Isolation and Characterization of a Photorepair-Deficient Mutant in *Drosophila melanogaster*

James B. Boyd and Paul V. Harris

Department of Genetics, University of California, Davis, California 95616

Manuscript received October 13, 1986
Revised copy accepted March 5, 1987

ABSTRACT

A mutation abolishing photorepair has been localized to map position 56.8 centimorgans on the second chromosome of *Drosophila melanogaster*. Strains homozygous for the *phr* allele are totally devoid of photorepair and partially deficient in excision repair. Both defects map to the chromosomal region between *pr* and *c*. Since a homozygous *phr* stock exhibits reduced photoreactivation, the corresponding wild-type allele plays a significant role in UV resistance.

ONE of the prominent lesions introduced into DNA by sunlight is the *cis-syn* cyclobutyl pyrimidine dimer (KITTLER and LÖBER 1977; ELLISON and CHILDS 1981). Accordingly, most if not all organisms possess an enzymatic photoreactivation mechanism (FRIEDBERG 1985) which specifically repairs this unique class of damage (SETLOW and SETLOW 1972). These enzymes, termed photolyases, bind tightly to DNA containing pyrimidine dimers and monomerize the fused bases with energy provided by light having a wavelength greater than 300 nm (SUTHERLAND, 1981). Since photolyases provide only one of several cellular defenses against pyrimidine dimers, their precise role and importance in cellular resistance is important to assess. In the absence of specific inhibitors to abolish their function, a mutational analysis is of particular value in pursuing this question. The best characterized mutations affecting this process have thus far been isolated in *Escherichia coli* (HARM and HILLEBRANDT 1962), yeast (RESNICK 1969) and Chlamydomonas (COX and SMALL 1985). Of particular relevance to the Drosophila study is the recent observation that the *phr* gene in *E. coli* is not only required for photorepair but influences excision repair as well (YAMAMOTO, SATAKE and SHINAGAWA 1984; HAYS, MARTIN and BHATIA 1985; SANCAR, FRANKLIN and SANCAR 1984). A possible interaction between these two repair mechanisms has also been suggested by studies of the human disorder xeroderma pigmentosum (SUTHERLAND, RICE and WAGNER 1975). In this report we describe the isolation and characterization of a photorepair defective mutant in Drosophila which further implicates the photorepair system in the mechanism of excision repair. The existence of this mutant in Drosophila should facilitate a combined genetic and molecular analysis of photorepair in this higher eukaryote. An abstract describing this study has appeared (BOYD and HARRIS, 1985).

Abbreviations: BSS, balanced saline solution; ESS, endonuclease sensitive sites; OTC, organ tissue culture.

MATERIALS AND METHODS

Drosophila stocks and culture: The mutant allele identified in this study is designated *phr*. Additional mutations and chromosomes are described by LINDSLEY and GRELL (1968). These include:

- bw^{V1}—brown—Variegated (dominant second chromosome marker)
- *D*—Dichaete (dominant third chromosome marker)
- *Cy0* (Second chromosome balancer)
- *TM3* (Third chromosome balancer)
- *C(1)DX, y f* (each attached *X* chromosome carries both a yellow and a forked mutation)
- *al dp b pr c px sp* (multiply marked second chromosome)

Culture conditions and mutagen treatment are described in BOYD *et al.* (1981). The symbol ♀ in the figures designates virgin females.

Radiation source: Far UV irradiation was supplied by a mercury vapor lamp as described by HARRIS and BOYD (1980). Near UV irradiation [produced by an FS40 sunlamp (Westinghouse)] was filtered through a plastic petri dish lid (0.4% transmission at 285 nm) to transmit predominantly wavelengths of 295–400 nm.

Analysis of photorepair: Three tissue sources have been employed to estimate the photorepair capacity of a mutant stock. Primary tissue cultures were used to obtain quantitative data (BOYD and SETLOW 1976; BOYD and HARRIS 1981). In order to establish the presence or absence of a phr^+ allele in a stock, larval brain ganglia (BOYD and SHAW 1982) or adult ovaries were subjected to the same protocol employed for detecting pyrimidine dimers in primary cultures. Organs were dissected in BSS (CHAN and GEHRING 1971) and transferred to 0.25 ml of OTC medium (NARACHI and BOYD 1985) containing 2 μCi of [methyl-^{3}H]thymidine (Amersham Corporation). Following overnight incubation at 25°, the radioactive medium was replaced with BSS lacking bovine serum albumin and incubation was continued for at least 1 hr. The organs were irradiated for 2–3 min with near-UV radiation. As measured by the procedure described below, this dose produces approximately 4–6.5 UV-endonuclease-sensitive sites per 10^8 dal of DNA. Organs

receiving photoreactivating light were exposed to fluorescent light for 40–60 min (BROWN *et al.* 1981). DNA was isolated from organs and cell cultures as previously described (HARRIS and BOYD 1980). Photorepair is then monitored by an enzymatic assay for pyrimidine dimers. Isolated DNA is exposed to an endonuclease from *Micrococcus luteus* which hydrolyzes single strands at pyrimidine dimers. Alkaline sucrose gradients are then employed to monitor DNA molecular weights and thereby measure the density of DNA nicks which in turn reflect the frequency of persistent pyrimidine lesions. This approach is employed to monitor photorepair by determining the influence of visible light (BROWN, HARRIS and BOYD 1981) on the disappearance of pyrimidine dimers from DNA.

Analysis of excision repair: Removal of pyrimidine dimers in the dark was monitored as described for photorepair with two exceptions: (1) cells were handled briefly under yellow lights to avoid photorepair and then incubated in the dark, and (2) residual damage was determined after longer incubation periods.

Incision capacity was analyzed by monitoring the accumulation of single-strand DNA breaks following UV exposure. UV irradiation was provided by exposure to an FS40 sunlamp for 30 sec. Interruptions in single strands were detected by a modification of the alkaline elution procedure of Kohn (HARRIS and BOYD 1980). In the presence of inhibitors of DNA synthesis this assay monitors the first step of excision repair in Drosophila cells. The variation between experiments observed with a particular strain (Table 2) arises from several factors such as culture density, culture age, and tissue culture medium (J. B. BOYD and P. V. HARRIS, unpublished observations). These factors have been controlled within a single experiment as much as possible to allow for accurate relative comparisons between strains.

***In vivo* photoreactivation:** The biological effect of the *phr* mutation was monitored as survival of irradiated larvae. Third instar larvae were isolated by floating them in 8% NaCl. They were rinsed in water and weighed damp to estimate their numbers. UV irradiation was performed as described by HARRIS and BOYD (1980) under a quartz plate which had transmission values of 87% at 254 nm, 92% at 295 nm and 93% at 313 nm. The UV dose stated in the text has been corrected for the transmission loss at 254 nm. Visual illumination (BROWN, HARRIS and BOYD 1981) was filtered through an additional soft glass plate with 0.3% transmission at 313 nm to avoid extensive reintroduction of pyrimidine dimers by short wavelengths. Larvae were subsequently cultured at 25° in the dark on standard medium supplemented with yeast paste. Cultures were analyzed on days 11 through 15 or 17.

RESULTS

Among several approaches which have been employed to assay photorepair in Drosophila (BOYD *et al.* 1980), we have selected one that utilizes an endonuclease activity from *M. luteus* to detect UV-induced pyrimidine dimers (BOYD, GOLINO and SHAW 1976; HARRIS and BOYD 1980). In that assay isolated DNA is incubated with the enzyme and its single-stranded molecular weight is then determined by alkaline sucrose gradient centrifugation. The presence of pyrimidine dimers in DNA is detected as a reduction in molecular weight after enzyme exposure. In addition to analyzing primary cell cultures with this technique

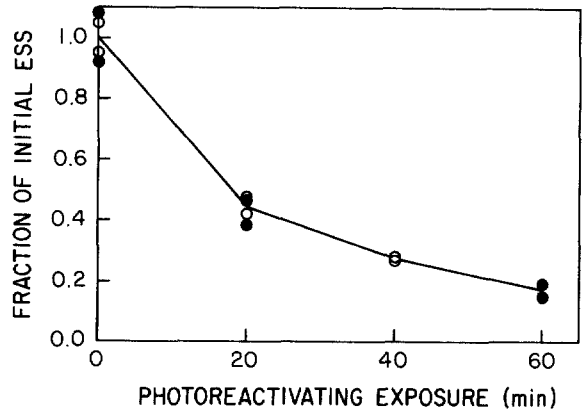

FIGURE 1.—Photorepair of pyrimidine dimers in primary cultures. Cell cultures derived from a control stock (*w*) were irradiated with either far-UV (predominantly 254 nm) or with a filtered FS40 sunlamp (295–400 nm) at doses that yielded approximately 6.1 endonuclease-sensitive sites per 10^8 daltons. Cultures were then exposed to photoreactivating light for the times indicated. DNA was isolated and analyzed for endonuclease-sensitive sites (ESS). Sunlamp, O; far UV, ●.

(HARRIS and BOYD 1980), we have also employed adult ovaries or larval brain ganglia in a more qualitative assay.

In this study we have employed a near UV source which largely overcomes the problem of poor penetration of far UV irradiation in organs and cell clumps, which are frequently found in primary cell cultures. This source produces more uniform damage within an experiment and more consistent yields of pyrimidine dimers between experiments (J. B. BOYD and P. V. HARRIS, unpublished observations). Since the proportion of pyrimidine dimers relative to other types of DNA photo-damage is markedly different after near- and far-UV exposure (RAHN 1979), it was necessary to demonstrate that the two UV sources give comparable results when used to examine photorepair. Figure 1 shows photorepair kinetics in control cultures given either far- or near-UV radiation at doses which yield similar densities of sites susceptible to nicking by the UV-endonuclease. Sites introduced by either UV source are presumably pyrimidine dimers since they are largely removed by exposure to visible light. These data demonstrate that the increased amount of non-dimer photodamage produced by longer wavelength radiation does not significantly alter the photorepair of pyrimidine dimers. The near-UV source was therefore used throughout this study.

Identification and localization of the *phr* gene: The mutant *phr* allele was discovered in an unrelated study during attempts to employ photorepair as an analytical tool. Selected stocks carrying third-chromosomal mutagen-sensitive (*mus*) mutations (BOYD *et al.* 1983) were found to be totally or partially devoid of photorepair. Since only selected alleles of a given *mus* locus appeared to exhibit this phenotype, an explanation was sought in the common genetic back-

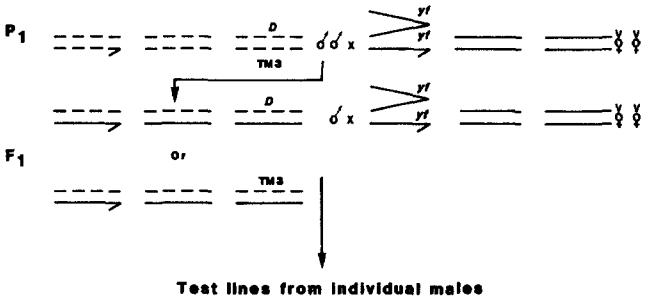

FIGURE 2.—Isolation and testing of individual *X* chromosomes. Individual lines carrying single *X* chromosomes from the photorepair deficient *D/TM3* stock were tested for photorepair with the brain-ganglia assay described in MATERIALS AND METHODS. Dotted lines designate major chromosomes derived from the repair deficient stock. The attached *X* chromosomes carry the mutations forked and yellow.

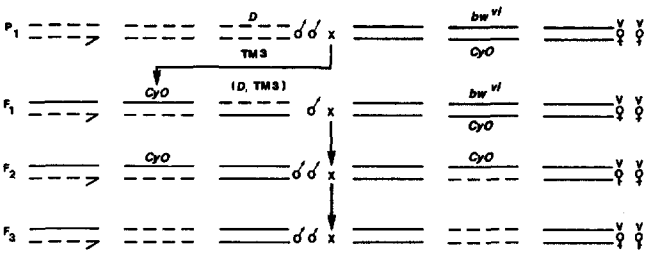

FIGURE 3.—Isolation and testing of individual second chromosomes. Single F_1 males were selected to produce separate lines homozygous for individual second chromosomes derived from the photorepair deficient *D/TM3* stock. Each line was tested for photorepair with the brain-ganglia assay described in MATERIALS AND METHODS.

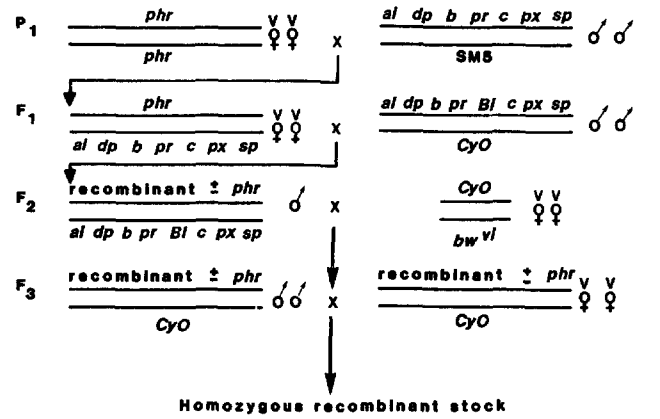

FIGURE 4.—Mapping procedure. The indicated crosses were employed to generate stocks homozygous for unique recombinant chromosomes. Recombinant second chromosomes generated in F_1 females were identified in individual F_2 males and tested for photorepair after expansion in separate stocks.

ground of the stocks. In generating those stocks ⅔ of the mutagenized *X* chromosomes and all of the mutagenized second chromosomes had been replaced with chromosomes from the balanced *D/TM3* stock (BOYD *et al.* 1981). When that balanced stock was tested for photorepair, it was also found to be strongly deficient (J. B. BOYD and P. V. HARRIS, unpublished observations), although it had previously been selected as being relatively insensitive to chemical mutagens (BOYD *et al.* 1981). That stock was therefore employed to analyze the genetic basis of the photorepair deficiency.

The *X* chromosomes of the *D/TM3* stock were tested by crossing males of that stock to repair-proficient attached-*X* females as indicated in Figure 2. Individual F_1 males were crossed with virgin attached-*X* females to establish four separate stocks in which the males of each stock carried a unique *X* chromosome derived from the *D/TM3* strain. Brain ganglia of three to four male larvae from each stock were tested for photorepair. Since all samples exhibited significant repair (J. B. BOYD and P. V. HARRIS, unpublished observations), the photorepair deficiency is autosomal recessive.

To test for linkage to the second chromosome, stocks were generated as outlined in Figure 3. Each stock carried a single second chromosome from the *D/TM3* stock and *X* and third chromosomes from the *CyO/bw*^{V1} stock. In independent tests the latter stock had been shown to be photorepair proficient. Four stocks carrying different second chromosomes from the *D/TM3* stock were each found to be photorepair deficient in an analysis of adult ovaries. This result excludes linkage to the *X* and third chromosomes. Fourth chromosomal linkage is also eliminated because the trait is recessive. One of the tested stocks was therefore selected for second chromosomal mapping studies.

Mapping was performed using the crosses depicted in Figure 4. In this scheme the F_1 females, which are heterozygous for the photorepair defect and a multiply marked second chromosome, produce recombinants that are identified phenotypically in F_2 males. One hundred and twelve different recombinant chromosomes were initially isolated over a balancer, and homozygous stocks were generated for each chromosome to be tested. An analysis of one recombinant from each of the six detectable intervals (Table 1) initially localized the photorepair trait to the *pr-c* interval. Continued analysis of the 18 recombinants recovered within that interval established a map position of 56.8 with a 95.8% confidence interval from 54.5 to 60.3 (based upon binomial probability). This result indicates that the photorepair defect is due to a single allele which has been termed *phr*.

The *mus205*^{A1} mutation maps near the *phr* locus in the *pr-c* interval (P. D. Smith, personal communication) and homozygous stocks are also deficient in photorepair (J. B. BOYD and P. V. HARRIS, unpublished observations). However, the *mus205*^{A1} allele is associated with a defect in postreplication repair (BOYD and SHAW 1982) whereas the *phr* stock is not (J. B. BOYD and P. V. HARRIS, unpublished observation). In addition, the parental *cn bw*-bearing chromosome upon which *mus205*^{A1} was induced confers

TABLE 1

Genetic mapping of *phr* on the second chromosome

A. Location of visible markers

Marker	*al*	*dp*	*b*	*pr*	*c*	*px*	*sp*
Map position	0.01	13.0	48.5	54.5	75.5	100.5	107.0

B. Photorepair capacity carried by recombinant chromosomes

Map interval tested	Second chromosomal genotype	No. of stocks tested	Photorepair capacity
	al dp b pr c px sp	1	+
	+ ++ ++ + +	1	−
al-dp	*al* ++ ++ + +	1	−
dp-b	*al dp*+ ++ + +	1	−
b-pr	*al dp b* ++ + +	2	−
pr-c	*al dp b pr*+ + +	1	−
pr-c	*al dp b pr*+ + +	4	+
pr-c	+ ++ + *c px sp*	1	+
pr-c	+ ++ + *c px sp*	12	−
c-px	+ ++ ++ *px sp*	2	−
py-sp	+ ++ ++ + *sp*	1	−

Individual stocks carrying unique recombinant chromosomes were generated as depicted in Figure 4. Larval brain ganglia were assayed for the disappearance of endonuclease-sensitive sites following exposure to visible light.

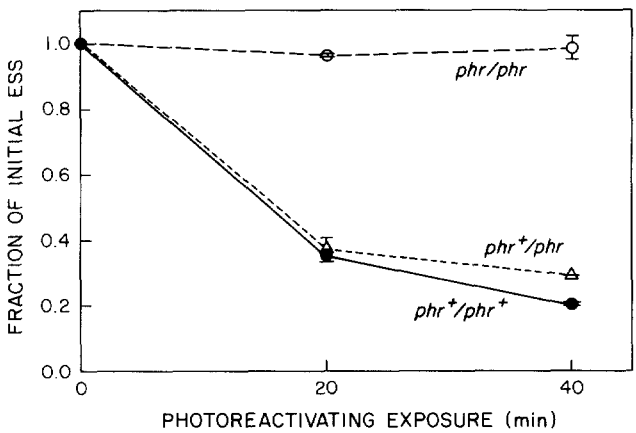

FIGURE 5.—Influence of mutant gene dosage on photorepair. Primary cell cultures of embryos carrying 0, 1, or 2 copies of the wild-type phr^+ allele were exposed to long wavelength UV irradiation for 2 min. The cultures were subsequently exposed to fluorescent illumination for the indicated times. The remaining pyrimidine dimers were assayed (HARRIS and BOYD 1980). All data have been corrected for simultaneous excision repair which was assayed in parallel cultures incubated in the dark. Each value is the mean of duplicate determinations with error bars representing one standard error.

photorepair deficiency. We therefore conclude that the $mus205^{A1}$ allele was induced on a chromosome already bearing a *phr* allele and that the two are not allelic.

Repair capacity of the *phr* mutant: A kinetic analysis of photorepair in strains carrying various doses of the *phr* allele is presented in Figure 5. In the wild-type strain a 40-min exposure to fluorescent light results in a loss of nearly 80% of the UV-induced pyrimidine dimers. Under identical conditions the

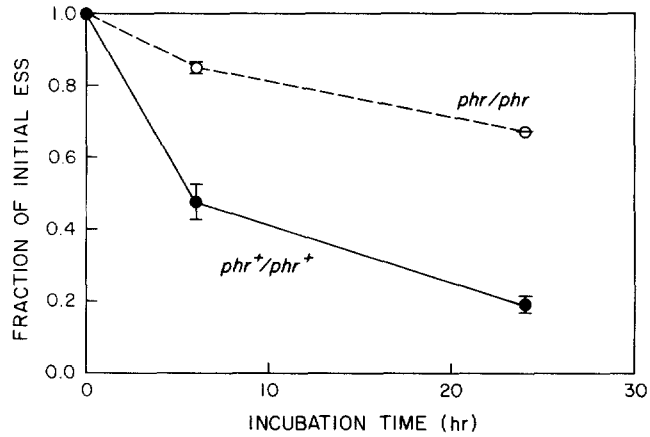

FIGURE 6.—Excision of pyrimidine dimers by wild-type and photorepair deficient strains. Primary cell cultures were analyzed as described in Figure 5 with the exception that all cultures were incubated in the dark for the indicated times. The control stock, which is photorepair proficient, carries the marker *w*. The error bars represent one standard error for duplicate determinations.

homozygous mutant strain is incapable of light-induced reversal of pyrimidine dimers. Since cells heterozygous at the *phr* locus exhibit a nearly complete repair capacity, the mutant allele is recessive under these assay conditions. This result implies that the UV dose employed is not sufficient to saturate the repair capacity of the wild type strain.

The capacity of the homozygous *phr* stock to perform excision repair has been monitored in two ways. Initially the loss of pyrimidine dimers was monitored after extended incubation in the dark. The data presented in Figure 6 reveal that the photorepair deficient mutant exhibits a strong reduction in excision repair under these conditions. The alkaline elution assay was then applied to determine if that block occurs in the incision step of excision repair. In that assay the accumulation of single-strand DNA breaks appearing after UV exposure is determined by measuring the rate with which cellular DNA is eluted from a filter. Such breaks accumulate because the post-incision steps of excision repair are blocked by DNA synthesis inhibitors (COLLINS and JOHNSON 1984). The data in Figure 7 reveal that at low UV doses the homozygous *phr* stock exhibits a low incision capacity as revealed by a reduced accumulation of single-strand interruptions. In order to determine if this deficiency in dark repair is also associated with the *phr* locus, it has been mapped using selected recombinant chromosomes described in Table 1. Four homozygous recombinant strains of genotype *al dp b pr* were assayed for incision with the alkaline elution assay (Table 2). Three of these recombinants were photorepair proficient and one was deficient. In each case we observed a coincidence between the photorepair and incision capabilities of these strains in that the rate of incision of the phr^+ strains varied between 2.3 and 3.3, whereas those of the *phr* strains were

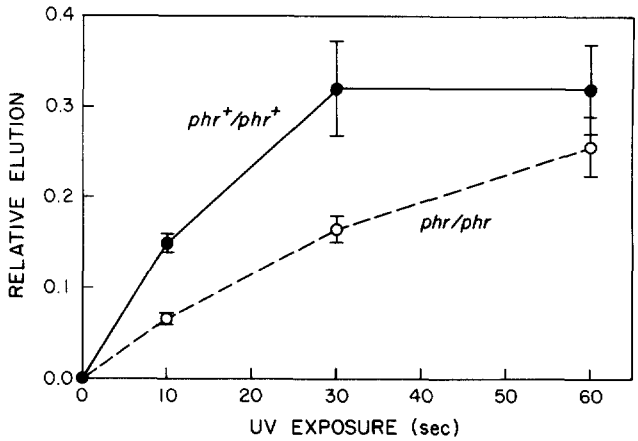

FIGURE 7.—Accumulation of single-strand DNA breaks in primary cultures of mutant and control cells after UV exposure. Following irradiation, the cultures were exposed to DNA synthesis inhibitors for 15 min and the DNA was analyzed by alkaline elution as described in MATERIALS AND METHODS. Relative elution values are proportional to the density of DNA single-strand interruptions. Error bars reflect one standard error for triplicate (control) or quadruplicate (mutant) determinations.

TABLE 2

Genetic mapping of the excision deficiency—incision capacity of *al dp b pr* recombinants

	Photorepair capacity (from Table 1)	Incision capacity (breaks/10^{11} dal/min)
Experiment A		
Recombinant 1	−	1.16 ± 0.17 (2)
Recombinant 2	+	2.28 ± 0.18 (4)
Experiment B		
Recombinant 1	−	1.50 ± 0.16 (5)
Recombinant 3	+	2.79 ± 0.21 (5)
Experiment C		
phr stock	−	0.57 ± 0.11 (4)
Recombinant 4	+	3.28 ± 0.28 (5)

Primary tissue cultures were prepared from homozygous embryos and analyzed for the DNA breaks which accumulate during 10–20 min of incubation after 30 sec of near-UV irradiation (HARRIS and BOYD 1980). The rates of formation of DNA single-strand breaks are presented as the means ± SE with the number of replicates in parentheses. Comparisons between experiments are less precise for reasons described in MATERIALS AND METHODS.

between 0.6 and 1.5. Due to variations between experiments, comparisons of values within a single experiment are more valid. Since we have been unable to separate the photorepair and excision repair defects by recombination, the phr^+ gene product potentially participates in both processes.

Biological responses of the *phr* mutant: Photoreactivation studies were performed to establish the relative importance of the *phr* gene in UV resistance (Table 3). Larvae were exposed to short wavelength ultraviolet light and then exposed to photoreactivating illumination for 1 hr. Under these conditions visible light enhances the survival of wild-type larvae. Photorepair can, therefore, provide a significant defense against high acute doses of UV irradiation in third instar larvae as has recently been shown in first instar larvae (RYO and KONDO 1986). Since little or no photoreactivation is observed in the corresponding photorepair deficient strain, the phr^+ function is required for the enhanced survival seen in the wild-type stock.

TABLE 3

Photoreactivation of UV-irradiated larvae

	Relative Survival	
Treatment	phr^+	phr^-
A − UV − light	1.00 (2869)	1.00 (2590)
B − UV + light	1.04 (2983)	0.90 (2335)
C + UV − light	0.21 (618)	0.30 (783)
D + UV + light	0.53 (1528)	0.35 (910)
Photoreactivation, D/C	2.47	1.16

Equal weights of homozygous third instar larvae were irradiated and cultured as described under MATERIALS AND METHODS. Total surviving adults are presented in parentheses. UV-irradiated larvae received 138 Jm^{-2}. Visible illumination was administered for 1 hr.

DISCUSSION

The discovery and analysis of a photorepair deficient mutant in Drosophila complements and extends early demonstrations of photoreactivation and photorepair in this organism (BOYD *et al.* 1980). BECK and SUTHERLAND (1979) have purified a photolyase from Drosophila tissue culture cells which requires an RNA cofactor and exhibits assay requirements similar to those of the *E. coli* enzyme (BECK 1982). Since the *phr* gene is absolutely required for photorepair activity, there is a strong possibility that it encodes a polypeptide of that enzyme. FERRO (1985) has also performed biochemical studies with a Drosophila strain which exhibits reduced photoreactivation (YEGOROVA *et al.* 1978). Although he identified a slightly reduced photorepair capacity in that strain, the phenotype in our hands is too weak to permit complementation studies with the *phr* mutant reported here (J. B. BOYD and P. V. HARRIS, unpublished observations). The *phr* allele is, therefore, the first Drosophila mutation expressing a photorepair deficiency strong enough to permit definitive genetic studies.

Interaction between photorepair and excision repair: In this analysis, a complete photorepair defect and a partial excision-repair defect have been mapped to a common chromosomal region. If a single mutation is, in fact, responsible for both phenotypes, the *phr* gene product participates in excision repair as well as in photorepair. In this regard, the *phr* phenotype is analogous to complementation group B of xeroderma pigmentosum (SUTHERLAND, RICE and WAGNER 1975) in which a complete photorepair defect is

associated with a partial deficiency in excision repair. In both organisms, however, the converse association between these phenotypes is not as strong. In man, for example, complementation group A of xeroderma pigmentosum is devoid of excision repair and yet possesses over one-third of the normal photorepair capacity (SUTHERLAND, RICE and WAGNER 1975). Likewise, in Drosophila the *mei-9*^{a} mutant, which is devoid of excision repair, possesses a strong photorepair capacity (BOYD, GOLINO and SETLOW 1976; BROWN, HARRIS and BOYD 1981). Therefore, among higher eukaryotes, genes thus far identified as being essential for photorepair appear to influence excision repair, although the converse may not be true.

An analysis of photorepair in *E. coli* offers a possible explanation for these observations., In 1962 HARM and HILLEBRANDT noted that in the dark the *phr*$^{-}$ mutant is more UV sensitive than the parental strain. More recently it has been shown that post UV survival in recA cells is enhanced by increasing the copy number of a cloned photorepair gene (YAMAMOTO, FUJIWARA and SHINAGAWA 1983; YAMAMOTO, SATAKE and SHINAGAWA 1984). This increased resistance is seen in the dark and is dependent upon the presence of a functional excision repair system. Both genetic (YAMAMOTO and SHINAGAWA 1985) and biochemical studies (HAYS, MARTIN and BHATIA 1985) of UV-irradiated bacteriophage λ further suggest that the *phr*$^{+}$ gene product potentiates excision repair mediated by the uvrABC system. Finally, a stimulation of the uvrABC nuclease by purified photolyase has been demonstrated *in vitro* (SANCAR, FRANKLIN and SANCAR 1984).

Extrapolation of the observations in *E. coli* to higher eukaryotes offers a potential explanation for the coincident defects that have been observed between photorepair and excision repair in man and Drosophila. Since both of these repair systems act on pyrimidine dimers, mechanisms may have evolved to avoid unproductive competition between them. Earlier suggestions (HAYNES *et al.* 1978) of "complex and functionally coordinated macromolecular structures" in DNA metabolism are becoming increasingly realistic as a solution to that problem. Participation of the *phr* gene product in a complex which includes excision repair can explain a variety of observations. In such a complex, distortions produced by a mutant photorepair enzyme could potentially influence associated excision activities. Interactions of this nature have been observed among bacterial suppressor mutations which restore a functional configuration to a multienzyme DNA synthetic complex (MAURER, OSMOND and BOTSTEIN 1984). Regardless of whether the eukaryotic photorepair system interacts directly with the excision mechanism or whether that interaction is more indirect, such observations provide a new dimension to studies of UV repair. The accumulating battery of eukaryotic mutants, including the *phr* allele described here, is likely to play a key role in the analysis of such interactions as they have in prokaryotes (ALBERTS 1985).

We are indebted to Drs. JAMES M. MASON and ROBERT K. BRODBERG for assistance with the genetic analysis. JEANNETTE ROBERTSON and ANGELA WAPNER ROSENSTEIN provided excellent technical assistance. This study has been supported by United States Public Health Service grants (GM32040 and GM22221) and by a contract from the U.S. Department of Energy (EV70210).

LITERATURE CITED

ALBERTS, B. M., 1985 Protein machines mediate the basic genetic processes. Trends Genet. **1.** 26–30.

BECK, L. A., 1982 Studies on a photoreactivating enzyme from *Drosophila melanogaster* cultured cells. Ph.D. dissertation, University of California, Irvine.

BECK, L. A. AND B. M. SUTHERLAND, 1979 Purification of a photoreactivating enzyme from *D. melanogaster* (meeting abstract). Am. Soc. Photobiol. **7:** 130.

BOYD, J. B. and P. V. HARRIS, 1981 Mutants partially defective in excision repair of five autosomal loci in *Drosophila melanogaster.* Chromosoma **82:** 249–257.

BOYD, J. B. and P. V. HARRIS, 1985 Isolation and characterization of a photorepair-deficient mutant in *Drosophila melanogaster* (meeting abstract). Genetics **110** (Suppl): S85.

BOYD, J. B. and R. B. SETLOW, 1976 Characterization of postreplication repair in mutagen-sensitive strains of *Drosophila melanogaster.* Genetics **84:** 507–526.

BOYD, J. B. and K. E. S. SHAW, 1982 Postreplication repair defects in mutants of *Drosophila melanogaster.* Mol. Gen. Genet. **186:** 289–294.

BOYD, J. B., M. D. GOLINO and R. B. SETLOW, 1976 The *mei-9*^{a} mutant of *Drosophila melanogaster* increases mutagen sensitivity and decreases excision repair. Genetics **84:** 527–544.

BOYD, J. B., P. V. HARRIS, C. J. OSGOOD and K. E. SMITH, 1980 Biochemical characterization of repair-deficient mutants of Drosophila. pp. 209–221. In: *DNA Repair and Mutagenesis in Eukaryotes*, Edited by W. M. GENEROSO, M. D. SHELBY and F. J. DE SERRES. Plenum Press, New York.

BOYD, J. B., M. D. GOLINO, K. E. S. SHAW, C. J. OSGOOD and M. M. GREEN, 1981 Third-chromosome mutagen-sensitive mutants of *Drosophila melanogaster.* Genetics **97:** 607–623.

BOYD, J. B., P. V. HARRIS, J. M. PRESLEY and M. NARACHI, 1983 *Drosophila melanogaster*: a model eukaryote for the study of DNA repair. In: *Cellular Responses to DNA Damage*, Edited by E. C. FRIEDBERG and B. A. BRIDGES. Alan R. Liss, New York.

BROWN, T. C., P. V. HARRIS and J. B. BOYD, 1981 Effects of radiation on the survival of excision-defective cells from *Drosophila melanogaster.* Somatic Cell Genet. **7:** 631–644.

CHAN, L.-N. and W. GEHRING, 1971 Determination of blastoderm cells in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **68:** 2217–2221.

COLLINS, A. R. S. and R. T. JOHNSON, 1984 The inhibition of DNA repair. Adv. Radiat. Biol. **11:** 71–129.

COX, J. L. and G. D. SMALL, 1985 Isolation of a photoreactivation-deficient mutant in Chlamydomonas. Mutat. Res. **146:** 249–255.

ELLISON, M. J. and J. D. CHILDS, 1981 Pyrimidine dimers induced in *Escherichia coli* DNA by ultraviolet radiation present in sunlight. Photochem. Photobiol. **34:** 465–469.

FERRO, W., 1985 Studies on mutagen-sensitive strains of *Drosophila melanogaster.* V. Biochemical characterization of a strain

(ebony) that is UV- and X-ray sensitive and deficient in photorepair. Mutat. Res. **149:** 399–408.

FRIEDBERG, E. C., 1985 *DNA Repair.* Freeman, New York.

HARM, W. and B. HILLEBRANDT, 1962 A non-photoreactivable mutant of *E. coli* B. Photochem. Photobiol. **1:** 271–272.

HARRIS, P. V. and J. B. BOYD, 1980 Excision repair in Drosophila: analysis of strand breaks appearing in DNA of *mei-9* mutants following mutagen treatment. Biochim. Biophys. Acta **610:** 116–129.

HARRIS, P. V. and J. B. BOYD, 1987 Pyrimidine dimers in Drosophila chromatin become increasingly accessible after irradiation. Mutat. Res. **183:** 53–60.

HAYNES, R. H., L. PRAKASH, M. A. RESNICK, B. S. COX, E. MOUSTACCHI and J. B. BOYD, 1978 DNA repair in lower eucaryotes. In: *DNA Repair Mechanisms*, Edited by P. C. HANAWALT, E. C. FRIEDBERG and C. F. FOX. Academic Press, New York.

HAYS, J. B., S. J. MARTIN and K. BHATIA, 1985 Repair of nonreplicating UV-irradiated DNA: cooperative dark repair by *Escherichia coli* Uvr and *Phr* functions. J. Bacteriol. **161:** 602–608.

KITTLER, L. and G. LÖBER, 1977 Photochemistry of the nucleic acids. Photochem. Photobiol. Rev. **2:** 39–131.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of Drosophila melanogaster. Carnegie Inst. Wash. Publ. 627.

MAURER, R., B. C. OSMOND and D. BOTSTEIN, 1984 Genetic analyses of DNA replication in bacteria: DNAB mutations that suppress DNAC mutations and DNAQ mutations that suppress DNAE mutations in *Salmonella typhimurium.* Genetics **108:** 25–38.

NARACHI, M. A. and J. B. BOYD, 1985 The *giant* (*gt*) mutants of *Drosophila melanogaster* alter DNA metabolism. Mol. Gen. Genet. **199:** 500–506.

RAHN, R. O., 1979 Nondimer damage in deoxyribonucleic acid caused by ultraviolet radiation. pp. 267–330. In: *Photochemical and Photobiological Reviews*, Vol. 4, Edited by K. C. SMITH. Plenum Press, New York.

RESNICK, M. A., 1969 A photoreactivationless mutant of *Saccharomyces cerevisiae.* Photochem. Photobiol. **9:** 307–312.

RYO, H. and S. KONDO, 1986 Photoreactivation rescue and hypermutability of ultraviolet-irradiated excisionless *Drosophila melanogaster* larvae. Proc. Natl. Acad. Sci. USA **83:** 3366–3370.

SANCAR, A., K. A. FRANKLIN and G. B. SANCAR, 1984 *Escherichia coli* DNA photolyase stimulates uvrABC excision nuclease *in vitro.* Proc. Natl. Acad. Sci. USA **81:** 7397–7401.

SETLOW, R. B. and J. K. SETLOW, 1972 Effects of radiation on polynucleotides. Annu. Rev. Biophys. Bioeng. **1:** 293–346.

SUTHERLAND, B. M., 1981 Photoreactivating enzymes. pp. 481–515. In: *The Enzymes*, Vol. XIV. Academic Press, New York.

SUTHERLAND, B. M., M. RICE and E. K. WAGNER, 1975 Xeroderma pigmentosum cells contain low levels of photoreactivating enzyme. Proc. Natl. Acad. Sci. USA **72:** 103–107.

YAMAMOTO, K. and H. SHINAGAWA, 1985 Weigle reactivation of phage lambda in a recA mutant of *Escherichia coli*: dependence on the excess amounts of photoreactivating enzyme in the dark. Mutat. Res. **145:** 137–144.

YAMAMOTO, K., Y. FUJIWARA and H. SHINAGAWA, 1983 Evidence that the phr^+ gene enhances the ultraviolet resistance of *Escherichia coli* recA strains in the dark. Mol. Gen. Genet. **192:** 282–284.

YAMAMOTO, K., M. SATAKE and H. SHINAGAWA, 1984 A multicopy phr^- plasmid increases the ultraviolet resistance of a recA strain of *Escherichia coli.* Mutat. Res. **131:** 11–18.

YEGOROVA, L. A., V. L. LEVIN and M. A. KOZLOVA, 1978 Heritability of UV sensitivity and photoreactivation ability in Drosophila embryos. Mutat. Res. **49:** 213–218.

Communicating editor: V. G. FINNERTY