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DNA DAMAGE AND REPAIR IN EUKARYOTIC CELLS[1]

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ABSTRACT

Damage in DNA after irradiation can be classified into five kinds: base damage, single-strand breaks, double-strand breaks, DNA–DNA cross-linking, and DNA-protein cross-linking. Of these, repair of base damage is the best understood. In eukaryotes, at least three repair systems are known that can deal with base damage: photoreactivation, excision repair, and post-replication repair. Photoreactivation is specific for UV-induced damage and occurs widely throughout the biosphere, although it seems to be absent from placental mammals. Excision repair is present in prokaryotes and in animals but does not seem to be present in plants. Post-replication repair is poorly understood. Recent reports indicate that growing points in mammalian DNA simply skip past UV-induced lesions, leaving gaps in newly made DNA that are subsequently filled in by *de novo* synthesis. Evidence that this concept is oversimplified or incorrect is presented.———Single-strand breaks are induced by ionizing radiation but most cells can rapidly repair most or all of them, even after supralethal doses. The chemistry of the fragments formed when breaks are induced by ionizing radiation is complex and poorly understood. Therefore, the intermediate steps in the repair of single-strand breaks are unknown. Double-strand breaks and the two kinds of cross-linking have been studied very little and almost nothing is known about their mechanisms for repair.———The role of mammalian DNA repair in mutations is not known. Although there is evidence that defective repair can lead to cancer and/or premature aging in humans, the relationship between the molecular defects and the diseased state remains obscure.

IN the last 10 years, much has been learned about DNA repair, but very little is known of its role in induction of mutation, or even in reversing damage that might otherwise lead to cell death. In this talk I will discuss the kinds of damage that occur and how different eukaryotic repair systems interact with each of them.

The repair of base damage is probably better understood than the repair of any other kind of DNA damage. This follows from the fact that the pyrimidine dimer, which is a kind of base damage and one of the main photoproducts induced in DNA by ultraviolet (UV) light, is an extremely stable compound that can be isolated and manipulated without change (BEUKERS and BERENDS 1960). Recently a method for studying DNA thymine damage induced by ionizing radiation has been developed (HARIHARAN and CERUTTI 1972). Base damage also occurs after exposure of cells to many kinds of mutagenic chemicals.

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Eukaryotic cells have at least three repair systems for handling base damage. One is photoreactivation (PR): the light-dependent, enzyme-catalyzed, *in situ* conversion of UV-induced pyrimidine dimers back to the original, undamaged monomers. This repair system is widely distributed in the animal kingdom (see review by Cook 1970) and occurs in higher plants as well (Saito and Werbin 1969). One large group of animals in which PR does not seem to occur at all, however, is placental mammals, including humans (Cook and McGrath 1967), although it does occur in marsupials (Cook and Regan 1969). This is an important repair system for base damage, and it can be considered the epitome of an error-free process.

A second and perhaps the most important repair system for base damage is excision repair. This seems to be a relatively accurate, almost error-free system that rarely causes mutation (Witkin 1968). This system requires at least five steps: (1) recognition of the damage—it is possible that some kinds of base damage are not detected; (2) incision, wherein an endonuclease specific for base damage induces a "nick" next to the damaged base(s)—the incision enzyme may be responsible for recognition as well; (3) excision of the damaged base(s) —this may or may not require an exonuclease specific for damaged DNA; (4) repair replication, i.e., the insertion of undamaged bases into the region where excision has occurred—this may occur essentially simultaneously with the excision step, although there is evidence that in bacteria repair replication follows excision (Kaplan, Kushner and Grossman 1969); and (5) ligation—the final sealing of the newly inserted bases to the extant part of the same strand. Excision repair has been found in many kinds of animal cells from *Tetrahymena* (Brunk and Hanawalt 1967) to man (Rasmussen and Painter 1966; Cleaver 1968; Cleaver and Painter 1968; Regan, Trosko and Carrier 1968), but it seems to be lacking among plant cells (Painter and Wolff 1973; Swinton and Hanawalt 1973; Wolff and Cleaver 1973). Its importance for survival of UV-irradiated human cells has been dramatically demonstrated in studies of people with the hereditary disease, xeroderma pigmentosum (XP). Cells from most people with this condition have reduced or zero ability to perform excision repair (Cleaver 1968, 1970) and also are much more sensitive to UV than normally repairing cells in terms of colony-forming ability (Cleaver 1970; Goldstein 1971). The relationship of repair defect to cancer induction, however, remains obscure, because cases of XP with no indication of repair defect have been reported (Burk *et al.* 1971). The lack of this repair system in plant cells is surprising and evokes many questions about DNA repair in plants.

A third repair system for base damage is post-replication or bypass repair. In bacteria this repair system seems to be "error prone" and may be responsible for many of the mutations observed (Witkin 1968). Although the evidence in bacteria is good that post-replication repair involves recombinational events between newly formed and parental DNA (Rupp *et al.* 1971), no evidence for this has been forthcoming in eukaryotic cells.

This repair system is involved with events occurring in newly formed strands of DNA produced by semiconservative synthesis after the insult to DNA has

occurred. It is well established that single strands of DNA made after UV-irradiation are of lower molecular weight than their control counterparts. The accepted part of the procedure is that DNA replication occurs at the normal rate up to the base damage, which acts as a block, but the step of circumventing the block is poorly understood in eukaryotes. According to LEHMANN (1972a), the growing point skips past the dimer, leaving a gap averaging about 1000 nucleotides long, and resumes replication, presumably at the normal rate, until it encounters another dimer. The gap supposedly exists for an appreciable time, an hour or so, and then, by some means or another, is filled by *de novo* synthesis. This model has gained widespread acceptance in a relatively short time. Because I am almost certain it is wrong, I want to spend some time discussing it further.

The "gap" concept comes from bacterial work, and the evidence for its existence in mammalian cells comes from a single kind of experiment (LEHMANN 1972a). Mouse L5178Y cells were irradiated with 254 nm light and incubated for 30–60 min (to allow growing points to reach the first dimers), and then tritium-labeled thymidine (^{3}H-TdR) (about 10^{-6} M) was added. After about an hour's incubation, the ^{3}H-TdR medium was removed and replaced with one containing 10^{-5} M bromouracil deoxyriboside (BrUdR). Several hours later the cells were washed and one aliquot was put straight into the lysis solution on top of an alkaline sucrose gradient while another aliquot was exposed to 313 nm light before lysis. Light at 313 nm specifically causes breaks in BrUdR-containing regions of DNA with almost no effect on DNA not containing this analog. When the molecular weights were computed after sedimentation of the DNA through the gradients, it was found that the DNA from cells not treated with 313 nm light was of high molecular weight, but DNA from 313-nm-treated cells was of low molecular weight—indeed, about the same as DNA from UV-irradiated cells that were lysed on sucrose gradients immediately after the ^{3}H-TdR pulse and not chased in BrUdR. DNA from cells not irradiated with 254 nm light before BrUdR incubation showed some degradation but not nearly so much as that from irradiated cells (Figure 1). The amount of 313 nm light required to re-form the low-molecular-weight DNA was compared with a plot of molecular weight of DNA *uniformly* labeled with BrUdR *versus* exposure to 313 nm light; from this comparison came the estimate that there were about 1000 total nucleotides per "gap".

There are three major considerations that throw doubt on the "gap" interpretation of these experiments. The first is that if lesions in DNA are not severe blocks to DNA replication (i.e., if the growing points simply skip past the dimers), one would expect very little effects of UV on the rate of DNA synthesis; but this is not true. UV is a potent inhibitor of DNA synthesis (POWELL 1962; RASMUSSEN and PAINTER 1964; CLEAVER 1967) in all mammalian cells thus far tested. EDENBERG (1973) has developed a model that explains the effects of UV on HeLa cell DNA synthesis by assuming that dimers are absolute blocks for semiconservative synthesis.

The second consideration is that LEHMANN's results, as well as those of BUHL, REGAN and SETLOW (1973) with human cells, are based on comparing the 313

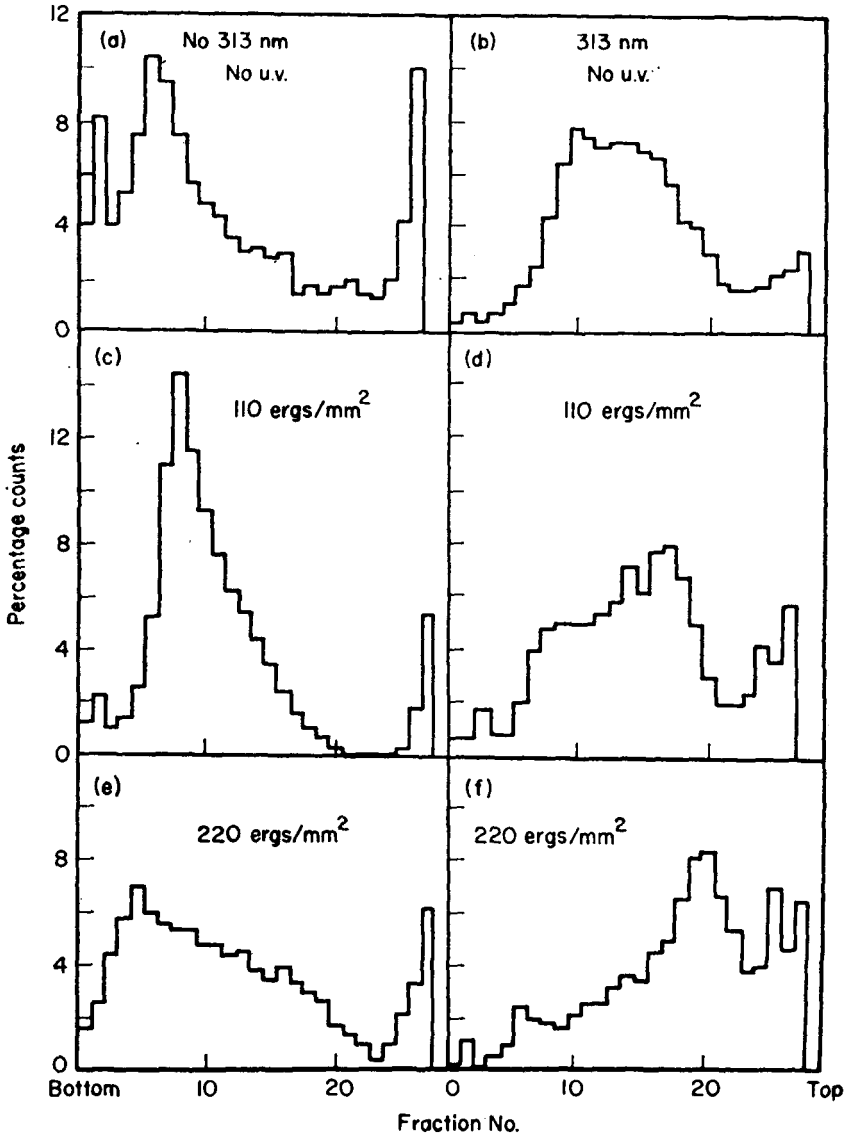

FIGURE 1.—Sedimentation profiles from UV-irradiated cells after 313 nm exposure. Cells were irradiated with 254 nm light, incubated for 45 min, pulse labeled, chased with BrUdR for 4–6 hr (left), and then exposed to 3.4×10^5 ergs/mm^2 at 313 nm (right). (a) and (b) No initial irradiation, 30-min pulse, 4.5-hr chase. (c) and (d) 110 ergs/mm^2, 30-min pulse, 4.5-hr chase. (e) and (f) 220 ergs/mm^2, 30-min pulse, 6-hr chase. (From LEHMANN, 1972a, with permission of Academic Press).

nm light-breaking efficiency for DNA made in the presence of BrUdR after 254 nm irradiation with the efficiency for DNA uniformly labeled with BrUdR. We have found that, due to an effect of UV on thymine precursor pools, DNA formed in the presence of BrUdR after UV irradiation is substituted with the

analog to a lesser extent than is DNA made in unirridated cells (by about 25%). This means that the "gap" must be larger than the estimated 1000 nucleotides. Depending on the relative efficiency of 313 nm light for breaking maximally substituted DNA to less fully substituted DNA, this could mean that the average "gap" is no more than an average of the distances between adjacent replicons whose operation was completely blocked by dimers before the BrUdR incubation.

The third consideration indicating that the "gap" concept is incorrect is that we have been unable to detect any evidence for gaps using a density gradient method. Briefly, HeLa cells were irradiated with 75 ergs/mm^2 and incubated first in medium containing BrUdR and caffeine for 2½ hr and then in ^{3}H-TdR-containing medium for 2 hr. Caffeine is said to prevent gap filling, so if gaps occur, they should accumulate during the 2½ hr incubation with caffeine (CLEAVER and THOMAS 1969). If gaps averaging 1000 nucleotides in length were formed during the BrUdR incubation and filled during the ^{3}H-TdR incubation, tritium should have been detected in heavy strands of DNA after extraction and analysis on an alkaline CsCl-Cs_2SO_4 equilibrium density gradient. When this was done there was absolutely no tritium in excess of that expected as a result of trailing from normal density in the region of the gradients occupied by heavy single DNA strands (Figure 2).

Equilibrium density gradient experiments (unpublished) have also shown that recombinational events between newly formed and previously existing DNA strands cannot be responsible for "gap filling". I am thus led to the conclusion that gaps of the kind found in bacteria do not occur in mammalian cells. Instead, I think that base damage acts as a block to DNA replication for a relatively long time (which in some cells may be long enough for excision to occur), and that the blocks are later bypassed, allowing adjacent, partly finished replicons to be completed, after which they can join to form the fully completed DNA molecules. The mystery in this idea is how, in cells that show little or no excision of dimers, these lesions are finally circumvented; that enigma will have to be the subject of further experimentation.

Another kind of damage that has been relatively well characterized is the "single-strand (SS) break". Strand breaks are not formed to any appreciable extent after biological doses of UV, but they are the best studied lesion in DNA caused by ionizing radiation; they are also formed by some chemicals. The big problem with SS breaks is that their chemistry is almost completely unknown. COQUERELLE, BOPP, KESSLER and HAGEN (1973) have shown in thymocytes that about 15% of SS breaks formed by X-radiation have 5′ phosphoryl groups at the end, while another 15% have 5′ OH groups. The remaining 70% are completely unaccounted for at this time. Moreover, it is not known whether or how often base and sugar damage accompanies (or precedes) the breaks, nor is it known whether base release sometimes or always accompanies break formation. It is already obvious that there must be several kinds of SS breaks.

Repair of SS breaks occurs rapidly after doses of ionizing radiation that kill over 99.99% of the cells, (LETT *et al.* 1967), and so there has been a tendency to dismiss SS breaks as unimportant in cell killing. Unfortunately, however, the

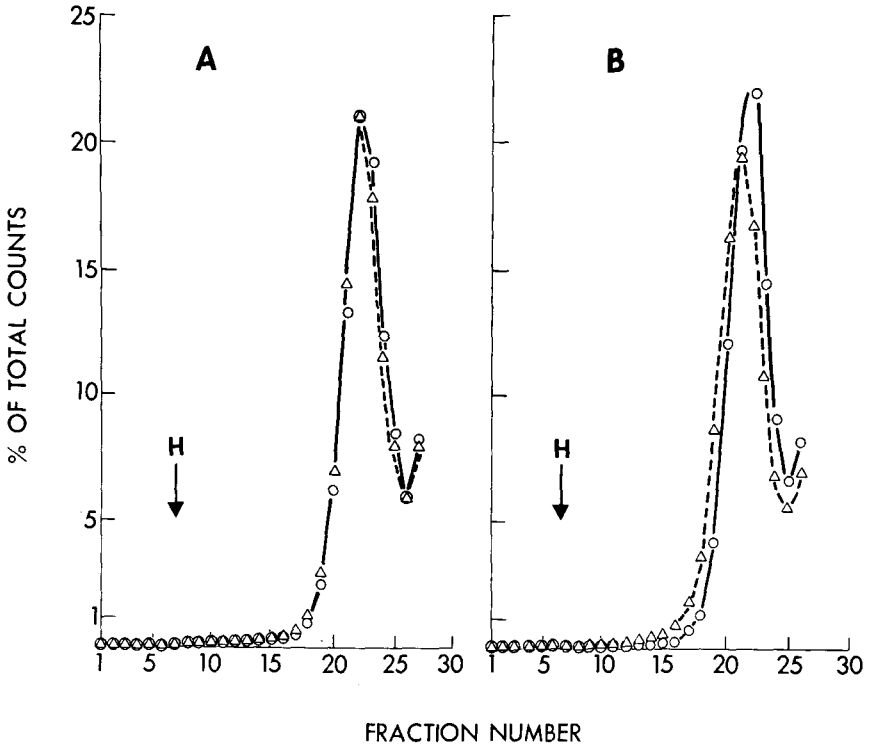

FIGURE 2.—Alkaline equilibrium density gradient profiles of DNA from cells incubated with BrUdR (10^{-5} M) and fluorouracil deoxyriboside (FUdR) ((10^{-6} M) for ½ hr, then for 2 hr with the same medium plus 2×10^{-3} M caffeine, then for ½ hr in medium with caffeine only, and then for 2 hr with 10 μCi/ml ^{3}H-TdR (50 Ci/mmole). (a) Control. (b) Cells irradiated with 75 ergs/mm^2 UV light before incubation with BrUdR and FUdR. Before centrifugation, ^{3}H-labeled DNA was mixed with DNA from unirradiated cells that had been incubated with ^{14}C-TdR instead of ^{3}H-TdR during the previous 2 hr. This ^{14}C-labeled DNA acts as a marker for normal density DNA. If "gaps" averaging 10^3 nucleotides in length were formed during incubation with caffeine in irradiated cells and later filled during incubation with ^{3}H-TdR, a peak of tritium counts should appear at or near fraction 7, the position where heavy strands band in the CsCl-Cs_2SO_4 alkaline gradient.

methods used will fail to detect a low percentage of unrepaired breaks, which, if they occur, might very well be a major cause of cell death. Misrepair is also not detectable by present-day techniques, and this could be the basis for or a component of chromosome aberration formation. If one looks at the reverse side of the coin, i.e., a system in which SS break rejoining does not occur, it is easy to infer that unrepaired SS breaks are important. We have recently studied the formation and repair of SS breaks induced by ^{125}I within the DNA. Very little repair of these breaks occurs, and the cells harboring them are very efficiently killed (BURKI *et al.* 1973).

Double-strand breaks are formed about 10% as efficiently as SS breaks by irradiation of mammalian cells with X-rays (SAWADA and OKADA 1970), but nothing is known of their chemistry and little is known about their repair.

Conflicting reports are current, and because methods for their analysis are still not well worked out, evaluation of these reports is difficult.

I will touch only briefly on the last two kinds of lesions that probably occur in mammalian cell DNA. The first is DNA–DNA cross linking, which occurs after treatment with bifunctional alkylating agents (LAWLY and BROOKES, 1963). This kind of cross linking occurs so rarely at biological doses of X-radiation and UV radiation that it is not considered to be an important lesion for these agents. Repair of cross-linked DNA occurs in mammalian cells (ROBERTS, CRATHORN and BRENT 1968) but the mechanisms are not understood.

DNA-protein cross linking has long been considered a potentially important UV-induced lesion in bacteria (SMITH 1967) and there is evidence that it also occurs after UV irradiation to mammalian cells (HABAZIN and HAN 1970). In the latter case, where lowered extraction of DNA from UV-irradiated cells was used as a measure of cross linking, there was no evidence for repair for several hours after irradiation. It is impossible at this time to estimate the relative importance of either kind of cross linking in cell death and mutagenicity.

The importance of DNA repair systems is obvious from their ubiquity, but puzzles remain. The apparent absence of repair replication after X-irradiation in plants (PAINTER and WOLFF 1973) is difficult to understand. In mammalian cells, repair replication after X-irradiation occurs (PAINTER and CLEAVER 1967; BRENT and WHEATLEY 1971) to such an extent that about 1–5 bases are inserted per radiation-induced SS break (PAINTER and YOUNG 1972; FOX and FOX 1973). This suggests that bases are lost and/or that "trimming" of the break is necessary before reunion can occur. It is highly unlikely that the chemistry of X-ray-induced strand breaks is greatly different in plant DNA than in mammalian DNA, so we are left with the question of how plants cope with this damage. Thus far, to my knowledge, no work on SS break formation or repair in plants has been reported, probably because of the difficulty with cell walls in preparing high-molecular-weight DNA for analysis. With techniques now available, preparation of spheroplasts of plant cells (CARLSON, SMITH and DEARING 1972) seems feasible, and we will probably soon find out that SS breaks in plants are reparable. Then we will have to deal with this dilemma.

Another unresolved question concerns the importance of pyrimidine dimers in placental mammals. In mice, hamsters, and other rodent cells there is no PR, and excision repair occurs to a very small extent, if at all (KLIMEK 1965; TROSKO, CHU and CARRIER 1965). Moreover, although low-molecular-weight DNA is synthesized immediately after UV-irradiation of mouse L5178Y cells (LEHMANN 1972a), a few hours later, pulses with ^{3}H-TdR and sucrose gradient analysis show that the DNA made in the irradiated cells is of the same high molecular weight as that made in controls, even though no dimers have been removed (LEHMANN 1972b). These kinds of results suggest that during evolution an efficient means for bypassing dimers developed so their excision was no longer necessary and PR was not required either. This attribute may simply be the ability of the replication complex to ignore dimers. The delays observed in rodent cells may be due to lesions other than dimers that can be excised. Such lesions could also be the causes

of the relatively large amount of repair replication observed in some (Chinese hamster) cells that do not excise dimers (CLEAVER 1970).

In summary, then, although research on DNA repair in the last decade has been rewarding, it is easy to see that a lot more work must be done before we fully understand how DNA repair systems prevent the lethal effects of DNA damaging agents, let alone the role of eukaryotic repair systems in mutation.

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