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UV PHOTOBIOLOGY: POSTREPLICATION REPAIR

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We have discussed two repair modes: 1) photoreactivation, in which dimers are reversed in DNA; and 2) excision repair, in which lesions are removed from the DNA. What happens if it is time for the cell to synthesize a whole new copy of DNA before the lesions are reversed or removed? One might image a number of scenarios. 1) The DNA repair enzymes may stop at the damage and never start again. 2) The DNA repair enzymes might go on inserting a few bases, not necessarily the right ones, and then copy as usual. 3) Finally, the DNA repair enzymes might fall off the DNA and resume synthesis further downstream.

What does happen in biological systems? There is evidence that our second possibility, insertion of a few bases at random, can happen in vitro. This might also happen in vivo. There is also good evidence from a number of systems, both bacterial and mammalian, that the third possibility can happen in cells; that is, that the DNA repair enzymes fall off the DNA, restart later downstream and leave a gap.

The evidence for the second possibility, the insertion of incorrect bases is as follows. Setlow and Bollum tested the ability of DNA polymerase to copy ultraviolet irradiated DNA (1). They found that the product DNA had a decreased number of A's incorporated into the new DNA. Thus the original parental strand of DNA which contained thymine dimers could not code

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correctly for the AA sequence which should have been inserted opposite. They also found that there was a decrease in the number of AA nearest neighbor sequences, thus implying that the incorporation by the DNA polymerase across from a pyrimidine dimer was inaccurate. They also found that there was a decrease in the total amount of synthesis as if the enzyme got to the damaged region and spent much longer than usual than "deciding" what to do before it could proceed down the DNA.

From E. coli comes major evidence that the third possibility can happen; that is, that the replicating enzymes might fall off the DNA and restart leaving a gap. Much of this evidence comes from the laboratory of Howard-Flanders and his colleagues (2). They found first that gaps were made in DNA synthesized just after UV. This was done by taking an excision-defective E. coli which was prelabeled with ^{14}C thymidine. After ultraviolet irradiation the cells were exposed to ^{3}H thymidine for 60 minutes and then sedimented in alkali to separate the DNA strands. They found that the newly synthesized DNA resulting from the UV irradiated cells had a smaller molecular weight and that from un-UV'd cells. They also found distance between the gaps was approximately equal to the distance between dimers in the DNA. (They knew the average molecular weight of the pieces, the UV exposure and thus the number of dimers in the genome.) They also have shown that pyrimidine dimers are opposite gaps in the newly synthesized DNA. To do this, they used bacterial episomes, small pieces of DNA which can exist in the bacterial cells independent of the cellular chromosomes. They UV'd a bacterial cell which was excision^{-} and which contained an episome with a genetic marker. They transferred the episome to excision-proficient or excision-deficient hosts, and found that the capacity of the recipient for excision repair made no difference. This implies that the structure which was being transferred into the cells was not able to be repaired by excision repair. However, when the episome was transferred to a cell and then photo-reactivated (and the control kept in the dark), it was found that the photoreactivated samples gave much more biological activity as recognized by the presence of the marker of the episome. This implies the structure which was being transferred was susceptible to photoreactivation. As you can tell, a dimer in a DNA opposite a gap can be acted on by photoreactivating enzyme since no incision and thus breakage of the DNA would be involved. Finally, they have estimated the size of the gaps by chromatography to be about 1,000 nucleotides.

What happens after the gap is created in the cell? Howard-Flanders has shown in bacterial cells there is slow gap filling after ultraviolet irradiation. If the E. coli, which

we used in the experiment just discussed above (that is, excision minus, ^{14}C thymine labeled, then ultraviolet-irradiated, allowed to incorporate ^{3}H thymidine and then sedimented in alkali) had been allowed to grow longer and had been sampled at different times of incubation after the UV, it would be found that immediately after the UV molecular weight of the DNA is large. (Remember that these bacteria are excision deficient.) After a few minutes the molecular weight is smaller; that is, during DNA synthesis the synthesis on the damaged template gives rise to DNA of lower molecular weight than if the synthesis were occurring on undamaged template. After a few hours, the molecular weight of the DNA became large again implying that somehow the gaps were filled. The exact mechanism by which the gap filling occurs is still not well understood. It is thought that somehow the E. coli can use information from the multiple copies of the bacterial chromosome present in the cell to provide the correct information for synthesis of the DNA. Again we should point out that there are no ideas as to exactly which enzymes can participate in these repair processes, even though E. coli would seem the ideal system since the genetics are reasonably well worked out.

What happens in mammalian cells? The problems of examining postreplication repair in mammalian cells are many. First the molecular weight of the intact DNA is so large that the sedimentation of molecules is not as independent DNA molecules but aggregated, tangled masses. Thus in order to be able to examine the DNA at all, it must be fragmented into molecular weights less than about 5×10^8. Using this procedure, Alan Lehmann in England has found that there are gaps in newly replicated material formed after UV (3). The distance between the gaps is approximately equal to the distance between dimers. In marsupial cells it has been shown that after UV the newly synthesized DNA is smaller than the DNA synthesized on undamaged templates. If the cells are UV'd and then photoreactivated, the DNA is larger. This would imply that the gaps which made the DNA smaller were indeed opposite dimers. There is also gap filling after ultraviolet irradiation. However, there is disagreement about the size of the DNA made after ultraviolet irradiation, the interpretation of size changes, and even whether postreplication repair is an independent repair process were some aberrant excision.

XP cells are also deficient in postreplication repair. Lehmann has shown that if cells are exposed to ultraviolet light and then grown, the molecular weight of the DNA can be followed by fragmenting the DNA into pieces at least smaller than 5×10^8.

After UV-irradiation, normal cells show a reduction in the molecular weight of the newly synthesized DNA, then rapidly convert this DNA to the 5×10^8 maximum. However, XP cells reach this level much more slowly than do normal cells. The striking thing, however, about Lehmann's data is that the slopes of the lines (log molecular weight vs. time), are the same for XP and normal cells. However, XP cells begin the DNA synthesis period at a much smaller molecular weight than do the normal. This implies that perhaps the defect in the XP's is too much nicking of the DNA rather than not enough gap filling.

Another group of patients is diagnosed by clinical signs as xeroderma pigmentosum; however, cells from these XP "variants" excise dimers as well as do cells from normal individuals. They also have virtually as much unscheduled DNA synthesis as do normal cells. Lehmann finds that the XP variants are even slower than XP's to reach the limit molecular weight, again because the molecular weight is initially smaller but the rates of increase are the same as normal. XP variants are also deficient in photoreactivation. It is not yet clear exactly what is the molecular cause of the XP in these cells which are normal with regard to excision.

MAJOR PROBLEMS IN DNA REPAIR: PROSPECT OF SYNCHROTRON RADIATION STUDIES

During these lectures, I have pointed out a number of important areas in DNA repair which must be solved before we can correlate DNA repair deficiencies and human diseases. For example, what is the molecular cause of XP? Another important problem is the target for the induction of human skin cancer by sunlight. What is the molecular lesion which leads to skin cancer?

Synchrotron radiation offers advantages for all of these studies. For example, it can provide important information on the molecular target for skin cancer induction. One way for studying this is by determining the action spectrum. Are the wavelengths which inactivate DNA more important or are the wavelengths which inactivate protein more important? These action spectra could be performed on experimental animals or on cultured cells by looking at UV transformation.

Examining the molecular lesion would be more difficult. However, we can examine the role of pyrimidine dimers in producing these effects if we can show that a biological damage caused by ultraviolet light can be reversed by longer wavelength

light in a photoenzymatic reversal. Then we would suspect that dimers were an important lesion in the production of skin cancer. However, the proper controls must be done to show that photoreactivation and not a nonspecific light effect (for example, on cell growth), was reversing the lesions. Again here action spectroscopy is crucial. If we could show that the action spectrum of the light reversal event was the same as that for the action spectrum of the purified photoreactivating enzyme, we would have good evidence that the light mediated amelioration of damage was due to true photoreactivation.

Synchrotron offers two major advantages for such biological studies. The first is light intensity. Most biological studies have been limited to mercury lines in the ultraviolet. In most sources, for adequate wavelength purity, the irradiation times have been many minutes to hours. During these irradiation times, the cells' metabolic processes are proceeding; these may complicate the experiment or even lead to possible errors. The second is the advantage of a continuum. Most ultraviolet action spectra have been limited to the mercury lines. In many cases there are not enough for good resolution of action spectrum. (However, we should notice that the absorption spectrum of biologically important molecules; that is, molecules essentially in a water solution, is very broad so it would not be fruitful to do action spectra, say at 1 Å spacing.)

In addition to discussing the advantages that the synchrotron now offer to biological studies, we must consider some possible problems. The first is the timing of the synchrotron beam. The pulsed photons from the synchrotron may give entirely different photochemistry, and this must be examined with regard to the possibility of multi-photon effects. This might lead to entirely different photobiology - which, while of possible interest in itself, might tell us nothing about cells and their responses to solar insults. Finally, just as in any biological studies, we must have careful and informed biology, as well as sophisticated light sources, so they both can be used to the maximum advantage.

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