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UV PHOTOBIOLOGY: EXCISION REPAIR*

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In addition to photoreactivation, the reversal of damage produced in DNA by ultraviolet light, there is another repair process, excision, in which the damaged regions are cut out of the DNA. Although we will deal mostly with damage inflicted on DNA by ultraviolet light, there are also excision systems for chemical damage as well. Most of what we know about excision comes from E. coli, but we can extrapolate to mammalian systems; how well the extrapolation fits the actual case is not yet clear.

There are two and perhaps three types of excision repair. The first is nucleotide excision in which a few to many bases, including the damaged area, are removed. There is base excision in which only the damaged base is severed from the DNA backbone, then nucleotide excision proceeds just as usual. Finally, in a very recent study, there is a hint that damage may be removed directly from a base leaving the DNA intact without any need for incision into the DNA backbone or any new synthesis. (Note that both nucleotide excision and base excision do require new synthesis into the DNA.)

I. Nucleotide Excision

We are gradually developing a good idea of the major steps in nucleotide excision. First is the recognition of damage by

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an enzyme called a UV-endonuclease. The next is incision by the nuclease into the phosphodiester backbone, generally at a site near the damage of damaged base. Then there is polymerization by covalent extention of the damaged strand using the complementary undamaged strand as template. Virtually simultaneously with polymerization there is excision of the damaged region. Finally, there is ligation of the newly synthesized region to the parental repaired strand.

Even in E. coli exactly which enzyme participates in which step is still largely unknown. For the endonuclease step there have been several reports of isolation and characterization of enzymes which seem to have had some properties expected of the enzyme carrying out the incision step. However, none of these enzymes has turned out to have definitive evidence in its favor. DNA polymerase I seems to participate in both polymerization and excision although it is possible there are other enzymes in the cell which can serve as backup enzymes should polymerase I be defective. Finally, DNA ligase is responsible for joining the newly-synthesized strand to the parental repaired strand.

An important tool in studying any multi-step pathway is complementation. First let us see how we could use the complementation to study DNA repair in E. coli and then later we will see how this can be used in mammalian cells. We know that in excision repair, damaged DNA is acted upon by a endonuclease, then by a polymerase and finally by a ligase. If we had a cell which was a mutant in the endonuclease, the repair pathway could not proceed. If we had another cell which was defective in the polymerase, the repair pathway still could not proceed. However, if we could combine the properties of these cells, we would have at least one good copy of the endonuclease, the polymerase, and the ligase, and thus the two mutants could be said to complement each other. This complementation approach has been used both in vivo and in vitro. Most recently it has been used by Erling Seeberg to isolate a UV-endonuclease from E. coli. We shall see later in this lecture how this approach can be used also in mammalian cells.

What happens in higher organisms with regard to nucleotide excision repair? They do seem to remove damage from their DNA by cutting out; however, the number of enzymes, their identity and the possible alternative pathways, are not yet known. The bacterial model provides just that: a good working model, although not necessarily reality.

How would we measure excision in higher organisms? First, we could look for a damaged piece which would be cut out and might appear in the smaller molecular weight portions of DNA.

We could look for the insertion of new bases. Two major procedures have been used to look for the insertion of new bases into DNA. The first of these is called unscheduled DNA synthesis. Ordinarily cells synthesize new DNA only during a limited portion of the cell cycle called S or synthesis. However, if the cells have been exposed to UV, one gets DNA synthesis even though the cell is not in the normally scheduled period of the cell cycle for synthesis. Thus this incorporation of new DNA is called unscheduled DNA synthesis. It seems to represent the insertion of the new bases corresponding to the portion of the DNA which were replaced due to the removal of damaged bases.

The second method of looking for the insertion of new DNA bases is by bromodeoxyuridine photolysis. In this method cells which are undergoing repair synthesis are supplied with bromodeoxyuridine (which is an analog of thymidine). It is incorporated into the DNA; when the cells are exposed to long-wavelength UV (e.g. 313 nm) the bromodeoxyuridine absorbs the light producing free radicals which make breaks in the DNA. So whenever there is new synthesis in the presence of bromodeoxyuridine, by the long wavelength photolysis one can induce DNA breakage and thus cells which have undergone DNA repair can be recognized by smaller size of the DNA.

Nucleotide excision repair provided the first correlation of DNA repair defect and possible human disease. Cleaver first noticed that xeroderma pigmentosum cells underwent unscheduled DNA synthesis at a lower level than did normal cells (1). These cells also show decreased repair synthesis as detected by bromodeoxyuridine photolysis.

Complementation analysis has also been carried out on xeroderma cells in culture. Cells from two individuals are fused using heat inactivated sendai virus. The fused product of the two cells is called a heterokaryon. Just as in complementation in E. coli discussed above, there is complementation of unscheduled DNA synthesis if the two xeroderma cells are from individuals with different defects in the same repair pathway.

There are at least five and perhaps seven complementation groups in XP, as defined by the fusion method (2). However, there are no data on which function is missing in which XP cells. Furthermore, our understanding of the proteins which function in DNA repair in normal humans is fragmentary at best.

In the past few years Tanaka and his associates and Hanawalt and his associates have been able to put a dimer-specific endonuclease into XP cells (3,4). When this is done, the cells then undergo unscheduled DNA synthesis at roughly the normal level.

Surprisingly enough, all XP cells tested, no matter what the complementation groups are complemented by the exogenous dimer-specific endonuclease! One possibility is that an endonuclease is missing in all known XP's. Perhaps defects in other repair enzymes would be lethal to cells. On the other hand, cells which show complementation by the UV-endonuclease are might be undergoing an alternate excision pathway initiated by the UV-endonuclease and thus the process might not relate to normal excision at all.

II. Base Excision Repair

The second kind of excision repair is called "base excision" in which the bond between the sugar and the damaged base is severed (5). Since this bond is the N-glycosyl bond, the enzyme releasing the damage base is called an N-glycosylase. The result of the action of the enzyme is just the release of the damaged base from the DNA. After the release of the damaged base, there is a nick inserted into the sugar-phosphate backbone at the site of the sugar which lacks the base. The enzyme performing this step has been termed an "apurinic endonuclease" for historical reasons: the first DNA which was generated with missing bases was prepared by removing purines from the DNA, thus the origin of the terms apurinic DNA and "apurinic endonuclease" (6). After the nicking of the sugar-phosphate backbone, there is excision and resynthesis just as before. Both the endonuclease and the N-glycosylase have been found in bacterial and in human cells. For example, for the uracil N-glycosylase the substrate is DNA damage in which thymine in DNA is demethylated to produce uracil. The N-glycosylase breaks the N-glycosyl bond in between the base uracil and the rest of the DNA, leaving the sugar-phosphate backbone intact and releasing the uracil from the DNA. Then the endonuclease makes a nick at the damaged site but it would not make release the damaged base, for example, uracil, from the DNA.

Are there defects in this mode of excision repair in humans? Indeed, some xeroderma cells show deficiencies in the level of the apurinic endonuclease. Linn and his group have been able to purify partially such an enzyme from human cells and thus can determine the source of this deficiency: is it due to a decrease in the number of enzyme molecule or to defective enzymes (6)? Ways of distinguishing these possibilities involve the characterization of the physical, chemical and kinetic properties of the enzyme; in fact, Linn and his associates have found that the apurinic endonuclease present in some XP cells shows altered kinetic properties. This implies a change in enzyme structure rather than a control mutation. This poses a problem: XP cells

have been shown to be deficient in photoreactivating enzyme, in excision repair, and in apurinic endonuclease. Now one might think that these deficiencies might be due to a common control mechanism which would just decrease the levels of several repair enzymes. But the evidence from Linn's lab indicates that the apurinic endonuclease, at least, is not merely present in lower numbers but is actually an altered enzyme. If these kinetic studies on partially purified enzymes are valid, this means that there are multiple defects in XP. However, the level of XP in the population (1/200,000) is too high for XP to result from a requisite three or four separate mutations. (If this were the case, one would expect to have almost no XP's in the human population!) Thus this poses one of the important problems in DNA repair and human disease today: exactly what is the molecular origin of XP?

III. Damage Removal

The third kind of excision repair is simple removal of damaged region of a base in DNA without excision of a stretch of DNA and without excision of the base. An example of this seems to be the removal of methyl groups which have been added to DNA bases by methylating agents. The cells could excise the entire region (nucleotide excision), they could just remove the base (base excision), but both these repair pathways are costly in terms of energy to resynthesize, and to ligate the new strand back to the parental strands. Pegg has some evidence that rat liver contains an activity which can merely remove the methyl groups from the damaged base (7). This enzyme has not been purified and it remains to be seen if the activity can be isolated and characterized. It is also not known if its activity exists in human cells, nor is it known if XP's are deficient in this enzyme. Problems in determining these points will include the insensitivity of the assay (which require many grams of substrate of cellular material for a few assays, each assay requiring many milligrams of DNA).

Thus although excision repair has been one of the most studied of the repair mechanisms, there are still many important questions which remain to be answered. First, it is not really known exactly which enzymes in bacteria, for which we may take E. coli to be a prime example, participate in excision repair. If there are deficiencies in our knowledge in bacteria, one might say that our knowledge in human cells is in chaos. There is really no complete or partial idea as to exactly which enzymes participate in excision repair in normal cells, much less which enzymes are deficient in xeroderma cells.

There are really no good explanations at this point as to the molecular cause of such apparent DNA repair deficiency diseases, such as XP. Indeed we are suffering an embarrass of riches, with too many molecular defects. Finally the possibility of a new and intriguing repair system in which only the damaged portion of base is removed without any new synthesis is an intriguing one which deserves further study.

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