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).

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 - Variegated (dominant second chromosome marker) D - Dichaete (dominant third chromosome marker) C(1)DX, yf (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 5 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-3H]thymidine (Amersham Corporation). Following overnight incubation at 25°C, 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 10^6 dal of DNA. Organs

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

Genetics 116: 253-259 (June, 1987)
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 incision capacity 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 (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-
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 F1 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 Cy0/bw" 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 F1 females, which are heterozygous for the photorepair defect and a multiply marked second chromosome, produce recombinants that are identified phenotypically in F2 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" 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" 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" was induced confers

---

**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 F1 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 F1 females were identified in individual F2 males and tested for photorepair after expansion in separate stocks.
TABLE 1
Genetic mapping of phr on the second chromosome

<table>
<thead>
<tr>
<th>A. Location of visible markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
</tr>
<tr>
<td>Map position</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Photorepair capacity carried by recombinant chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map interval tested</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0.11</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0.26</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0.80</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

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.

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.

homoygous 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 homoygous 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

photorepair deficiency. We therefore conclude that the mus205 † 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
inhibitors for 15 min and the DNA was analyzed by alkaline elution.

Following irradiation, the cultures were exposed to DNA synthesis

Error bars reflect one standard error for triplicate (control) or

RIS (from Table 1) (breaks/10^9 dal/min)

**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 quaduplicate (mutant) determinations.

**TABLE 2**

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

<table>
<thead>
<tr>
<th>Photorepair capacity (from Table 1)</th>
<th>Incision capacity (breaks/10^9 dal/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment A</td>
<td></td>
</tr>
<tr>
<td>Recombinant 1</td>
<td>–</td>
</tr>
<tr>
<td>Recombinant 2</td>
<td>+</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
</tr>
<tr>
<td>Recombinant 1</td>
<td>–</td>
</tr>
<tr>
<td>Recombinant 2</td>
<td>+</td>
</tr>
<tr>
<td>Recombinant 3</td>
<td>–</td>
</tr>
<tr>
<td>Experiment C</td>
<td></td>
</tr>
<tr>
<td>phr stock</td>
<td>–</td>
</tr>
<tr>
<td>Recombinant 4</td>
<td>+</td>
</tr>
</tbody>
</table>

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 de-

**TABLE 3**

Photoreactivation of UV-irradiated larvae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>A − UV − light</td>
<td>phr+</td>
</tr>
<tr>
<td>B − UV + light</td>
<td>phr-</td>
</tr>
<tr>
<td>C + UV − light</td>
<td>phr+</td>
</tr>
<tr>
<td>D + UV + light</td>
<td>phr-</td>
</tr>
</tbody>
</table>

_phr_ stock. 2.47 1.16

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

fense 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.

**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" 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, FUJISAWA 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 ROBSON 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


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.


Communicating editor: V. G. FINNERTY