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Excision Repair of UV Radiation-Induced DNA Damage in *Caenorhabditis elegans*

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ABSTRACT

Radioimmunoassays were used to monitor the removal of antibody-binding sites associated with the two major UV radiation-induced DNA photoproducts [cyclobutane dimers and (6-4) photoproducts]. Unlike with cultured human cells, where (6-4) photoproducts are removed more rapidly than cyclobutane dimers, the kinetics of repair were similar for both lesions. Repair capacity in wild type diminished throughout development. The radioimmunoassays were also employed to confirm the absence of photoreactivation in *C. elegans*. In addition, three radiation-sensitive mutants (*rad-1*, *rad-2*, and *rad-7*) displayed normal repair capacities. An excision defect was much more pronounced in larvae than embryos in the fourth mutant tested (*rad-3*). This correlates with the hypersensitivity pattern of this mutant and suggests that DNA repair may be developmentally regulated in *C. elegans*. The mechanism of DNA repair in *C. elegans* as well as the relationship between the repair of specific photoproducts and UV radiation sensitivity during development are discussed.

THE small nematode *Caenorhabditis elegans* possesses a number of features which make it attractive for the study of many basic biological processes (KENYON 1988; WOOD 1988). These attributes have also rendered *C. elegans* a valuable model for the study of DNA repair. Accordingly, radiation-sensitive and mutagen-sensitive mutants have been isolated (HARTMAN and HERMAN 1982; N. MUNAKATA, personal communication; J. REDDY and P. S. HARTMAN, unpublished). Several of these have been analyzed for their effects on recombination, radiation-induced and spontaneous mutability, meiotic nondisjunction, and aging (HARTMAN and HERMAN 1982; HARTMAN 1984a,b; HARTMAN *et al.* 1988; JOHNSON and HARTMAN 1988; COOHILL *et al.* 1989; D. BAILLIE, personal communication). Epistatic interactions between the radiation-sensitive (*rad*) mutations indicate that *C. elegans* possesses at least two DNA repair pathways (HARTMAN 1985). In addition, several nucleases from this nematode have been detected and characterized MUNAKATA and MOROHOSHI 1986; HEVELONE and HARTMAN 1988).

Beyond these descriptive studies, recent efforts have focused on the determination of specific DNA repair mechanisms. Three basic types of DNA repair systems have evolved in a wide variety of organisms (FRIEDBERG 1985). In the first, various enzymes recognize and catalyze the direct reversal of specific DNA damages. Photoreactivation is a well-characterized example of direct reversal wherein an enzyme utilizes energy provided by visible light to directly split cyclobutane dimers induced by UV radiation. Various data, including some presented in this communication, indicate this nematode does not photoreactivate UV radiation-induced pyrimidine dimers (KELLER *et al.* 1987). A second type of DNA repair can best be described as a series of DNA damage tolerance mechanisms. The initial damage may not be removed, but various processes act to lessen its cytotoxic consequences. Because replication through the damaged area is often requisite for this, these processes are classically termed post-replication repair. Postreplication repair has been demonstrated in wild-type *C. elegans* (J. REDDY and P. S. HARTMAN, unpublished data).

This communication describes the status of the third major DNA repair system, excision repair. Here damage is enzymatically recognized and excised, and the complementary strand is used as a template to direct the synthesis of a new, undamaged strand. We have analyzed the abilities of staged populations of wild-type and four *rad* mutants to repair cyclobutane dimers and pyrimidine(6-4)pyrimidone photoproducts [(6-4) photoproducts], the most prominent lesions induced by UV light. These data indicate that *C. elegans* has a DNA repair profile unique among the eukaryotic systems analyzed to date. They suggest substantial developmental regulation of DNA repair capacity. Moreover, the regulation observed in the *rad-3* mutant indicates that such developmental regulation of DNA repair can directly impact organismal survival.

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MATERIALS AND METHODS

Strains and general procedures: The wild-type strain N2 was maintained at 20° as described by BRENNER (1974). A strain bearing the temperature-sensitive mutation *ts151*, which prevents gonad formation at the nonpermissive temperature (HIRSH and VANDERSLICE 1976) was reared at 15°. The following genes and alleles, which conform to the system described by HORVITZ *et al.* (1979), were used: *rad-1(mn155)*, *rad-2(mn156)*, *rad-3(mn157)*, and *rad-7(mn161)* (HARTMAN and HERMAN 1982; HARTMAN 1985).

Large quantities of animals were grown on casserole dishes supplemented with a chicken egg (HARTMAN *et al.* 1988; HEVELONE and HARTMAN 1988). Animals were washed from the dishes with phosphate-buffered saline (PBS) and allowed to settle for approximately 15 min in a separatory funnel. Animals were then dissolved in alkaline bleach, which yielded a synchronous population of eggs (embryos) aged roughly 30 min to 3 hr postfertilization (HARTMAN and HERMAN 1982; HARTMAN 1984a). Centrifugation and resuspension for three rounds in PBS served to remove the alkaline bleach, with 0 time corresponding to that of the final wash. Animals were plated on seeded NGM plates containing 10 g/liter peptone and 25 g/liter agar (BRENNER 1974) until they were irradiated. N2 and the *rad* mutants were incubated at 20°, whereas the temperature-sensitive mutant was incubated at the nonpermissive temperature of 25° to prevent gonadogenesis. Dauer larvae were isolated on the basis of their resistance to sodium dodecyl sulfate as described previously (HARTMAN 1984b).

Irradiation protocols: Radiation was delivered by a germicidal bulb (G15T8) to animals suspended in phosphate buffered saline. The fluence rate was 0.5 Wm^{-2} as determined by ferrioxalate actinometry (JAGGER 1967). For most experiments, animals were then plated on a series of unseeded NGM plates, incubated in the dark for the appropriate time, washed off the plate, and frozen in liquid nitrogen. In experiments designed to detect photoreactivation, 254-nm irradiated animals were exposed to two black-light bulbs (F15T8 BLB) at a distance of 55 cm for 30 min. This irradiation was at approximately 22°.

DNA isolation: DNA was isolated by first thawing animals and lysing at 60° for 30 min in 100 mM Tris (pH 8), 50 mM EDTA, 200 mM NaCl, 750 μg/ml proteinase K (Sigma Chemical, St. Louis, MO) and 1% sodium dodecyl sulfate. DNA was extracted once with an equal volume of buffer-saturated phenol and dialyzed against two changes of 30 mM Tris, 40 mM NaCl and 1 mM EDTA (pH 8). DNA concentrations were determined by a fluorescence assay (HEVELONE and HARTMAN 1988) and adjusted to a final concentration of 25 μg/ml.

DNA repair assays: Preparation of the antiserum and details of the radioimmunoassays have been published previously (HARTMAN *et al.* 1988; MITCHELL, HAIPEK and CLARKSON 1985a, b). In brief, a radiolabeled ligand, containing sites of photodamage, was competed against sample DNA extracted from UV-irradiated animals for binding to antisera raised against UV-irradiated DNA. The conditions employed have been shown to specifically quantify (6-4) photoproducts and cyclobutane dimers as judged by testing: (1) the refractivity of the binding site to enzymatic photoreactivation; (2) digestion with dimer-specific UV endonuclease; and (3) photolysis by mid-UV light. Each treatment was repeated at least twice and duplicate samples were taken in all cases.

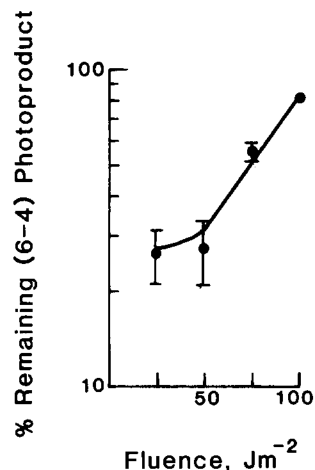

FIGURE 1.—Saturation of (6-4) photoproduct repair capacity. First stage larvae were irradiated with various fluences and allowed to repair for 24 hr.

RESULTS

A brief review of the developmental cycle of *C. elegans* may be helpful to the understanding of the experiments presented below (WOOD 1988). The life cycle of *C. elegans* is about 3.5 days in length at 20°. Roughly 30 min after fertilization, which occurs internally, a protective eggshell is deposited. Embryogenesis is divided into proliferative and morphogenesis phases, each lasting approximately 6 hr. Animals hatch with precisely 558 cells. Four well-defined molts punctuate the four larval stages L1 through L4, during which the cell number increases to exactly 810 in adult hermaphrodites. These are generated by largely invariant cell lineages which have been completely characterized (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON *et al.* 1983). Under harsh environmental conditions, such as starvation or overcrowding, animals molt to become dauer larvae rather than L3s (CASSADA and RUSSELL 1975). This senescent stage may survive for months with little effect on postdauer life span (KLASS and HIRSH 1976).

Repair in wild type (N2): One of us has developed a sensitive radioimmunoassay which is capable of quantitating both (6-4) photoproducts and cyclobutane dimers in sample DNA (MITCHELL, HAIPEK and CLARKSON 1985a,b). We have previously applied the assay to *C. elegans* to study the relationship between DNA repair and aging (HARTMAN *et al.* 1988).

To determine if excision repair could be saturated, 24-hr-old animals were irradiated with different fluences and either frozen immediately or incubated in the dark for 24 hr. In these experiments, animals were able to remove over 70% of (6-4) photoproducts at fluences up to 50 Jm^{-1}; however, a progressively smaller fraction was removed at higher fluences (Figure 1). Similar results were obtained when DNAs were

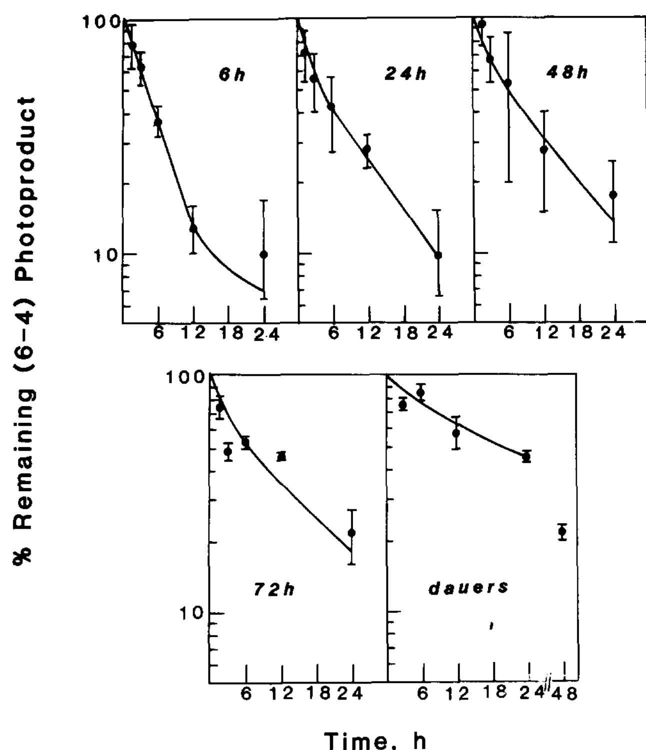

FIGURE 2.—Kinetics of repair of (6-4) photoproducts in animals of different developmental stages; 6, 24, 48, and 72 hr refer to the times elapsed after populations were synchronized and correspond to embryos, L1s, L4s and adults.

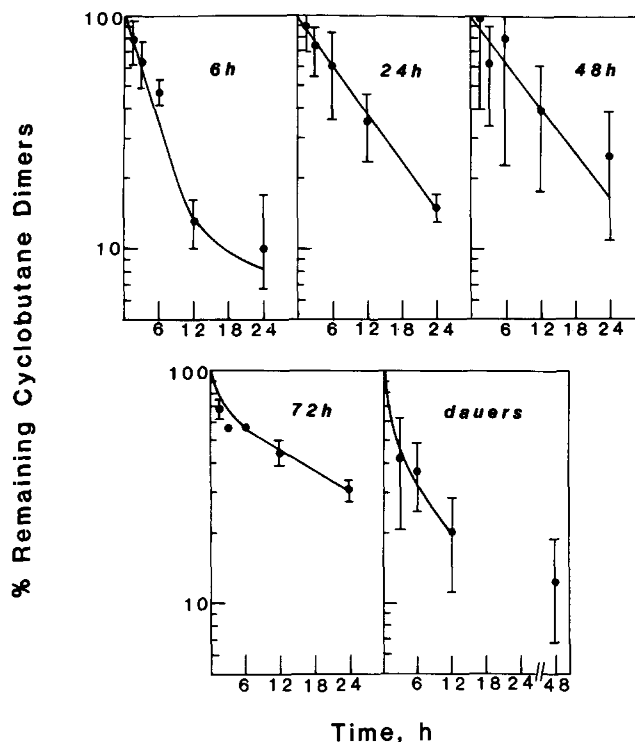

FIGURE 3.—Kinetics of repair of cyclobutane dimers in animals of different developmental stages; 6, 24, 48, and 72 hr refer to the times elapsed after populations were synchronized and correspond to embryos, L1s, L4s and adults.

assayed for cyclobutane dimers. Thus, excision repair in *C. elegans* is saturated at fluences greater than 50 Jm^{-2}. This correlates well with the inactivation kinetics obtained using this staged population (HARTMAN 1984a). In these inactivation profiles, where survival was measured as the ability of animals to develop into adults, there was little killing at fluences of 50 Jm^{-2} and smaller. Survival then decreased in a fluence-dependent fashion, with approximately 75% and 30% survival at 75 Jm^{-2} and 100 Jm^{-2}, respectively.

The kinetics of repair were examined in staged populations by synchronizing at embryogenesis and allowing development to the desired stage. Animals were exposed to 50 Jm^{-2}, incubated in the dark, and assayed for the presence of (6-4) photoproducts (Figure 2) and cyclobutane dimers (Figure 3). In these experiments, 6, 24, 48, and 72 hr after synchronization correspond to populations of embryos, L1s, L4s and adults, respectively. While the induction ratio of (6-4) photoproducts *vs.* cyclobutane dimers did not change, the total number of induced photoproducts declined throughout development (data not shown). This reduction, which totalled approximately 30% when comparing 6 *vs.* 72-hr animals, can be attributed to the increased shielding resulting from cellular growth during development.

Although survival was not quantitated in these experiments, previous data indicated 50 Jm^{-2} to be a biologically relevant fluence (HARTMAN 1984a). Radiation sensitivity changes dramatically in *C. elegans* throughout development such that young embryos are much more radiation sensitive than older stages (HARTMAN 1984a). These published data indicate survivals of between 75% and 95% after 50 Jm^{-2}, the fluence employed in Figures 2 and 3.

The following conclusions appear warranted by these data. First, both photoproducts were removed at roughly equal rates. The only exception occurred in dauer larvae, where (6-4) photoproducts were initially excised more slowly than cyclobutane dimers. For example, almost 70% of cyclobutane dimers whereas less than 30% of (6-4) photoproducts were excised at 12 hr after irradiation in dauers. Second, the initial excision rate for cyclobutane dimers was quite similar in all five developmental stages tested. Four of the developmental stages excised (6-4) photoproducts at the same initial rate, with only dauer larvae retarded in this capacity. Third, the number of both photoproducts retained after 24 hr increased with progression through development. For example, the percentages of cyclobutane dimers remaining after 24 hr were approximately 10% for embryos, 15% for L1s, 20% for L4s and 30% for adults. However, even "middle-aged" adults (72-hr-old) retained considerable DNA repair capacity.

These developmental stages were selected such that scheduled DNA synthesis was minimal; *e.g.*, 6-hr-old

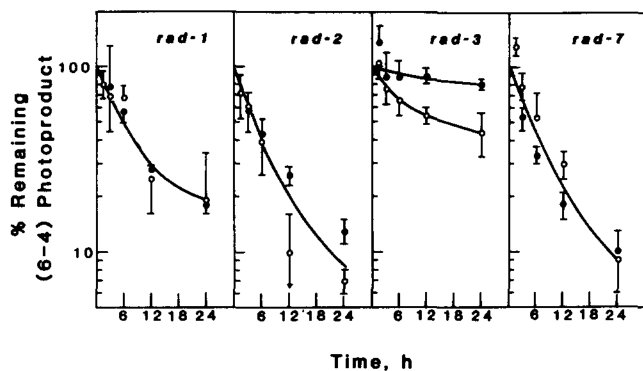

FIGURE 4.—Kinetics of repair of (6-4) photoproducts in four *rad* mutants. Staged populations were irradiated as either embryos (O) or first-stage larvae (●) and assayed at the indicated times.

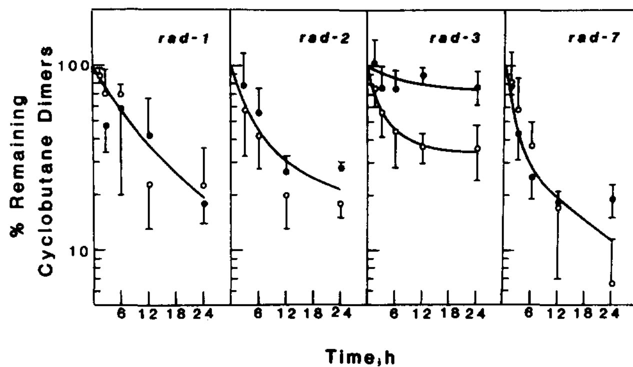

FIGURE 5.—Kinetics of repair of cyclobutane dimers in four *rad* mutants. Staged populations were irradiated as either embryos (O) or first-stage larvae (●) and assayed at the indicated times.

animals were embryos in the morphogenesis phase of embryogenesis, after completion of all embryogenic cell divisions. Had this precaution not been taken, newly synthesized DNA would have "diluted" the irradiated DNA, giving the false impression of removal of immunoreactive material. For the same reason, a strain bearing the *ts151* mutation was employed instead of N2 for measuring repair in 72-hr-old animals. These animals were reared at 15° instead of 20° to generate large populations. Synchronized populations were then shifted to 25°. The *ts151* mutation eliminated gonad formation at the non-permissive temperature. Thus, the 72-hr-old temperature-sensitive mutants underwent absolutely no scheduled DNA synthesis versus the extensive germ line synthesis characteristic of N2 during adulthood (HIRSH and VANDERSLICE 1976). Control experiments with this strain showed its repair kinetics were experimentally identical to N2 if assayed at 24 hr at 20°.

The ability to photoreactivate UV-radiation-induced DNA damage was assayed using the radioimmunoassay by exposing L1s to 50 Jm^{-2} germicidal light and either incubating in the dark for 30 min or exposing to photoreactivating light for the same time. Since photoreactivation is an enzymatic process, animals were maintained at approximately 22°. There were 97 ± 7.3% (6-4) photoproducts and 96 ± 9.9% cyclobutane dimers remaining in the DNA after photoreactivating treatments. Similar levels remained when animals were incubated in the dark. The absence of photoreactivation is consistent with previously reported data (KELLER *et al.* 1987).

Repair in the *rad* mutants: The radioimmunoassays were also employed to determine the excision repair capacities of four *rad* mutants. Since the radiation hypersensitivities of these mutants vary dramatically throughout development (HARTMAN 1984a,b), repair was measured in embryos (irradiated 6 hr after synchronization) and first-stage larvae (irradiated 24 hr after synchronization). With respect to (6-4) photoproduct removal, both embryos and L1s in *rad-1*, *rad-2* and *rad-7* genetic backgrounds displayed excision repair capacities (Figure 4) roughly equal to that of N2 (Figure 2). Conversely, while *rad-3* embryos were able to excise over 50% of all (6-4) photoproducts in 24 hr, *rad-3* larvae excised less than 25% of the same lesion in an equivalent time period (Figure 4). Similar kinetics were observed for cyclobutane dimer removal (Figure 5). Again, at both developmental stages tested, three of the mutants (*rad-1*, *rad-2* and *rad-7*) were normal or near normal in their repair capacities. In addition, the developmental variation in the repair capacity of *rad-3* animals was evident. In this case, embryos excised over 60% of the damage while first stage larvae removed only 20%.

DISCUSSION

The kinetics of cyclobutane dimer and (6-4) photoproduct removal were determined in several staged populations of the nematode *C. elegans* using a radioimmunoassay. These data can be compared with the repair kinetics of other organisms. Prokaryotes, at least *E. coli* (FRANKLIN and HASELTINE 1984) and *Micrococcus radiodurans* (VARGHESE and DAY 1970), excise both photoproducts very rapidly, within 2 hr, and at the same rate. Lower eukaryotes such as Saccharomyces (PRAKASH 1975; REYNOLDS and FRIEDBERG 1981) and Neurospora (MACLEOD and STADLER 1986) also act on cyclobutane dimers quickly such that repair is completed within several hours after induction. In contrast, established mammalian cell lines display slower repair kinetics (MITCHELL, HAIPEK and CLARKSON 1985a, b). Moreover, in mammalian cells, the two lesions are repaired at markedly different rates. For example, in both human and hamster cells, removal of (6-4) photoproducts is very fast, with 75% completed within 4-hr postirradiation. In contrast, cyclobutane dimers are removed much more slowly, resulting in a 70% loss from human cells and 50% loss from hamster cells only after 17 hr of postirradiation incubation. The repair of UV-induced photoproducts in *C. elegans* resembles that in mammalian cells, except

with reduced (6-4) photoproduct repair. The observed difference in the repair of these two lesions in mammalian cells suggests that either different enzymes are involved in the initial stages of excision repair or that the enzyme(s) responsible have a higher affinity for the (6-4) photoproduct. Such a system does not appear to be operative in *C. elegans*.

The kinetics of cyclobutane dimer removal were corroborated in wild-type first-stage larvae (data not shown). In these experiments, DNA was extracted and digested with a cyclobutane dimer-specific endonuclease from *Micrococcus luteus*. Samples were then sized on alkaline agarose gels. The repair kinetics were consistent with the radioimmunoassay data. In addition, we examined for repair synthesis by pulsing staged populations of *C. elegans* with tritiated thymidine after graded fluences of UV radiation. Although care was taken to select developmental stages when scheduled DNA synthesis was minimal, unscheduled DNA synthesis (UDS), reflecting repair synthesis, was not detected. In addition, attempts to reproduce the observation of UDS in the nematode (*Turbatrix aceti* (TARGOVNIK, LOCKER and HARIHARAN 1984; TARGOVNIK *et al.* 1984) were unsuccessful.

The repair of specific lesions in dauer larvae is particularly intriguing from a mechanistic point of view. In these estivating animals, metabolic functions have been reduced to basal levels, yet cyclobutane dimer repair as measured by the radioimmunoassay is similar to the rate observed in embryos. On the contrary, (6-4) photoproduct repair is greatly reduced. The selective loss of this repair capacity suggests that the enzymes involved in the repair of this lesion are different from those involved in dimer repair. Alternatively, a common repair complex must have a lower affinity for (6-4) photoproducts. The preferential repair of cyclobutane dimers is opposite that of mammalian cells in which more efficient (6-4) photoproduct repair is observed (MITCHELL, HAIPEK and CLARKSON 1985a,b). Using the ability to develop into an adult as the criterion of survival, dauer larvae are as resistant to UV irradiation as animals in other developmental stages (HARTMAN 1984b). Thus, (6-4) photoproducts must be relatively nontoxic in this nonreplicating system.

The RIA was also applied to demonstrate that photoreactivation is not operative in this organism. The latter conclusion is consistent with the finding that exposure to photoreactivating light did not modulate UV-radiation killing (KELLER *et al.* 1987). In addition, nematode extracts could not photoreactivate UV-irradiated transforming DNA prepared from *Haemophilus influenzae* (KELLER *et al.* 1987). Thus, three independent lines of evidence indicate photoreactivation to be absent in *C. elegans*. Of the eukaryotic systems analyzed for cyclobutane dimer and (6-4) photoproduct removal, including human, rodent and frog cells, *C. elegans* is the only one which does not have a demonstrated or putative photoreactivation mechanism. Recently, it has been shown that a repair-deficient human cell line (XP-A) transformed with the yeast *phr* gene has an enhanced ability to repair (6-4) photoproducts in the dark (D. L. MITCHELL, unpublished data). Hence, the absence of both preferential (6-4) photoproduct repair and photoreactivation in *C. elegans* may not be coincidental.

Although no significant differences in UV sensitivity are evident in wild-type *C. elegans* as it progresses through its life cycle (HARTMAN 1984a,b), the capacity to repair cyclobutane dimers and (6-4) photoproducts decreased. Whereas the initial rate of repair in embryonic and adult animals was similar up to six hr, approximately twofold more lesions remained in the DNA of the UV-irradiated adults after 24 hr. These data indicate that significant developmental variation in the ability to repair specific photoproducts is tolerated by these organisms.

A striking phenotype of the *rad* mutants has been their stage-specific variations in hypersensitivity (HARTMAN 1984a,b). For example, *rad-1* animals were extremely UV-radiation hypersensitive if irradiated as embryos, but they displayed wild-type resistance when irradiated as first-stage larvae. Conversely, *rad-3* animals were moderately hypersensitive when irradiated as embryos, but L1s were highly hypersensitive. In fact, of the four *rad* mutants so characterized, only *rad-1* and *rad-7* displayed a similar developmental pattern of hypersensitivity. It is possible that these developmental differences reflect the animal's stage-specific dependence upon different DNA repair pathways. For example, rapidly proliferating embryonic cells might be more dependent upon postreplication repair than larval cells, which are mostly postmitotic. The extreme embryonic hypersensitivity coupled with wild-type larval resistance of *rad-1* could, therefore, be explained by supposing that the Rad-1 gene product participates in postreplication repair. In a similar fashion, a greater dependence upon excision repair in larvae versus embryos could account for the *rad-3* pattern of hypersensitivity, since *rad-3* animals are excision-repair defective.

An alternative explanation for this stage-dependent variation is that DNA repair is developmentally regulated in the nematode. Temporal variation of the Rad gene expression could easily account for the different patterns of hypersensitivity observed with the *rad* mutants. Direct support for this hypothesis comes from the observation that the excision repair capacity of *rad-3* animals varies substantially throughout development; specifically, the excision repair defect in the mutant is much more pronounced in larvae than in embryos (Figures 4 and 5). The fact that larvae

are much more hypersensitive than embryos to the lethal effects of UV radiation (38 *vs.* 4.6 times more sensitive, respectively; HARTMAN 1984a) indicates the biological relevancy of this developmental regulation.

Epistatic interactions, determined by comparing the relative UV and γ-radiation sensitivities of various single and double mutants, indicate that *C. elegans* possesses at least two dark DNA repair pathways, one controlled by *rad-1* and *rad-2* and the other under the control of *rad-3* and *rad-7* (HARTMAN 1985). These assignments are consistent with the observation that *rad-1* and *rad-2* mutants are hypersensitive to both UV and ionizing radiation, whereas *rad-3* and *rad-7* mutants are hypersensitive only to UV radiation (HARTMAN and HERMAN 1982). It was anticipated that the RIA data would illuminate the molecular nature of these two epistasis groups. To a certain extent this has been true. For example, the Rad-3/Rad-7 epistasis group likely controls excision repair, since *rad-3* mutants were so defective (Figures 4 and 5). However, this carries the strong prediction that *rad-7* embryos should also be excision-repair defective. They were not (Figures 4 and 5). While this discrepancy can be explained by supposing that the Rad-7 gene product controls a step in excision repair subsequent to lesion removal, such as resynthesis or ligation, other inconsistencies remain with the epistasis groupings as described above. First, the model does not explain the developmental fluctuations in hypersensitivity patterns. If *rad-7* and *rad-3* control excision repair, they would be expected to show the same hypersensitivity patterns throughout development. They do not, as *rad-3* hypersensitivity increases throughout development whereas *rad-7* hypersensitivity is completely lost by the first larval stage. Second, UV radiation-induced mutation frequencies argue against combining *rad-3* and *rad-7* into a single functional group. COOHILL and coworkers (1989) have recently employed the *eT1*-balanced tester system developed by BAILLIE and coworkers (ROSENBLUTH, CUDDEFORD and BAILLIE 1983) to determine that *rad-3* was hypermutable when UV irradiated. Excision-repair defective mutants in bacteria (WITKIN 1976) and yeast (LAWRENCE 1982) show similar hypermutability, presumably due to their greater dependence upon other, error-prone repair processes. Conversely, a mutation in the *rad-7* gene conferred UV-radiation hypomutability, suggesting that this gene controls an error-prone DNA repair system (COOHILL *et al.* 1989). Thus, a variety of data argue that *rad-7* and *rad-3* participate in different DNA repair systems, despite their epistatic groupings. There are examples in both yeast (ECKARDT-SCHUPP, SIEDE and GAME 1987) and Drosophila (P. D. SMITH, personal communication) where genes cannot be neatly assigned into a single epistasis group. It is hoped that future studies will illuminate the nature of the two epistasis groups.

Survival in this organism has been measured as the ability to attain adulthood (HARTMAN and HERMAN 1982; HARTMAN, 1984a,b, 1985). Since postembryonic cell divisions are not absolutely necessary for development into an adult [see HARTMAN (1984a,b) for a more complete discussion], survival of *C. elegans*, at the developmental stages examined in this study, is not DNA-replication dependent. This is as opposed to mammalian cell data, in which the inhibition of the initiation and progression of DNA replication is primarily responsible for death, as measured by reductions in colony-forming ability. Thus, in *C. elegans*, other mechanisms besides those that inhibit DNA synthesis are determinative for survival, in particular those involved with the modulation of gene regulation or transcription. In this regard, it may be particularly noteworthy that dimer and nondimer photoproducts can inhibit the transcription of an exogenous gene transfected into human cells (PROTIC-SABLJIC and KRAEMER 1987).

It is important to note that these experiments with *C. elegans* measure the effects of radiation on intact organisms, composed of cells with a variety of developmental fates. Conversely, most DNA repair studies involving metazoa employ disassociated cells, usually immortalized, in tissue culture. It is now quite apparent that the developmental status of a cell (*e.g.*, DEHAZYZ and SIROVER 1986) as well as the transcriptional state of a given gene (*e.g.*, BOHR *et al.* 1985) may profoundly affect DNA repair capacity. The complex developmental behavior of radiation-sensitive mutants in *C. elegans*, coupled with the organism's invariant mode of cellular development, suggest that further studies with this nematode may provide unique insights into factors which regulate DNA repair in a developmental context.

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