RECOMBINATION IN RELATION TO ULTRAVIOLET SENSITIVITY IN *CHLAMYDOMONAS REINHARDI*

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ABSTRACT

A mutant strain of *Chlamydomonas reinhardi* which is UV sensitive as a result of a single-gene chromosomal mutation has also been found to exhibit reduced recombination. In crosses homozygous for the mutant allele, a reduction in recombination frequency was demonstrated in two different linkage groups and in three different genetic backgrounds. Thus a single mutation affecting UV sensitivity also has a possible effect on recombination. Such a mutant could be analogous to the *rec*⁻ mutants discovered in *E. coli* and as such be useful in the study of recombination mechanisms. Three additional UV-sensitive isolates were tested. Recombination was not altered in these mutant strains.

THE most detailed evidence now available concerning dark repair of ultraviolet (UV) irradiation damage to DNA comes from recent studies with *E. coli* mutants sensitive to UV light. Two classes of mutant have been isolated: *uvr* mutants (HOWARD-FLANDERS, BOYCE and THERIOT 1966) and *rec*⁻ mutants (CLARK and MARGULIES 1965; VAN DE PUTTE, ZWENK and RORSCH 1966). The *uvr* mutants appear to be deficient in an enzyme system that replaces a damaged region of one DNA strand with DNA copied from the intact complementary strand (BOYCE and HOWARD-FLANDERS 1964; PETTIJOHN and HANAWALT 1964). In these mutants genetic recombination is not affected. In *rec*⁻ mutants, on the other hand, UV sensitivity appears to be a direct consequence of a deficiency in the recombination mechanism: the mutant cells are unable to form by recombination one complete genome from two damaged genomes (HOWARD-FLANDERS, THERIOT and STEDEFORD 1969).

Dark repair of the kind deficient in *uvr* mutants has been clearly demonstrated in eucaryotic organisms (REGAN, TRASKO and CARRIER 1968; WHITSON, FRANCIS and CARRIER 1968). In the UV-sensitive mutants so far studied, meiotic recombination frequency is not altered; mitotic recombination frequency, in contrast, is sometimes increased (SNOW 1967), sometimes decreased (HOLLIDAY 1967; SCHROEDER 1970). No eucaryotic mutants clearly analogous to *rec*⁻ bacterial mutants have been reported.

UV-sensitive mutants of the eucaryote *Chlamydomonas reinhardi* have been reported (DAVIES 1967). The presence of these mutations did not alter meiotic

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recombination frequency. In this paper we report four independently isolated mutant strains of Chlamydomonas sensitive to UV light. One of the mutations appears analogous to *rec*$^-$ (*E. coli*) by consistently causing a decrease in meiotic recombination frequency when homozygous in a cross.

MATERIALS AND METHODS

Strains: Haploid cultures of *C. reinhardi* (strain 137C) were used in this study. The mutant markers were *ac-51* (requires acetate for growth at pH 8.3), *sr-1* (resistant to streptomycin), *pab-2* (requires para-aminobenzoic acid for growth), *msr* (resistant to methionine sulfoximine), and *uvsE1*, *uvsE4*, *uvsE5* and *uvsE6* (sensitive to UV light). *msr* is approximately 11 map units distal to *pab-2* in linkage group I (GILLHAM unpublished); *sr-1* is approximately 22 map units proximal to *ac51* in linkage group IX (GILLHAM and LEVINE 1962); and the mating-type locus (*mt*+ or *mt*−) is located in linkage group VI (EBERSOLD, LEVINE and OLMSTED 1962).

Media: Minimal medium (M) (EBERSOLD 1956) was supplemented with 2.0 gm/l sodium acetate (Ac), 5 mg/l para-aminobenzoic acid (P), 100 μg/ml streptomycin sulfate (S), and 450 μg/ml *dl*-methionine *dl*-sulfoximine (Ms) as required. The pH of this medium was raised to 8.3 by substituting 6 gm/l Tris-(hydroxymethyl)-aminomethane for the pH 7 phosphate buffer, usually used in M.

Isolation of UV-sensitive mutants: Wild-type cells were grown to a concentration of 1×10^6 cells/ml in liquid M medium and treated with either 5 μg/ml ICR-170 for 2 hr or with UV light for 90 sec using a Westinghouse Sterilamp Type SB-30 with a single 17 watt WL 78230 cold cathode tube. The distance from the plate to the light was 165 mm and the energy at the plate surface was 25.21 ergs/mm^2/sec. Both mutagenic treatments result in approximately 90–95% killing. The treated cells were incubated in liquid M medium for 48 hr and diluted to a concentration of 1500 cells/ml. Samples (0.1 ml) were then plated onto M agar medium. After incubation for four days, colonies were replica-plated in duplicate to M agar medium and each replicate was irradiated for 20 sec (at 25.21 ergms/mm^2/sec). One plate was allowed to remain in darkness for 18 hr to prevent photoreactivation and then transferred into the light ("dark plate"). The other was placed directly into the light ("light plate"). Both places were incubated until colonies were macroscopically visible. Colonies which grew on the "light plate" but failed to develop on the "dark plate" were classified as UV-sensitive.

UV dosage-survival curves: A 10-ml suspension (10^6 cells/ml) in liquid M medium was exposed to UV irradiation in a 15×60 mm Petri dish. The cell suspension was stirred continually during irradiation. 0.3 ml samples were removed from the culture after 0, 15, 30, and 60 sec. All samples were diluted serially (10^{-1}, 10^{-2}, and 10^{-3}) using liquid M medium. A 0.1 ml portion of each diluted cell suspension was spread onto the surface of an M agar plate. The plates were placed in darkness for 18 hr and then illuminated. The colonies on each plate were counted five days later.

Genetic analysis: Two methods used for determining the recombination frequency between linked genes (tetrad analysis, and single-strand analysis by zygote plating) have been described previously (LEVINE and EBERSOLD 1958). In tetrad analysis, all products from a zygote were examined, thus making this technique an accurate and unambiguous test for recombination. In order to test for recombination by single-strand analysis, colonies formed by germination of zygotes (each colony containing all 4 meiotic products) were replica plated to test media which allowed detection of one recombinant type. Clearly this method was not as powerful as tetrad analysis since only *one* recombinant product can be detected, and that product is sometimes lost due to mechanical errors in replica plating. However, single-strand analysis does allow testing of many zygotes in a short time and is especially useful when recombination data for closely linked genes is desired.

RESULTS

UV sensitivity: Approximately 5×10^4 colonies were tested for UV sensitivity,

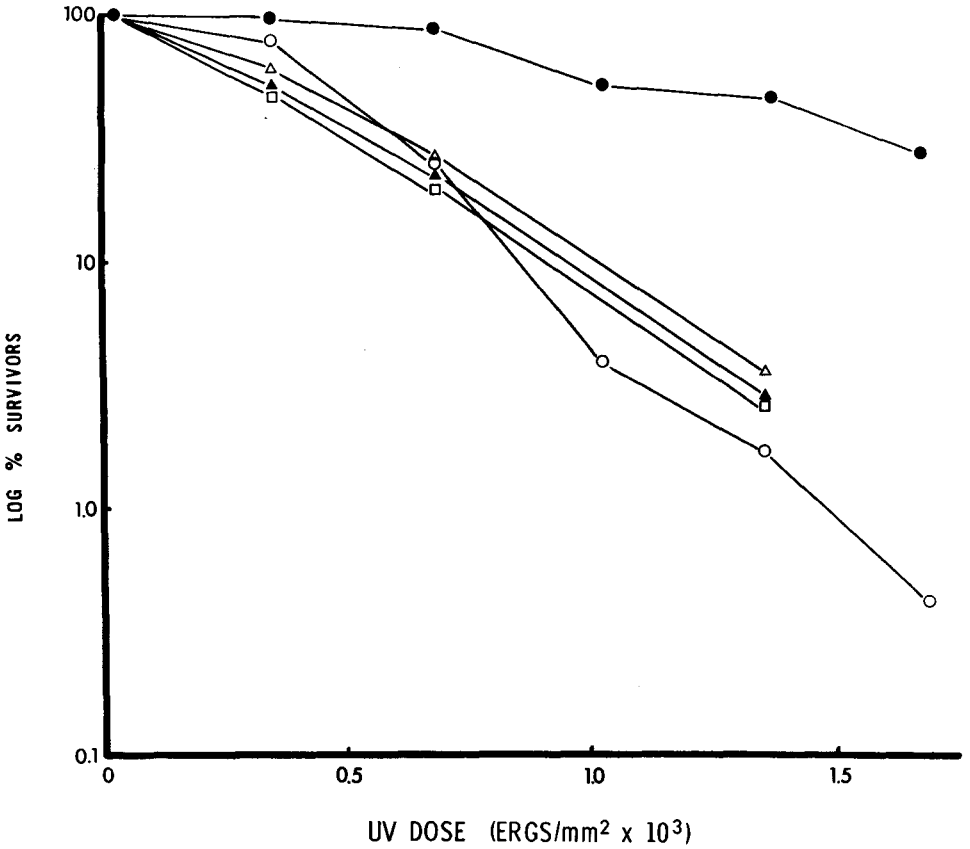

FIGURE 1.—Survival curves for wild type and UV-sensitive mutant strains. (● = wild type, ○ = *uvsE1*, △ = *uvsE5*, ▼ = *uvsE6*, and □ = *uvsE4*.

and four independently isolated UV-sensitive strains were obtained. A comparison of the UV dosage-survival curves of the wild-type strain and the four UV-sensitive (*UVS*) mutant strains is shown in Figure 1. At a dose of 1300 ergs/mm^2 the survival of each mutant was approximately one tenth that of the wild type. Since the degree of sensitivity of each mutant was approximately the same at the three doses used, it was not possible to distinguish the mutants from one another on the basis of UV sensitivity alone. Photoreactivation was normal in all four mutants.

Genetic basis for UV sensitivity: Each *uvs* mutant was crossed to a *uvs*$^+$ *pab-2 msr* strain and segregation of each *uvs* allele was determined by the analysis of approximately 100 tetrads. *Pab-2* and *msr* were included in the cross to demonstrate that all of the tetrads were complete and also to develop strains utilized in later experiments. In reciprocal crosses (with respect to mating type) in which 90% of the tetrads obtained were complete, *uvs* and *uvs*$^+$ segregated 1:1 as did *pab-2* and *msr*. Thus *uvsE1*, *uvsE4*, *uvsE5* and *uvsE6* represent single-gene chromosomal mutations. Tetrad analyses of the above crosses as well as subse-

TABLE 1

Recombination frequencies between pab-2 *and* msr *from zygotes homozygous for the wild type* (uvs+) *and for each UV-sensitive mutation*

	Zygote genotype and cross number	Total zygotes	Number of *pab-2+ msr* recombinants
(1)	*uvs+ pab-2+ msr+* / *uvs+ pab-2 msr*	1613	328 (20.3%)
(2)	*uvsE1 pab-2+ msr+* / *uvsE1 pab-2 msr*	1449	239 (16.5%)
(3)	*uvsE4 pab-2+ msr+* / *uvsE4 pab-2 msr*	1533	309 (20.2%)
(4)	*uvsE5 pab-2+ msr+* / *uvsE5 pab-2 msr*	1486	298 (20.1%)
(5)	*uvsE6 pab-2+ msr+* / *uvsE6 pab-2 msr*	1192	235 (19.7%)

quent crosses indicated: 1) that each of the four *uvs* mutations was located in a different linkage group; 2) that none of the *uvs* mutations was located in linkage group I, or appeared to be linked to *mating type* (linkage group VI); 3) and that *uvsE1* was not located in linkage group IX. Recent information (unpublished) however, indicates that *uvsE1* is located in right arm of linkage group VI.

Effect of uvs *mutations on the frequency of recombination between linked genes:* The frequency of recombination between *pab-2* and *msr* was determined by single-strand analysis of crosses homozygous for each UV-sensitive mutation. Results from a cross homozygous for the wild-type alleles served as a control. Zygotes from each cross were plated onto P agar medium. After germination and incubation for four days, the resulting colonies were replica-plated to PMs and Ms agar media to permit detection of *pab-2+ msr* recombinants. The results are presented in Table 1. The recombination frequency between *pab-2* and *msr* in the control cross was 20.3% (Cross 1). When zygotes were homozygous for *uvsE4*, *uvsE5* and *uvsE6* (Crosses 3, 4, & 5), the recombination frequencies were 20.2%, 20.1% and 19.7% respectively. It was concluded, therefore, that these *uvs* mutant genes had no effect on the frequency of recombination. In crosses homozygous for *uvsE1* the frequency of recombination between *pab-2* and *msr* was 16.5% (Cross 2) which is significantly lower than the frequency found for Cross 1 ($P < 0.005$).

uvsE1 pab-2 msr was introduced into the mutant strain *ac-51 sr-1*, in order to ascertain whether the presence of the *usvE1* had a similar effect on recombination frequency in a different linkage group. In addition, the effect on recombination frequency between *pab-2* and *msr* could be determined in a cross reciprocal (with respect to *mt*) to Cross 3. The recombination frequency between *ac-51* and *sr-1* was determined by tetrad analysis in the following crosses:

TABLE 2

Summary of tetrad data from zygotes homozygous for uvsE1+ *and* uvsE1

Cross 6 Zygote genotype: $\frac{uvsE1+\ ac\text{-}51\ \ sr\text{-}1}{uvsE1+\ ac\text{-}51+\ sr\text{-}1+}$

PD	NPD	T	Total	Percent recombination
741	3	591	1338	44.6
Cross 7 Zygote genotype: $\frac{uvsE1\ \ ac\text{-}51\ \ sr\text{-}1}{uvsE1\ \ ac\text{-}51+\ sr\text{-}1+}$				
1005	1	577	1584	36.6

PD = Parental ditype tetrad, NPD = Non-parental ditype tetrad.
T = Tetratype tetrad.

Cross 6. *uvsE1*+ *ac-51 sr-1 pab-2*+ *msr*+ × *uvsE1*+ *ac-51*+ *sr-1*+ *pab-2 msr.*

Cross 7. *uvsE1 ac-51 sr-1 pab-2*+ *msr*+ × *uvsE1 ac-51*+ *sr-1*+ *pab-2 msr.*

The frequency of recombination between *ac-51* and *sr-1* in the control cross (Cross 6) was 44.6%. In Cross 7 the frequency was 36.6% (Table 2). Therefore, in crosses homozygous for *uvsE1*, the recombination frequency between *ac-51* and *sr-1* was significantly reduced ($P < 0.001$). Using a different sample of zygotes from Crosses 6 and 7 the frequency of recombination between *pab-2* and *msr* was determined by single-strand analysis. The recombination frequency was 19.7% in 4335 zygotes from Cross 6 and 16.4% in 2746 zygotes from Cross 7 ($P < 0.001$). Therefore, in crosses homozygous for *uvsE1* the recombination frequency between *pab-2* and *msr* was significantly reduced.

Dominance of the uvsE1+ *allele:* The following cross was analyzed by single-strand analysis of 1104 zygote colonies in order to determine whether *uvsE1* or *uvsE1*+ was expressed during meiosis in crosses heterozygous for these alleles.

Cross 8. *uvsE1 pab-2 msr mt*- × *uvsE1*+ *pab-2*+ *msr*+ *mt*+.

The recombination frequency between *pab-2* and *msr* was 20.6%. This is the same as the recombination frequency between these two loci obtained in crosses homozygous for *uvsE1*+. Therefore, during meiosis, *uvsE1*+ is dominant over *uvsE1*.

Effect of uvsE1 *on the frequency of recombination in a different genetic background:* Tetrad analysis of the original isolates of *uvsE1* yielded approximately 50% (117/238) incomplete tetrads in which only two of the four products survived. This aberrant pattern could be caused by a chromosomal inversion in one of the parents, and the genetic data for all markers scored in these crosses were consistent with this interpretation. The data also showed that none of the markers involved were linked to the "inversion." Individual *uvsE1* products were isolated from incomplete tetrads and were tested for the presence or absence of the presumed inversion. The frequency of recombination between *pab-2* and *msr* was analyzed in crosses homozygous or heterozygous for the presumed inversion (I^+ = wild type, I = presumed inversion) and which were homozygous for either *uvsE1*+ or *uvsE1* (Crosses 9, 10, 11, and 12).

TABLE 3

Recombination frequencies between pab-2 *and* msr *zygotes heterozygous and homozygous for an inversion and homozygous for* uvsE1 *or* uvsE1+

Cross number	Inversion	uvs Alleles	Number of tetrads	Percent recombination between *pab-2* and *msr*
6	*I+/I+*	*uvsE1+/uvsE1+*	4334	19.7
7	*I+/I+*	*uvsE1/uvsE1*	2746	16.4
9	*I+/I*	*uvsE1+/uvsE1+*	250	16.0
10	*I+/I*	*uvsE1/uvsE1*	1440	12.4
11	*I/I*	*uvsE1+/uvsE1+*	237	22.4
12	*I/I*	*uvsE1/uvsE1*	3475	15.1

I = Inversion, *I+* = wild type.

Cross 9. *uvsE1+ I+ pab-2+ msr+ × uvsE1+ I pab-2 msr.*
Cross 10. *uvsE1 I+ pab-2 msr × uvsE1 I pab-2 msr.*
Cross 11. *uvsE1+ I pab-2+ msr+ × uvsE1+ I pab-2 msr.*
Cross 12. *uvsE1 I pab-2+ msr+ × uvsE1 I pab-2 msr.*

The results of these crosses were compared to Crosses 6 and 7 (Table 3). The recombination frequency between *pab-2* and *msr* in Cross 9 which is heterozygous for the presumed inversion shows an apparent decrease of 25% when compared to Cross 6, due to the loss of two products in 50% of the zygotes. Similarly, Cross 10 shows an apparent decrease when compared to Cross 7. This apparent decrease in recombination frequency was not found in crosses homozygous for the presumed inversion (Crosses 11 and 12). In comparing Cross 9 with Cross 10 and Cross 11 with Cross 12, the differences in recombination frequency were not statistically significant because of the relatively low number of products in Crosses 9 and 11. However, the results were consistent with a decrease in the frequency of recombination in crosses homozygous for *uvsE1*. Thus in spite of differences in genetic background, *uvsE1* had the same effect when homozygous in the cross.

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LITERATURE CITED

BOYCE, R. P. and P. HOWARD-FLANDERS, 1964 Release of ultraviolet light induced thymine dimers from DNA in *E. coli* K-12 Proc. Natl. Acad. Sci. U.S. **51**: 293–300.

CLARK, A. J. and A. D. MARGULIES, 1965 Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S. **53**: 451–459.

DAVIES, D. R., 1967 UV-sensitive mutants of *Chlamydomonas reinhardi*. Mutation Res. **4**: 765–770.

EBERSOLD, W. T., 1956 Crossing over in *Chlamydomonas reinhardi*. Am. J. Botany **43**: 408–410.

EBERSOLD, W. T., R. P. LEVINE and M. A. OLMSTED, 1962 Linkage maps in *Chlamydomonas reinhardi*. Genetics **47**: 531–543.

GILLHAM, N. W. and R. P. LEVINE, 1962 Studies on the origin of streptomycin resistant mutants in *Chlamydomonas reinhardi*. Genetics **47**: 1463–1474.

HOLLIDAY, R., 1967 Altered recombination frequencies in radiation-sensitive strains of Ustilago. Mutation Res. **4**: 275–288.

HOWARD-FLANDERS, P., R. P. BOYCE and L. THERIOT, 1966 Three loci in *Escherichia coli* K-12 that control the excision of Pyrimidine dimer and contain other mutagen products from DNA. Genetics **53**: 1119–1136.

HOWARD-FLANDERS, P., L. THERIOT and B. STEDEFORD, 1969 Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J. Bacteriol. **97**: 1134–1141.

LEVINE, R. P. and W. T. EBERSOLD, 1958 Gene recombination in *Chlamydomonas reinhardi*. Cold Spring Harbor Symp. Quant. Biol. **23**: 101–109.

PETTIJOHN, D. and P. HANAWALT, 1964 Evidence for repair-replication in ultraviolet damaged DNA in bacteria. J. Mol. Biol. **9**: 395–410.

REGAN, J. D., J. E. TROSKO and W. L. CARRIER, 1968 Evidence for excision of ultraviolet-induced pyrimidine dimers from the DNA of human cells *in vitro*. Biophys. J. **8**: 319–325.

SCHROEDER, A. L., 1970 Ultraviolet-sensitive mutants of Neurospora. Genetic basis and effect on recombination. Molec. Gen. Genetics **107**: 291–304.

SNOW, R., 1967 Increased mitotic recombination induced by ultraviolet light in UV-sensitive strains of yeast (*Saccharomyces cerevisiae*). Genetics **56**: 591–592.

WHITSON, G. L., A. A. FRANCIS and W. L. CARRIER, 1968 Ultraviolet-induced pyrimidine dimers in Tetrahymena. I. Removal in the dark. Biochim. Biophys. Acta **161**: 285–290.

VAN DE PUTTE, P., H. ZWENK and A. RORSCH, 1966 Properties of four mutants of *Escherichia coli* defective in genetic recombination. Mutation Res. **3**: 381–392.