DNA Repair: New Clues to Carcinogenesis

All cells are exposed to a constant barrage of chemicals and radiation—the ultraviolet radiation in sunlight, for example—that cause alterations in the structure of DNA, the cells' genetic material. Since a single change in DNA structure may be harmful or even lethal, it is not surprising that an elaborate array of mechanisms for repairing damaged DNA has evolved.

Bacteria are known to have several distinct repair mechanisms, and analogous systems appear to exist in mammalian, including human, cells. In fact, research on the mammalian systems has been growing by leaps and bounds recently, stimulated by results suggesting that defects in DNA repair may increase an individual's susceptibility to cancer.

Because of the strong correlation between the ability of an agent to produce changes (mutations) in DNA and its ability to cause cancer, investigators have long suspected that damage to DNA plays a causal role in carcinogenesis. The discovery that certain rare genetic diseases of humans—diseases that are accompanied by an increased incidence of cancer in the patients—are associated with defects in DNA repair provides strong evidence for this hypothesis in the view of many investigators. Moreover, there are indications that the increased risk of cancer is not just limited to the few persons having the diseases in question but may also affect the symptomless carriers of the defective genes causing the conditions.

The postulated DNA repair diseases, which include xeroderma pigmentosum (XP), ataxia telangiectasia (AT), and Fanconi's anemia, are very rare. They are also autosomal recessive conditions that only produce symptoms in individuals who receive two copies of the defective gene. Such individuals are called homozygotes. Persons with only one copy are gene carriers who may pass the gene on to their progeny but who do not themselves develop symptoms.

Even though the homozygotes are rare, the carriers may be relatively common in the population. Provided that certain assumptions hold, an equation called the Hardy-Weinberg equation can be used to calculate the frequency of carriers in a population from the known frequency of homozygotes. Michael Swift of the University of North Carolina has calculated the carrier frequencies for the repair deficiency diseases. They range from about 0.5 percent for most of the conditions to 1 percent for AT. For comparison, the homozygote frequency for AT is one in 40,000 persons.

By comparing the incidence of cancer in relatives of AT patients (for whom the probability of being carriers can be easily estimated) with the incidence in the general population, Swift determined that the carriers were at increased risk of dying from cancer. He estimates that for carriers less than 45 years of age the risk of dying from cancer is some five times the risk for the general population. Swift says that AT carriers may comprise more than 5 percent of all persons dying from cancer before age 45. He had previously performed a similar epidemiological study with blood relatives of Fanconi's anemia patients and found that carriers of the Fanconi's anemia gene were about three times more likely than noncarriers to die of cancer.

Carriers of the XP gene are not at increased risk of dying from cancer, according to Swift, but they do appear to have a higher incidence of skin cancers, which occur very frequently in XP patients themselves, than noncarriers. (With the exception of the relatively rare melanomas, skin cancers are not usually fatal.) Thus, even though they do not have the other characteristic symptoms of the diseases in question, the carriers, like the homozygotes, appear to be at increased risk of developing malignancies.

Because of the proposed link between defective DNA repair and cancer, the most tempting hypothesis to explain Swift's findings is that the carriers have some subtle defect in DNA repair that predisposes them to cancer. There is as yet, however, no evidence to support that hypothesis. Swift himself thinks that, if the conditions he is studying are to be useful in understanding the genetic predisposition to cancer in man—and he thinks they will be—the principal goal of current research should be to understand the basic biochemical defect or defects underlying each of the conditions.

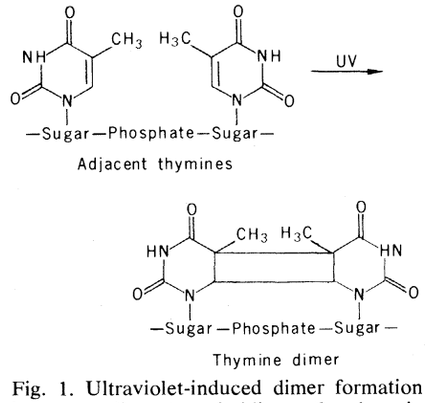

Fig. 1. Ultraviolet-induced dimer formation between adjacent pyrimidines, thymines in this case, on a DNA chain. Dimers may also form between adjacent cytosines (cytosine is the other pyrimidine base found in DNA) or between cytosine and thymine.

Thus far the best understood of the conditions—and the one for which there is the strongest evidence linking inadequate DNA repair and cancer—is XP. This condition is characterized by a high degree of sensitivity to sunlight, freckling, and, most important, a very high incidence of skin cancer on those body surfaces exposed to sunlight. Some XP patients have had more than 100 skin cancers, including many malignant melanomas, which are much more rare in the general population. In addition, the patients often have neurological abnormalities such as mental retardation and problems in coordinating their movements. About 150 XP patients have currently been identified worldwide.

Ten years ago, James Cleaver and his colleagues at the University of California in San Francisco found that skin cells from XP patients could not repair the DNA damage produced by irradiating them with ultraviolet light, which has long been suspected of being a cause of skin cancer in humans. Cleaver's findings suggested that the skin cancers of XP patients resulted from the inability of their cells to repair ultraviolet-induced changes in DNA.

The most important of these changes is the formation of chemical linkages between adjacent pyrimidine bases on the nucleic acid chain, thus producing dimers (Fig. 1). The dimers distort the DNA molecule and, if not repaired before replication begins, they interfere with the normal synthesis of the daughter molecules. Even one dimer, if it is in a critical location, may be lethal to the cell. Alternatively, the cells might survive and divide but the presence of distorted regions on the DNA could lead to errors in base-pairing during replication and a high mutation rate.

The mechanism by which bacteria remove the dimers from DNA is reasonably well understood, although not all of the enzymes have been isolated and characterized (Fig. 2). In the first step an enzyme (called an endonuclease) recognizes the distorted region and puts a nick in the affected chain. Another enzyme begins at the nick and clips out the nucle-

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otides (nucleotides are the building blocks of nucleic acids), including those containing the dimer, in the distorted segment. This leaves a gap in one of the two strands of the double-stranded DNA molecule. The gap is filled by the appropriate nucleotides that pair in the normal manner with the complementary nucleotides on the intact strand. The nucleotides are joined together enzymatically and the gap is sealed. This process is called excision repair because the altered nucleotides are actually clipped out of the DNA. Most investigators think that excision repair is an error-free process.

Researchers have shown by a variety of techniques that normal mammalian cells can also excise pyrimidine dimers and carry out excision repair. Most XP cells cannot, however. The problem appears to be in the first step—the one in which the endonuclease nicks the distorted DNA chain. For example, Allen Smith and Philip Hanawalt of Stanford University treated XP cells to make them permeable to an endonuclease of viral origin. After the treated cells took up the enzyme, which recognizes and nicks DNA chains only in the vicinity of dimers, they gained the capacity to carry out excision repair. Thus, it appears that once the block to the first step of excision repair is circumverted, the remaining steps can proceed.

Irradiating cells with ultraviolet light is not the only way to damage their DNA. Chemicals—many of which are carcinogens—also damage the nucleic acid. Both bacterial and mammalian cells have the capacity to repair alterations induced in their DNA by chemicals. Some of these changes are apparently repaired by an excision process similar to that for removing ultraviolet damage, although work from the laboratory of Richard Setlow at Brookhaven National Laboratory indicates that the mechanism for excising chemical damage to DNA may differ, at least in part, from that for cutting out ultraviolet-induced dimers.

Nevertheless, several investigators have shown that XP cells that are deficient in excision repair of ultraviolet damage are also incapable of repairing the changes induced by some chemicals. The XP cells can repair damage caused by other chemicals and by x-rays, however. All in all, the results suggest that DNA repair in mammalian cells is a complex business, possibly involving several pathways.

Another finding that supports the theory that DNA mutations are involved in the development of cancer is the observation by Veronica Maher and J. Justin McCormick of Michigan State University that cells from XP patients, who have such high susceptibilities to skin cancers, exhibit significantly higher frequencies of mutations than do normal cells exposed to the same dose of ultraviolet radiation. Moreover, the investigators found that the susceptibilities of the cells either to killing or to mutagenesis induced by the radiation correlate with the magnitude of their repair deficiencies.

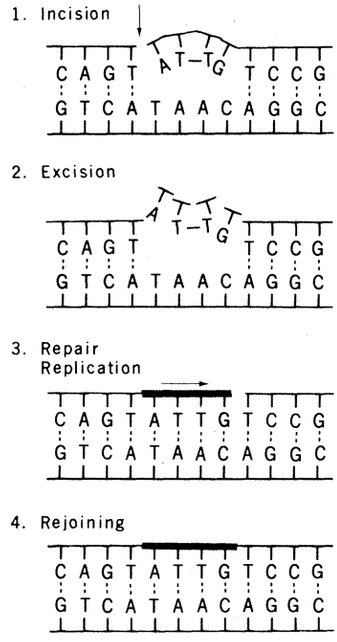

Fig. 2. Pathway for excision repair. In the first step, the endonuclease recognizes and nicks (arrow) a DNA region distorted by the presence of a thymine dimer. Another enzyme begins at the nick and removes nucleotides, leaving a gap in one strand of DNA. The gap is filled by nucleotides pairing with bases on the intact chain, and these nucleotides are joined by an enzyme (a DNA polymerase). In the last step, a fourth enzyme, called a ligase, makes the final connection and the process is completed.

Cells from different XP patients display varying rates of excision repair that range from less than 2 percent to more than 50 percent of normal values. Normal cells require the highest doses of ultraviolet light to kill a given percentage of cells or to induce a particular mutation frequency. Cells with almost no repair capability required the least. Cells with intermediate repair levels require intermediate radiation doses. This neat picture obtained with cells in culture is clouded, however, by the fact that there is no clear-cut correlation between the severity of the patients' XP symptoms, including occurrence of skin cancers, and defectiveness of DNA repair.

Maher and McCormick hypothesize that the cell-killing and mutagenic effects of the carcinogenic agents they have tested result from lesions in the DNA that the cells fail to excise before some critical event. They think that the critical event is replication of the DNA. In support of this suggestion is Maher and McCormick's observation that XP cells can repair their damaged DNA if they are prevented from replicating for a sufficiently long time. As the extent of repair increases with time, the survival of the cells improves and the number of mutations decreases. The investigators thus postulate that the role of excision repair is the error-free removal of DNA lesions before the damage becomes permanent during replication.

Most of the evidence linking defective DNA repair and cancerous transformations is inferential. Direct evidence for the relationship has been harder to come by, but there is one experiment indicating that damaging the DNA of cells by subjecting them to ultraviolet radiation gives the cells the ability to cause tumors when they are injected back into animals. The experiment was performed by Setlow and his colleagues, who used a small fish, the Amazon molly, as their experimental animal. This fish reproduces in an unusual manner such that the offspring of a single female constitute a clone. Since the tissues of the clone members are identical there is no problem with rejection of transplanted tissues among the members.

The Brookhaven investigators found that all of the fish injected with irradiated fish cells developed invasive thyroid tumors; untreated cells did not produce this effect. As an additional control, the irradiated cells were exposed to visible light following the ultraviolet treatment and before injection into the fish. Visible light activates an enzyme, called the photoreactivating enzyme, that specifically repairs pyrimidine dimers. (Photoreactivation is different from excision repair; the photoreactivating enzyme splits the dimers directly without removing them from the DNA.) These cells did not produce thyroid growths, a result indicating that ultraviolet-irradiated cells lose their tumor-producing potential when the damage is repaired.

Despite the consensus that the first step of excision repair is defective in XP cells, the situation is turning out to be more complicated than it once appeared. One sign of the complexity is the existence of several XP "complementation groups." According to Dirk Bootsma of Erasmus University in Rotterdam and Jay Robbins and his colleagues at the National Cancer Institute (NCI), when cells from one XP patient were fused with cells from certain others, the hybrid progeny somehow acquired the ability to carry out excision repair. In other words, the cells complement one anoth-

er; each supplies to the hybrid a function missing in the other, a result indicating that each member of the fused pair is defective in a different function.

The existence of at least five different complementation groups has now been confirmed, and there are reports suggesting the existence of two more. Thus, several distinct genetic defects appear to give rise to the same end result, XP disease. Yet only the first step of excision repair is supposed to be inadequate in XP cells.

These results might be explained if the endonuclease, the enzyme catalyzing the first step, contained several components, as many enzymes do. In that case, a defect in any of the components could prevent the enzyme from working properly. Isolation of the mammalian enzymes would shed some light on this issue, but this has not yet been accomplished.

The endonuclease itself may not be at fault in XP cells, however. The DNA of mammalian cells occurs as a complex (called chromatin) with protein. The DNA may be inaccessible to the endonuclease unless there is some way to temporarily remove the proteins from it.

Results obtained by Christine Mortelmans, Errol Friedberg, and their colleagues at Stanford University indicate that the excision repair defect in XP cells might be the result of impaired access of the endonuclease to the chromosomal DNA and not the result of a defect in the enzyme itself. They demonstrated that extracts from XP cells cannot excise pyrimidine dimers from chromatin, but that the extracts do remove dimers from DNA that has been stripped of its protein. Thus, control factors of some kind may be needed to expose the DNA so that the endonuclease can act. The control factors, rather than the enzyme, may be defective in XP cells.

In related experiments, Michael Lieberman, Michael Smerdon, and Thea Tlsty of Washington University, have been investigating the role of chromatin structure in excision repair. Mammalian chromatin consists of beadlike structures called core particles containing both DNA and protein that are joined by linker regions consisting mostly of DNA. The investigators found that repair of ultraviolet damage to DNA proceeds in two phases, a rapid phase lasting 2 to 3 hours and a slower phase continuing for at least 35 hours. Most of the fast repair occurs in the more accessible linker regions of the chromatin whereas the slower repair is more uniformly distributed throughout the DNA, including the segments in the core particles. Other investigators, including Cleaver, have obtained similar results.

Lieberman says that they now have evidence that the chromatin structure undergoes a rearrangement during the repair process. As a result, many of the repaired segments move from the linkers into the core particles, possibly helping to produce the more uniform distribution of repaired DNA segments observed during the slow phase of repair. Such rearrangements may also expose inaccessible damaged sites, including those in the core particles, to repair enzyme activity.

Another indication of the genetic complexity of XP is the existence of the XP "variants." Robbins and his colleagues found that cells from one of their patients at NCI carried out excision repair just as well as normal cells, even though the patient had the classical XP symptoms. Since then at least four additional patients similar to the one identified by the NCI group have been found. The existence of these patients, termed XP variants, was puzzling to XP and DNA repair experts because at first it seemed to contradict the hypothesis attributing the high incidence of skin cancer in XP patients to defective DNA repair.

Defect in XP Variant Cells

Although excision repair is only one of several mechanisms by which cells repair damaged DNA, early experiments did not reveal any obvious defects in any of the mechanisms in XP variant cells. Finally, however, Alan Lehmann and his colleagues at the Medical Research Council Cell Mutation Unit at the University of Sussex, England, produced evidence that the variant cells are defective in a kind of repair usually called postreplication repair because it occurs during or after replication. If a DNA molecule containing dimers replicates before dimer excision, gaps occur in the newly synthesized strands, probably opposite the dimers. Additional repair mechanisms are thus needed to fill these gaps.

The presence of postreplication repair in mammalian cells is suggested by observations that the size of newly synthesized DNA in ultraviolet-irradiated cells is smaller than it normally is. With time—and presumably with the activity of postreplication repair systems—the DNA grows to normal sizes. Lehmann found that the time required for the newly synthesized DNA of irradiated XP variant cells to attain large sizes is much greater than for normal cells. Thus, the variants appear to be defective in DNA repair, although not in excision repair.

Of particular importance to the carcinogenesis question is the issue of the accuracy of the different repair pathways. An error-prone pathway could actually do more harm than good by introducing additional mutations. Investigators generally think that excision repair is accurate. The postulated mechanism, involving as it does base-pairing of the newly inserted nucleotides with those on the "good" DNA chain, predicts an accurate restoration of the damaged strand. And evidence garnered by Maher and McCormick supports this contention. They found that normal cells have a lower mutation rate after exposure to ultraviolet radiation than do XP cells, even though the normal cells carry out more extensive excision repair.

Whether postreplication repair is error-free or error-prone is not yet known. The accuracy of the repair depends on the mechanism by which it occurs. Both accurate and inaccurate mechanisms of postreplication repair can be postulated, but at this time there is little evidence about which one is correct.

Bacteria, however, apparently do have an error-prone postreplication repair pathway. The pathway does not operate all the time, but it is turned on by exposure of the cells to very low doses of mutagenic agents, including ultraviolet and x-irradiation and chemicals. These agents induce an enzymatic activity that can apparently replicate damaged DNA but only at the expense of an increased number of mutations.

Some investigators, such as Hanawalt and Setlow, have evidence that low doses of carcinogenic agents induce DNA repair activity in human and other mammalian cells. Arguing from analogy with the bacterial systems, they suggest that this repair activity may also be error-prone and thus contribute to the development of cancer. The hypothesis that low doses of carcinogens turn on an error-prone repair pathway is of obvious significance to the carcinogenesis problem. There is as yet no direct evidence for this hypothesis, however, and the issue is far from settled.

In fact, a great many issues concerning DNA repair in mammalian cells are far from settled. The genetic heterogeneity of XP is one of the problems that concerns investigators. Moreover, some researchers have questioned whether defective DNA repair can account for all of the symptoms of the postulated DNA repair diseases. For example, some XP patients have neurological problems, such as mental retardation, in addition to the skin symptoms. Robbins has suggested, however, that inability to repair the damaged DNA of nerve cells, especially early in development, may kill the nerve cells, which are not replaced, and account for the neurological problems.

Several investigators have also em-

phasized the great need for better understanding of the exact structural changes produced in DNA, especially by chemicals, and the correlation of specific DNA changes with particular cellular events, whether cell-killing, mutagenesis, or carcinogenesis. Chemicals and radiation exposure may produce several alterations in DNA structure, not all of which are necessarily harmful to the cell.

Peter Cerutti of the University of Florida is one of the investigators who is working to identify and characterize the DNA lesions produced by various carcinogens. He points out that persistence of the lesions may help to determine their biological significance. For example, he has found that a highly carcinogenic derivative of benz[*a*]pyrene, a suspected human carcinogen that is widespread in the environment, causes a persistent chemical lesion in the DNA of mammalian cells. Cerutti thinks that such persistent, but nonlethal, lesions may be responsible for the development of mutations and also cancers.

The other diseases thought to be caused by defects in DNA repair are AT, Fanconi's anemia and, possibly, Bloom's syndrome. All of these conditions are characterized by a high incidence of both cancer and chromosome abnormalities. The actual defects in DNA repair have not been as well characterized as those in XP cells, although several investigators have detected repair deficiencies in some, but not all, cells derived from AT and Fanconi's anemia patients. These diseases, like XP, appear to be genetically heterogeneous.

Thus, there are still many unanswered questions concerning the postulated connection between DNA repair pathways and cancer. Nevertheless, as research into the intricacies of the mammalian pathways grows, investigators think that they will see a payoff both in terms of basic knowledge about the systems and of a better understanding of how DNA repair affects human susceptibility to cancer.—JEAN L. MARX

Tidal Waves: New Method Suggested to Improve Prediction

Tidal wave, or more properly, tsunami (soo nah′ me) prediction is not a glamorous field of research today, as earthquake prediction is, but the stakes are still high. A large earthquake, the most common cause of tsunamis, in one part of the Pacific can create waves capable of suddenly inundating villages and cities on the coasts of both North and South America, Asia, and the islands in between. The destruction can be staggering. A tsunami in 1896 killed 27,000 people in Japan. One, in 1960, destroyed or severely damaged every Chilean town along 800 kilometers of coast. But tsunami prediction, a reality since 1948, has helped minimize the loss of life during the four major tsunamis that have crossed the Pacific since 1948.

The reliability of tsunami warnings has improved during the 30-year history of the Tsunami Warning System, now a cooperative international organization operated by the U.S. Weather Service, but problems still remain. Improvement has been due largely to increased experience and the expansion of the system's network of observation stations throughout the Pacific. Now, an innovation in the way that possible tsunami-generating earthquakes are monitored may soon help to reduce the number of false alarms and increase public cooperation in areas, such as Alaska and Hawaii, where the most rapid warnings are required.

The present observation network of the Tsunami Warning System (Fig. 1) allows experts to locate and measure the magnitude of large earthquakes that may cause tsunamis, as well as to detect a tsunami itself and follow its progress across the Pacific. The system includes seismographs and tide gauges that are installed throughout the Pacific and linked to the Tsunami Warning Center in Hawaii. If an undersea earthquake appears from seismological data to be large enough to cause a tsunami, a watch is issued, alerting threatened populations to the possibility that a tsunami has been created. If a tsunami is actually observed by the tide gauges in the system, a warning is then issued. Because most watches and many warnings are not followed by destructive tsunamis, the effectiveness of the system has at times been impaired.

One reason that some unnecessary alerts must be called is that a great deal remains to be learned about how tsunamis behave once they are formed. A tsunami, which is actually a series of waves, like ripples from a pebble thrown into a pond, moves quite predictably across the deep waters of the Pacific. Its great speed in the open ocean, about 800 kilometers per hour, contrasts with its small height of less than a meter. The variation of a tsunami's speed with water depth is well understood, so that arrival times thousands of miles from the earthquake can be accurately predicted.

Unfortunately, the size that a tsunami will be when it reaches land remains rather unpredictable. Eventually, it may rear up to as high as 30 meters within a few hundred meters of shore as it slows to about 50 kilometers per hour. Or it may disperse its energy before arriving at a particular beach and not cause any damage. Attempts to understand the basis of these differences have met with limited success.

The tide gauges of the warning system can record the passage of a tsunami (although it has nothing to do with the tides) and provide the first proof that a tsunami has been indeed created. But they cannot be used for predicting how large it will be at another location. In addition, initial tide gauge reports may not arrive at the warning center until 2 hours or more after the quake because of the travel time involved and the sometimes circuitous communication links with remote stations. Plans to link the network by a satellite have not yet progressed beyond a single experimental link.

An alternative to waiting for tide gauge reports would be to issue a warning solely on the basis of reports from several readily accessible seismograph stations. This is more rapid but considerably less reliable. It may take only a few minutes if the quake is within a dense network of instruments such as the one that comprises the Alaska Regional Tsunami Warning System. To predict whether or not a tsunami has been formed, the location and magnitude of the earthquake must be known. It is relatively easy to determine whether a quake is in the ocean, where it can cause a tsunami. Determining its magnitude is more difficult.

As a general rule, destructive tsunamis are generated by earthquakes of magnitude 8 or larger on the Richter scale. But not all tsunamis follow the general rule. For example, the 1946 Unimak Island quake in the Aleutians had a Richter magnitude of only 7.2 (about eight times smaller seismic wave amplitudes than magnitude 8.0), but it generated tsunami waves of 9 to 17 meters in Hawaii, killing 159 people.

Currently, the only way to avoid overlooking a dangerous earthquake is to put a conservative lower limit on the magnitudes that will trigger a watch. Any earthquake within the Pacific basin greater than magnitude 7.5 automatically initiates a watch. A warning is only issued for the Pacific if tide gauges detect a

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