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DNA REPAIR *

Richard Setlow
Biology Department
Brookhaven National Laboratory

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What is known about DNA repair in E. coli and most bacterial systems has already been discussed. The difficulty in extrapolating these data to mammalian systems is that the putative first enzyme in excision repair, the so-called "UV" endonuclease, really has not been isolated. So we have a profound conceptual difficulty that makes problems when we get to mammalian cells. Numbers will give an idea of the magnitude of the problem. In E. coli, there is good experimental evidence that UV-induced changes in DNA, called pyrimidine dimers, are the cause of much of the lethality and many mutations. The number of pyrimidine dimers per chromosome per mean lethal dose is shown in Table 1. For E. coli wild type, they are in the neighborhood of 3,000. Even though wild-type cells are very good at repairing such damage, they are ultimately affected by them. If you investigate mutants deficient in excision repair, so-called uvrA mutants, the numbers are around 50, so you can see that excision repair in itself results in about a 60-fold decrease in E. coli sensitivity. In a double mutant, recA uvrA, those numbers come down to about 1 dimer per bacterial chromosome.

The existence of numbers such as 50 rather than 1 for excision-deficient cells led to the search for other repair mechanisms described as postreplication repair. Even though wild-type cells are very proficient at repairing damage, they never can do it 100%. The important concepts are the kinetics of the processes of replication and repair and whether the replication is what has been termed error-free or error-prone. Obviously, if you blocked replication and permitted repair to take place, you would have a much happier state of affairs for a cell than if you let both go on and asked a cell to replicate past a lesion before it was repaired. In most systems the two processes

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go on at the same time. You do not say "here's an insult, repair it, and then I'll think about replicating it." Those are complications in E. coli.

The kinds of excision repair systems known at present are given in Table 2. Most of the information comes from data both in E. coli and in mammalian cells. My colleague James Regan and I (1974) categorized excision repair into two types. One type is called long patch, where a region of DNA of the order of 100 nucleotides is removed and replaced, even though the initial damage itself might only have been 1 or 3 nucleotides. It appears that the system cannot quite accommodate cutting out small pieces--as if it gets ahead of itself. This long-patch type of repair follows UV damage and many other bulky types of damage, presumably those caused by the polycyclic aromatic hydrocarbons, such as N-acetoxyacetylaminofluorene, nitroquinoline oxide, and many other compounds. The second category is short patch--on the order of 1-10 nucleotides. It is characterized by its existence in cells that are exposed to X-rays or some alkylating agents. Presumably, an alkylating agent can lead to a depurination that can be repaired by a simple break and rejoining.

A short time ago I heard of a third kind of excision

repair that applies to alkylation damage. It is felt that the major damaging alkylation product (not the principal numerical product, but the major alkylation product) is O-6 alkyl guanine (the major product numerically is N-7 alkyl guanine, but the N-7 seems to have very few biological consequences compared with the O-6). At a recent meeting Pegg (1978b) described evidence that there is an enzyme in rat liver that removes the alkyl group from the O-6 and gives back guanine (see also Pegg, 1978a). This repair does not affect the polynucleotide. You would not detect this repair by any easy physical test unless you happened to have a specific marker on that methyl group, and most people do not do that. It might be detected in a mutagenesis test. I call this repair a zero patch; zero nucleotides are removed and replaced, and it seems to be documented for the O-6 alkyl guanine.

In the other tests, long- and short-patch repair can be detected by various biochemical or biophysical means that look at changes in the parental DNA, such as the incorporation of nucleotides by repair replication or unscheduled synthesis. The big advances in this area have come from the recognition that there are mutants that are sensitive to various environmental agents. Of course, the point of all this is that the sensitivity of an organism to such an agent (chemical or physical) in the environment depends not only on what the agent is, but also on whether the cells can repair the damages. You can see that there can be big differences in the effectiveness of various agents; these differences are also reflected in the mutagenicity of various compounds. At a given dose, the uvrA mutant would be mutagenized much more than wild type.

The extension of these ideas to mammalian cells comes from the recognition that there are human mutants that are deficient in repair. James Cleaver (1968) had the bright idea to look at human mutants that got skin cancer at early ages--so-called xeroderma pigmentosum (XP) individuals. Such individuals, diagnosed clinically, have 100% cumulative incidence of skin cancer by the age of 10 or 12. They are characterized by the fact that their cells are deficient in one or more DNA repair mechanism. Veronica Maher (Maher and McCormick, 1976) and her collaborators have shown that XP cells are also mutagenized very readily by UV and that the cells themselves are also sensitive to UV. XP happens to be a very peculiar case in that we know a great deal about the disease and we know the etiologic agent--sunlight--very clearly. We know that the individuals are deficient in repair; their cells are mutagenized to a high rate in culture, and there is a high incidence of skin cancer. The concentration of homozygotes in the population is of the order of 1 in 10^5; the heterozygotes in the popu-

lation are estimated to be in the neighborhood of 1 in 100. The homozygotes are very sensitive; the heterozygotes, as far as has been determined epidemiologically, are close to normal. They do not seem to be at much greater risk than normal individuals.

It is of interest that xeroderma pigmentosum cells in culture are sensitive not only to UV, but also to many other agents. They are both mutagenized by and are more sensitive (killed) to these other agents. As far as the clinical disease is concerned, however, UV swamps out any other environmental agent that might affect such individuals.

Finding UV-repair defects among XP individuals has stimulated the search for other human genetic diseases in which there is a high propensity for cancer, and several have been found (Setlow, 1978). Ataxia-telangiectasia (AT) is also associated with a high cancer incidence; it is estimated that the cumulative incidence of cancers (most of which may be lymphatic) among such individuals may amount to 10%. The etiologic agent is not known; we just know that they are a high-cancer-risk population. What is known is that cells of such individuals, and the individuals themselves are very much more sensitive to X-rays, i.e., maybe fivefold. The idea to look at X-ray sensitivity came about because some AT individuals had very severe reactions to X-ray cancer therapy. Although the cells and the individuals are sensitive to X-rays, that does not mean that their cancer arises from ionizing radiation in the environment. There are a number of chemicals to which cells from these individuals are sensitive--alkylating agents, MNNG, and MMS, for example. But whether the people are sensitive to these agents is another matter. The interesting thing here is that the concentration of homozygotes is of the same order as found for XP--about 1 in 10^5--and they are also high risk.

The heterozygotes have been subjected to an epidemiological analysis by Swift and coworkers (1976), and among the heterozygote population they find a higher risk for cancer than in the normal population. Let me give you some examples from their data. Heterozygotes less than 45 years of age have a fivefold greater risk of cancer death than the average population, so they are a high-risk group.

EHRENBERG: Cancer of which sites?

SETLOW: Of several sites, if I remember.

The epidemiology was all done on mortality records. The biggest change is in young individuals. For the total population up to age 75, which is the highest age looked

at, cancer mortality was 50% higher than the normal population. It appears that AT heterozygosity is shifting the incidence curve to lower ages.

The molecular nature of AT is not clear. AT cells in culture are very sensitive to X-rays. Approximately one-half the cell strains looked at are defective in repair of one form or another. One-half of them seem to be proficient in repair, which indicates extreme heterogeneity (that these arise for different reasons) or we are not looking at the right kind of repair. Whether it is a repair that involves zero patch, and no one has looked at it, or whether they do not perform repair is just not clear. So, when I say "defective in repair," you have to remember that this is based on an experiment, and maybe people have done the wrong experiment.

But all the cells are sensitive (in terms of killing) to X-rays. We just do not know why. They are all also sensitive to alkylating agents.

There is a syndrome called Fanconi's anemia in which, according to Swift (1971), the heterozygotes are also at higher risk than the average population. The homozygotes die from numerous hematological abnormalities as well as face a high risk of leukemia. The heterozygotes in this population have been estimated by Swift to account for 5% of all leukemia deaths. They are present in the general population to the extent of 1 in 100, and therefore 5% is a fivefold excess over average.

As far as we know, the deficiency in this case is in a kind of a repair that is quite different from long-, short-, or zero-patch repair. It seems to be a deficiency in the repair of cross-links, something that joins two DNA strands together. So cross-linking agents such as mitomycin C, psoralen plus light, and so on affect such cells much more than they affect normal cells, and the cross-links persist for longer periods of time. The etiologic agent for the high incidence of cancer, however, is not known.

BREWEN: Are they also defective in repairing protein-DNA cross-links?

SETLOW: I have not seen anything on that.

The environmental agents, if any, involved in ataxia-telangiectasia or Fanconi's anemia are not known. Luckily for us, the etiologic agent of xeroderma pigmentosum is known, and that is why we have a lot of background information on it.

In summary, mammalian cells that lack repair systems are more sensitive to a number of agents. There is a correlation, but not a superb one, between such deficiencies and increased cancer, although for AT and Fanconi's

anemia we do not know what the environmental agents are. Incidentally, the models that most people like to use for human cells in culture are rodent cells, and rodent cells in culture are all (at least as far as UV is concerned) in the class of xeroderma pigmentosum--deficient in one way or another in excision repair. So even though they are easy to grow, they are not necessarily a good quantitative model.

WATSON: Is that because of their thick skin?

SETLOW: No, these are cells in culture.

RAY: And that does not apply to primary cultures, I would gather.

SETLOW: It applies to primary cultures if they are taken from mice after birth.

BREWEN: Mouse embryos are deficient?

SETLOW: Not if you get cells from very young embryos. It appears that in the rodent system the UV excision repair system is really there genetically; it gets turned off at some time, maybe because the animals realize they have a thick skin and do not need it. It is there, but it is turned off some time during late gestation.

RAY: When you say that, are you speaking about epithelial cells as a comparison between rodents and humans, or are you talking about all cells including specific organs such as the liver?

SETLOW: In the rodent system, epithelial cells and fibroblasts have been studied. With regard to human cells, most of the work has been done with fibroblasts. I know that normal human epithelial cells are proficient in excision repair. There were some early experiments done to look at the ability of the skin of XP individuals to do unscheduled synthesis after UV--experiments that would not be permitted nowadays. Such experiments presumably showed that epithelial cells are deficient, so there does not seem to be any tissue dependence for repair of UV damage. People have not looked carefully at the tissue dependence for repair of damage caused by many other agents, such as alkylating agents.

FLAMM: Lieberman and his colleagues (Ambacher, Elliott, and Lieberman, 1977) have done some of that work. Many of
the mouse species they looked at were not competent with respect to UV repair in somatic cells. They were

competent with respect to both long-patch and short-patch repair with the whole series of chemicals, in certain instances exceeding human cells in their ability to repair damage induced by certain types of chemicals.

SETLOW: The problem with chemicals, as with the alkylating agents, is that you are not really sure what product to study. You want to look at the deleterious product, and in the case of alkylating agents the major product is relatively nondeleterious, so you can look at that. You are not sure what its biological significance is. But you are right, it is not clear what goes on in rodent versus human cells except for UV.

I want to present some NIH data (Table 3) on xeroderma pigmentosum to emphasize what a dramatic difference there is between normal people and XP individuals. This is an analysis by Jay Robbins and his collaborators (1974); it indicates the fact that multiple tumors develop. The table also illustrates that XP individuals have a high incidence of malignant melanoma. Basal and squamous-cell carcinomas are relatively innocuous cancers to deal with, but that is not true of malignant melanoma. It is a serious disease in the white population and seems to be increasing at the rate of 5% or 6% per year. The XP data implicate damage to DNA, if you want to put it that way, in malignant melanoma. Individuals who are defective in that repair have a prevalence of 50% in this sample, whereas the normal incidence in the general population is just a few in 100,000 per year.

In a fit of frenzy I at one time (Setlow, 1978) made a compilation showing the various chemicals that have been tested with XP, AT, and Fanconi's anemia cells (Table 4). I was making the point that cells deficient with one agent, which is how these cells are characterized in the first place, end up being deficient in repair with a large number

Table 4

of other agents, most of which have no obvious connection with UV. AT cells are also sensitive to a wide variety of agents and proficient with a large number. It seems as though these repair-proficient anomalies in mammalian cells--the clues to which are taken from bacteria, whose science is in a bad state--span a wide range of chemicals. Agents that damage DNA are bad, and conceivably there exist sensitive subsets of the population that one has to worry about--perhaps, for example, heterozygotes of AT or of Fanconi's anemia.

NEEL: Your estimates of heterozygote frequencies are based on the assumption that the entity is genetically homogeneous. As we know, there are now about eight subgroups or eight complementation groups for XP, two at least for AT, and MacPaterson, at a recent symposium (1977), said he got tired of doing that kind of work. These are independent loci, and that fact could double these heterozygote frequencies. Earlier in this conference there was a question about mutable strains of people and these would be candidates.

SETLOW: These are mutable as cells. There was some question at a conference we attended as to whether fibroblasts of AT were mutable or not; some people said no, and I just was not wide awake enough to say "to what agents?" I do not know whether they really tested the right agent or the wrong agent.

RAY: The work you and others have done showing that the intrinsic or inherent repair capability of the same kind of cell taken from a variety of species can differ has been very interesting--the difference between human and rodent cells, for example. Has anyone any knowledge at this point about the inherent or intrinsic repair capability for organs with a very low mitotic index, such as the liver and bladder, compared with organs with a very high mitotic index, such as the villi of the intestines, hair follicles, and certain parts of the reticuloendothelial system? I have wondered whether this is known; has anyone looked at this?

EISENSTADT: Have liver and brain been compared with respect to repairing out of nitroso . . .?

RAY: Liver and brain?

EISENSTADT: Yes.

SETLOW: Yes in so far as repairing some kinds of alkylation. This is part of the evidence that leads to the circular kind of argument that we get into. Whichever

was the sensitive tissue--brain in this case--did not remove O-6 alkyl guanines as readily as liver and so this is some evidence that O-6 alkyl guanine is bad.

BREWEN: But what about the data of Strauss and collaborators (Scudiero et al., 1976) on leukocytes?

SETLOW: The data show that when leukocytes with a relatively low repair rate are stimulated to divide they have an increased repair rate.

BREWEN: For UV damage, but they do repair X-ray damage without stimulation.

SETLOW: Confluent cells are just as good for UV or a number of the chemical damages as growing cells, but, again, that is the same cell.

RAY: I guess the question at the back of that is whether or not some of this inherent or intrinsic repair capability could indeed be related to sensitivity or resistance to carcinogenic agents in those particular organs.

SETLOW: People attempted to guess at the lethal product or the mutagenic product, such as O-6 versus N-7 alkyl guanine, and determined that the O-6 in brain lasts for a long time, whereas it does not last for a long time in liver.. Therefore, the rate of repair is important. But obviously the rate of repair has to be related to the rate of proliferation in that tissue.

RAY: That is why I asked about mitotic index in the first place.

SETLOW: I cannot really answer that. For X-ray damage, almost all kinds of cells repair at reasonable rates. Let me add one point that came up in the previous presentation: the ability of cells to reactivate or to have some sort of inducible activity. In mammalian cells, there is a viral system that also has been used in the same sort of way. This is a system in which an irradiated virus shows a higher survival if it is plated on irradiated cells--or cells treated with a number of chemical carcinogens. There is reactivation of UV-irradiated viruses for a number of chemical types of damage to mammalian cells. It is not as dramatic as in bacteria. That is probably the best evidence for an induced repair system.

WALKER: It is probably worth mentioning that with XP, too, if you look for more than one biochemical effect, the different complementation groups seem to be lacking more than one activity. It is as though they were lacking some regulatory . . .

SETLOW: They lack one, they lack the other. It is a rather complex system. There are in the neighborhood of six to eight complementation groups. We do not have that number in E. coli where we cannot even analyze three, so to speak. We do not know what the defect is in E. coli--whether it is uvrA, B, or C. It seems that in xeroderma pigmentosum it could be A through H.

EHRENBERG: With reference to dose-response curves, very often people believe, at least on the quasi-scientific level, that if you have a repair system, there must be a threshold of the effects. That is, at very low doses the repair might be error-free and complete. I have seen an investigation quoted where the repair of O-6 alkylation had been determined with regard to dose response down to very low doses. I do not know who did that, but it was certainly a study in the United States.

SETLOW: I think Pegg (1978a) may have done that. It is true that if you go down to very low dose rates or doses (which is what we are exposed to, not acute rates as in all these experiments), you would expect much more efficacious repair. But in the case of UV, I would argue that this is a simplistic point of view for the following reason. Even though we all talk about pyrimidine dimers as being the big thing, they are not the only thing. There may be a number of other products that are not repaired as effectively. Even when you go to low dose, there are still other products. For example, just to put this in perspective, I put down the number of pyrimidine dimers per chromosome per mean lethal dose. For XP cells, for the whole chromosome set, this number is about 10^5.

There are lots and lots of products, most of which are ignored--after all, these are distributed among 40-odd chromosomes. This really means that there are appreciable numbers of other products about whose biochemical characteristics we know little--for example, DNA-protein cross-links. Unfortunately, we only measure the ones we know something about and can measure easily. Some might be at the level of 10, which you really would not detect by the means we have; they might not be effectively repaired. There is no way of my getting at that problem with mammalian cells.

LEE: May I make a comment about repair in germ-cell stages? I will consider this in historical order.

Rejoining of chromosomes is at least one method that would require repair--mechanism unknown and probably rather complex. But 30 years ago Muller showed from the kinetics of rejoining in mature sperm that chromosomes do not undergo rejoining until after the egg is fertilized. This process of chromosome rejoining is apparently turned off at the midspermatid phase. The latest spermatid and mature spermatozoa do not have the capability of chromosome rejoining, whereas the early stages do.

About four years ago Sega did work on unscheduled DNA synthesis. In this case, synthesis occurred normally, of course, by premeiotic replication. In the presence of a variety of mutagens, including alkylating agents, X-rays, and so forth, the unscheduled synthesis continued after meiosis until about midspermatid stage and then it shut off. In more recent work, Sega (1978) compares the stage of unscheduled DNA synthesis and the stage of maximum sensitivity of the germ-cell stage to dominant lethal formation. The patterns are quite different for different mutagens.

In the case of EMS, for example, the maximum sensitivity for dominant lethality occurs 7 to 9 days after treatment of the mouse, whereas the unscheduled synthesis does not appear until a few days later. They do not coincide. In fact, the beginning of the scheduled synthesis is the end of the mutation-detection system in that germ-cell stage. The other mutagens, however, have quite different patterns, so even with the four or five different systems we studied, there is not a consistent pattern between the time of germ-cell stage sensitivity and the time of unscheduled DNA synthesis.

A third type of experiment that may be related to repair would be to measure the loss of the labeled group on the DNA. An abstract by Janka is all there is in the published literature. Unpublished work on Drosophila has shown a loss of the labeled group in the early germ-cell stages. From a midspermatid stage only to mature spermatozoa, there is an accumulation of the alkyl group, and loss from the sperm stored in the female is at a rate that corresponds to the published rates of hydrolysis, with no indication of any enzymatic loss at all.

After fertilization, Janca (1977) has been able to determine two points. He has a level of labeling in the mature sperm, and he has the level 15 minutes later. He has not been able to get a point between those two. But there is a drop of some 40-50% of the

alkylation in that short period of time. The rate would require many times that of hydrolysis. Apparently there is a change in the loss of the labeled alkyl group upon fertilization. It appears that in the germ-cell stage (in these two metozoans and humans), each individual is a result of a cell that passed through stages where the repair systems were shut off and stages where they functioned. At early cleavage, apparently there is replication of DNA and a repair system going. An error-prone system at that point would be predicted.

NEEL: May I suggest that you have failed to quote what might be the most spectacular example of all repair mechanisms in humans--the well-known failure to recover induced mutations from late eggs in irradiated female mice.

LEE: That is in the mouse, not in humans.

NEEL: No, but we do not yet have that kind of evidence.

LEE: I think there is some question as to whether the mouse ovary is reflective of the human female ovary. Grant Brewen would be better able to discuss that than I. Yes, I have limited my discussion here to the male, but certainly there is a very rapid change in the female system there; exactly which model we should use for humans, perhaps Brewen will discuss.

ABRAHAMSON: In the Drosophila female, repair goes on from oogonial stages up to stage-14 oocytes. You have stage 7, and between stages 7 and 14 there is about an 8-hour period. So somewhere in there you lose repair. Dean Parker showed that, and we did also (Parker and McCrone, 1958; Parker and Hammond, 1958; Abrahamson, 1961). The stage-14 oocyte does not repair again until after fertilization, which is a stimulating process. Grant, do you want to discuss the female?

BREWEN: Actually, I was going to talk about it in my little presentation. But I will make this one point. The stage in the mouse oocyte that has 100% repair is stage 1; stage 2, the true dyctyate oocyte, does not exist in humans or for that matter in very many mammals.

PERSON: I want to criticize your phrase "100% repair." That is 0% survival.

BREWEN: Well, not quite, it is 0.1%. I was quoting Bill Russell when I said 100% repair (see review by Searle, 1974).

ABRAHAMSON: Its LD50 dose is 9 R and its LD99.9 dose is 50 rad X-rays. His lowest dose experiment was at 50 rad for the female for that stage, and it is true he got zero mutation.

BREWEN: Fifty rad is the only dose for which there are any data, because at any higher doses there are no surviving oocytes.

EHRENBERG: Does the similarity between bacteria and humans in various respects indicated here make it possible to determine which test is applicable to risk in humans? The bacterial systems are made more and more quantitatively sensitive simply to detect mutagenesis. But such changes could increase the difficulty of quantitation of risk. For instance, great increases in postreplication repair through these plasmids would be abnormal but very practical for detection.

WALKER: In terms of carcinogenesis, Ames has been looking at this correlation between carcinogenic potency and mutagenic potency, having gone through the world's cancer literature of the last 50 years or so where there were test data that satisfied criteria set up ahead of time. He found a correlation (about which he is not yet willing to say much) between carcinogenic potency and mutagenic potency covering a six-order-of-magnitude scale (Ames and Hooper, 1978; Meselson and Russell, 1978). In that system he put in things designed to pick up more and more of the known carcinogens. This he used as a yardstick for calibrating the system as he went along. What he ended up with seems to be not a bad predictor of potency, even though all he was looking for initially was a yes/no response. How that relates to mutagenicity, I don't know.

VALCOVIC: I would only add that this is for a relatively small number of points on that curve, though. A lot of carcinogens are not on there. It is a highly selected sample.

FLAMM: Sure. No one is saying that they biased it to come out that way. It is just that it has not covered the whole universe of carcinogens because the data have not been sufficient to meet those criteria. Once you have removed the nitrosamines and the nitro compounds that are in the middle--one being too high and the other being too low--you are left with points just at either end. It says essentially that things like chloroform and chlorinated alkenes and alkanes are

very weak carcinogens, whereas things like aflatoxin are very potent.

WALKER: Isn't it the other way around? There is a huge cluster in the middle and then there are a couple of things at each end.

FLAMM: Except if you remove the things that are producing problems--for instance, nitrosamines are not registering as strongly for carcinogenicity as they do in animals, whereas nitro compounds are registering as too potent. But there are a lot of nitro compounds and nitrosamines there because there are adequate carcinogenicity data on them. I think what we really need are people to do carcinogenicity experiments in ways that will generate some useful information on the potency in various animals.

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