair and has identified a number of the genes responsible for various forms of DNA repair. “The field has exploded,” he says. “What used to be the field of DNA repair is now cellular responses to damage. It spills into other fields all over the place.”

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BASES ASTRAY

Even during the normal process of cell proliferation, a few hundred of the 6 billion nucleotide bases—the A-, T-, C-, and G-designated



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chemical entities that unzip and synthesize new DNA during replication—are chemically altered or end up in the wrong place. Given the complexity of the process, the fact that some bases go astray or get damaged should not be surprising. Imagine what it would take to duplicate every item in a typical home and then identically arrange the new versions at another location. Mistakes, spills, and breakage would be virtually inevitable. Because the genome contains exponentially more items than any home could hold, “duplicating a cell is like duplicating a small city,” says HHMI investigator Stephen J. Elledge at Harvard Medical School and Brigham and Women’s Hospital. “It’s a humongous job.”

Fortunately, in most environments, base pairs go astray relatively few times during replication of the cell. According to HHMI investigator Paul Modrich at Duke University Medical Center, the error rate during the cell-division cycle typically amounts to no more than 100 to 1,000 mistakes per copy of the entire genome. That would be something like one typo in every 1 million to 10 million keystrokes on a computer keyboard.

Why worry about such a small number of errors? The reason is that in genomic replication even a single typo can be disastrous. “Even a single base change can lead to an inherited disease or predispose a cell to tumor development,” says Modrich.

Not only can mistakes occur during normal cell cycling, but DNA faces threats to its integrity all the time. Exposure to UV radiation, X-rays, and other environmental insults such as tobacco smoke, as well as oxidative free radicals and certain medications, can break DNA strands apart or cause a base to become chemically altered. A 1968 study by James E. Cleaver, a professor of dermatology at the University of California, San Francisco, alerted the scientific world that, without repair, these broken ends and mutations could undermine the stability of the entire genome, with untold results (see sidebar).



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Modrich and others followed Cleaver’s finding with the discovery that certain defective repair mechanisms were associated with a common hereditary form of colon cancer. Eventually, it became clear that failed DNA repair lay behind many other types of cancers as well as a variety of inherited neurodegenerative disorders. “We now know that a number of cancer syndromes involve defective repair mechanisms,” Modrich says.

GENOMIC EDITING

During the course of cell division, when DNA damage occurs in the cells of individuals who do not have rare genetic disorders, a “genomic editor” steps in to fix it most of the time. A complex series of steps unfolds, mustering a large but still not fully known number of genes and enzymes to detect and repair the

errors. While this goes on, the entire replication machinery of the cell comes to a momentary halt. “Cells invest a huge amount of energy to fix DNA,” says Elledge.

Highly specialized repair mechanisms deal with different types, sizes, and regions of damage. Some repairs chop out the bad base or bases on one strand and then patch them, using the intact strand as a template. (That’s called either base excision repair or nucleotide excision repair, depending on how the lesion gets detected and which pathway is used to remove it.) Other types of repair fuse broken ends back together, using a variety of tricks for uncoiling and splicing the strands. Still others use recombination mechanisms in which sections of a broken strand are repaired by using information located on an undamaged homologous chromosome. And other mechanisms chemically change a mutated base back to its original form without breaking the DNA strand. Each repair mechanism requires its own combinations of genes and enzymes.

Several separate repair systems may be required to complete a particular repair task. HHMI investigator David G. Schatz at the Yale School of Medicine notes that “each sys-

tem has its strengths and weaknesses. You can’t completely compensate for the loss of one just by having the other. They don’t completely overlap.” Much remains unknown, however, about how the genome orchestrates repairs to address different types of damage, leading Modrich, Elledge, and others to study not only the processes that underlie recognition and signaling of the problem but also the coordination mechanisms that determine which type of repair pathway is assigned to fix which type of damage.

For all its complexity, DNA repair seems remarkably effective. However, sometimes the correct restoration may not take place at all. Repairs can go awry or not occur quickly enough to ensure the cell’s survival. If such a cell does survive, it could replicate the damage. Fortunately, a failsafe system then comes

into play. At that point, says Schatz, a so-called checkpoint-pathway system acts “as a foreman on the job who says that the caretakers—the carpenters who rushed in to repair the genomic scaffold—didn’t do the job right and the cell has to die.”

The checkpoint genes—perhaps most famously the *p53* gene, termed a “tumor-suppressor gene”—initiate a ritualistic process of cellular dismantling called apoptosis, resulting in cell suicide. In normal skin cells, for example, sunburn leads to extensive apoptotic responses, and the skin sloughs off its dead cells as peeling, a necessary process for healthy remodeling of the skin and ridding the body of UV radiation-damaged cells that could mutate and become cancerous.

MECHANISM FOR SURVIVAL

Given the presence of DNA repair responses in even single-cell organisms such as yeast, it is likely such processes arose very early in the development of life, perhaps even simultaneously with life itself. “Repair mechanisms evolved a long, long time ago,” says Elledge. “When we were single cells floating out in the ocean, we were constantly bombarded by UV light and X-rays. We needed a repair mechanism for survival.”

Comparable systems therefore are found in all organisms, making it possible to study repair mechanisms in a wide variety of model systems—for example, by observing the effect of knocking out different parts of



Genes that David Schatz and colleagues discovered encode a protein complex that snips DNA like “molecular scissors.”

the damage-control response in yeast, mice, and other species. The findings can then be applied to exploring the more complex human genome.

Many of these animal studies shed light on fundamental mechanisms underlying

diverse diseases and conditions. For instance, studies of the *p53*, *BRCA1*, and *BRCA2* genes, which are part of the same checkpoint pathway, have shown that when cells with damaged DNA fail to be killed, a cascade of events may follow, setting off unchecked proliferation of damaged cells. “If you get rid of activation of *p53*,” says Elledge, “there’s no apoptosis, and that’s one of six or seven things that happen in the evolution of cells that are eventually going to turn into a tumor.”

While unchecked damage may be the first step in ultimately producing cancer, sometimes a cell will purposely initiate a form of DNA damage for the health of the organism. While working in the MIT laboratory of David Baltimore (who is now president of the California Institute of Technology), Schatz discovered two genes, *RAG1* and *RAG2*, that encode a protein complex—a kind of “molecular scissors”—to make cuts in DNA. The snipped ends of the DNA are joined to DNA segments in other parts of the chromosome, through recombination and end-joining repair processes, to form novel genetic combinations that encode B-cell anti-

Early Research

Early insights into DNA repair came from the study of children with xeroderma pigmentosum (XP), a rare, genetically inherited disease. For youngsters with this condition, brief exposure to even normal daylight rapidly leads to skin cancer and other malignancies. Some call this ultraphotosensitive group “moon children” because on the occasions when they venture outside they must wear head-to-toe UV-proof suits. No cure for XP exists, and few patients survive to adulthood.

In 1968, James E. Cleaver, a professor of dermatology at the University of California, San Francisco, discovered that XP patients lack an essential mechanism, known as excision repair, that in normal individuals acts to correct solar damage to the skin’s DNA. He showed that, absent this damage-repair mechanism, the skin cells of moon children continue to replicate their UV-damaged DNA. Those cells can then mutate into cancerous forms, leading to tumors or neurodegenerative disorders.

Cleaver’s finding surprised many geneticists. The traditional view—that DNA’s tightly bound double-helical structure remained stable and largely inviolable throughout the life of the cell—had already been challenged, but for the first time it was apparent that, without proper repair, propagation of cells with damaged DNA could lead to grave harm. —M.W.

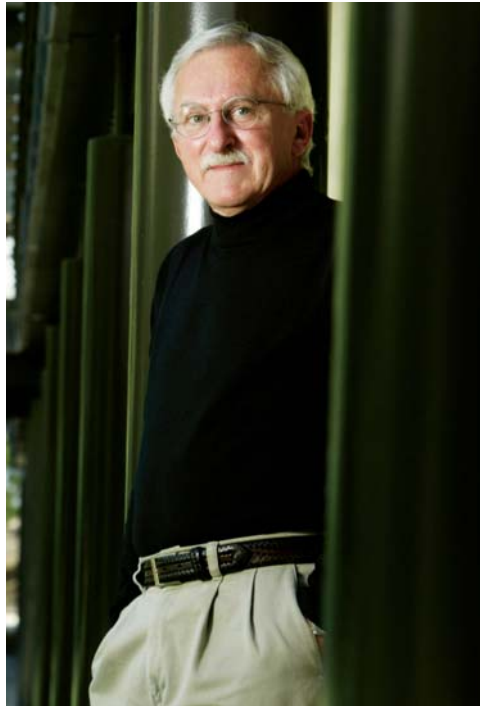
UP-CLOSE

bodies and T-cell receptors. These altered cells can then sense and lead the attack on invading pathogens.

HHMI investigator Frederick W. Alt at Children's Hospital, Boston, and Harvard Medical School; André Nussenzweig at the National Cancer Institute; and his brother Michel C. Nussenzweig, an HHMI investigator at the Rockefeller University, have been studying the machinery underlying the breaks and recombinations that enable an immune response. DNA is tightly bound in compact bundles, making the mechanics of its unwinding especially hard to study.

"The process [of breaking off and unwinding ends and then reattaching them in the right place]," says Michel Nussenzweig, "is like going into a huge tangle and making a break, and holding the ends together while you untangle and then repair it. It's incredibly complicated."

Lymphocytes somehow carry out this DNA chopping-and-rejoining process without recruiting their complete DNA checkpoint machinery and initiating apoptosis. But while the reshuffled DNA enables the immune cells to recognize and respond to foreign invaders, the chopping off and unwinding of DNA ends leaves them at least temporarily vulnerable to unwanted linkages with other loose ends. The ends float freely and may splice together with ends located in other chromosomes, sometimes with unfortunate results; certain genetic translocations



Why worry about DNA repair? Paul Modrich warns that even a single DNA base change can lead to disease or tumors.

unexpected therapies." Knowing that a person carries a defect in a repair mechanism might result in recommendations for beneficial lifestyle changes. For instance, up to 80 percent of women who inherit a damaged version of *BRCA1* or *BRCA2* will develop

of *BRCA1* makes breast cancer cells 10 to 1,000 times more resistant to one type of drug, which works by damaging DNA within cancer cells. However, the gene makes the cells over 1,000 times more sensitive to a second type of drug that works by blocking cell division. Screening breast cancer patients for the functioning of *BRCA1* before treatment could make choosing a more effective drug more likely.

Another strategy is to make tumors more sensitive to a therapeutic agent by knocking out part of their repair pathway. Yet another is to manipulate elements in the checkpoint pathway to initiate apoptosis in cancer cells. To address as many of these options as possible, Elledge is providing pharmaceutical companies with checkpoint-pathway genes he has identified; subsequent studies should show whether they might be effective targets for antitumor drugs in one way or another.

Investigators are also looking into gene-therapy techniques to help mimic natural DNA-repair mechanisms; such methods might correct the errors behind inherited disorders such as Huntington's disease, cystic fibrosis, and sickle cell anemia. A team led by Yale's Peter M. Glazer has already been able to introduce a specific DNA sequence into a target gene, where it corrects a mutation, in extracts of human cervical cancer cells. The researchers are now pursuing a similar strategy in animal models of the disease. "If you can bind something to the gene, maybe you can use that to change the gene," says Glazer. "If you change the gene to a new sequence, it is permanently fixed."

The routine therapeutic use of such genetic interventions remains a distant hope, however, because the DNA damage-control mechanisms still essentially remain a mystery. "Life," says Friedberg, "is necessarily a delicate balance between genomic stability and instability—and of mutation and repair." By understanding the mechanisms that keep life in balance, the possibility for repair of damaged human cells—perhaps even repair of the repair system itself—may come closer to reality. **11**

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of this type lead consistently to B-cell lymphoma, one of the most common forms of cancer. "The system rolls the dice," says Michel Nussenzweig, "because the value of immune protection is so high."

UNEXPECTED THERAPIES

Better understanding of faulty repair pathways that lead to genomic instability may yield new insights into treating cancer. "The more information we have," says Alt, "the more chance there is of stumbling onto

breast cancer before age 70. Studies are under way to see if treating these women with the drug tamoxifen before they develop cancer may have preventive benefits.

When people do develop diseases, it actually may be possible to tailor more effective treatments early on for individuals by using knowledge of their DNA repair deficits. "We could segment patients based on who will respond to a particular agent," says Elledge.

Already, scientists in the United Kingdom have found that the fully functioning version