JOURNAL OF BACTERIOLOGY, Oct. 1972, p. 632-634
Vol. 112, No. 1

Printed in U.S.A.

Interaction of Genes Controlling Ultraviolet Sensitivity in *Neurospora crassa*

R. W. TUVESON

Department of Botany, University of Illinois, Urbana, Illinois 61801

Received for publication 19 May 1972

Two independently segregating ultraviolet (UV) sensitivity genes in *Neurospora crassa* interact synergistically resulting in UV sensitivity approximately twice that expected based on an evaluation of the sensitivities of the individual mutants. The mutant genes singly and together reduce photoreactivation (PR) in vivo although a PR enzyme is produced which exhibits normal activity in vitro.

Interaction of mutant genes controlling excision-repair (*uvr*) and recombinational repair (*rec*) has been reported in *Escherichia coli* (2, 3). Although difficult to estimate from the ultraviolet light (UV) inactivation curves presented, it appears that the *uvr* and *rec* genes interact synergistically such that the double mutants are more sensitive to UV than would have been predicted from the sensitivities of the individual mutants. Interaction is expected for these genes since they govern repair systems that are independent. It has also been shown that double mutants in *E. coli* composed of a temperature sensitive *polA* mutant and specific *rec* mutants are more UV sensitive than the corresponding single mutants, but whether synergism was observed has not been reported (5). Although several UV-sensitive mutants have been isolated in *N. crassa* (1, 6, 8), interaction among such mutants leading to enhanced UV sensitivity has not been demonstrated. It has been shown that the double mutant *uvs-1, upr-1* in *N. crassa* is no more sensitive to radiation than the more sensitive of the single mutants (10). To demonstrate interaction between genes controlling UV sensitivity in *N. crassa*, we selected two UV-sensitive strains that differed in their ability to undergo (i) UV-induced mutation and (ii) meiosis and (presumably) crossing-over since *E. coli, rec* and *uvr* mutants differ in these properties (3, 4). It has been shown that the *uvs-3* mutant in *N. crassa* is resistant to UV-induced mutagenesis (F. J. de Serres, Genetics **68**:14s-15s, 1971) and that crosses between strains that are "homozygous" for *uvs-3* are sterile (6), whereas the *upr-1* mutant

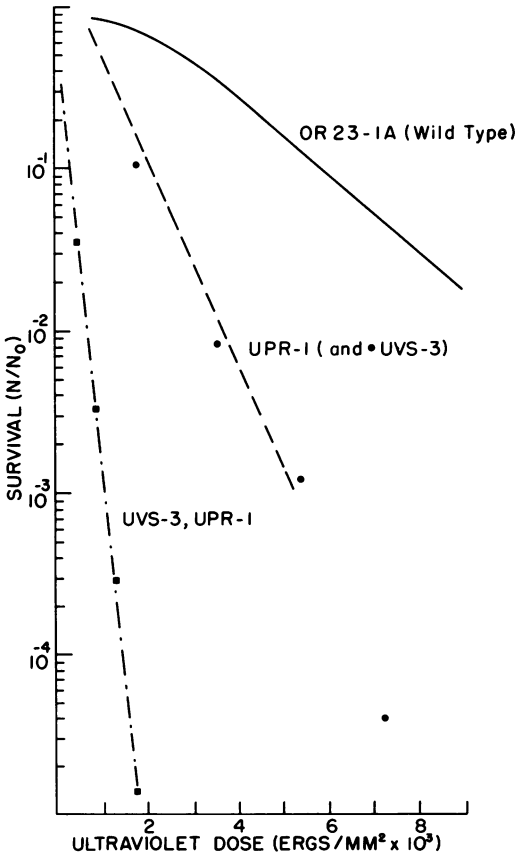

FIG. 1. *Ultraviolet inactivation curves for various Neurospora crassa strains. Curves for the wild type (solid line) and upr-1 (broken line) have been published (10, 12). Symbols: ●, uvs-3; ■, upr-1, uvs-3.*

in *N. crassa* is only slightly impaired for UV-induced mutagenesis (F. J. de Serres, Genetics 68:14s–15s, 1971) and is without any detectable effect on events at meiosis (13).

The *uvs-3* strain (ALS 11, FGSC no. 1627) was obtained from the Fungal Genetics Stock Center, Humboldt State College, Arcata, California. The sources of the other cultures and methods used for determining survival, carrying out photoreactivation (PR), and performing crosses have been presented (10, 12). Reactivation of UV-inactivated *Haemophilus influenzae* transforming deoxyribonucleic acid was used to estimate the activity of the PR enzyme in crude extracts of various *N. crassa* strains (9, 11).

The results of a UV-inactivation experiment with the *uvs-3* mutant are presented in Fig. 1. For comparison, we have presented UV-inactivation curves for the *upr-1* single mutant and the wild type which we have published previously (12). The *uvs-3* mutant is about as UV sensitive or perhaps slightly more than the *upr-1* mutant. The *upr-1* gene is located in linkage group IL near mating type (12), and *uvs-3* has been placed in linkage group IVL (6). We crossed the mutants and isolated linear tetrads. In the first tetrad investigated, all spore pairs appeared equally UV sensitive. In the second tetrad, two spore pairs were equivalent to the two parents in UV sensitivity, a single spore pair exhibited wild-type sensitivity, and the fourth pair was exceptionally sensitive to UV. This exceptional pair we interpret as resulting from independent assortment of the two radiation sensitivity genes giving us the double mutant (*upr-1, uvs-3*) in which the UV-sensitivity genes interact. The UV-inactivation curve for the double mutant is presented in Fig. 1. The dose enhancement factor (DEF = effect without the modifying factor/effect with the factor; the UV sensitivity genes are the modifying factors in this case) calculated at the 10^{-1} survival level for the *upr-1* mutant is 2.7 whereas for strain *uvs-3* it is about 2.9. The DEF for the double mutant is approximately 16.2 which is about twice what one might have expected given the DEF of the

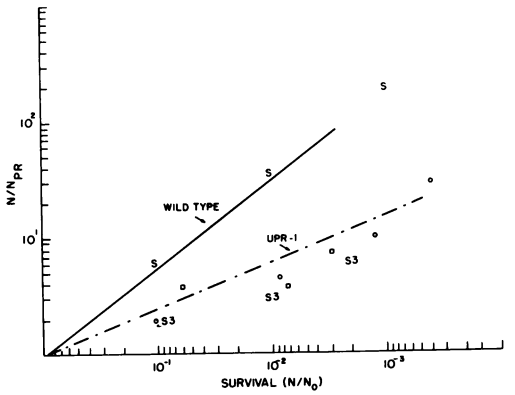

FIG. 2. *Photoreactivation (PR) of various N. crassa strains at equivalent survival levels. The efficiency of PR at a specific survival level is expressed as the ratio of survival subsequent to PR over survival without PR (N_{PR}/N). The lines in the figure which have been published previously (10, 12) represent PR in wild type (solid line) and upr-1 (broken line) strains. Symbols: S, points derived from published curves (7) for wild type; S3, points derived from published curves (7) for uvs-3; O, uvs-3 (our data); □, upr-1, uvs-3.*

TABLE 1. *Effect of various Neurospora crassa crude enzyme extracts on ultraviolet (UV)-inactivated Haemophilus influenzae transforming DNA*[a]

Components of the transformation mixture					Str transformants/ml
Competent cells[b]	0.015γ UV-DNA	Source of crude enzyme	0.015γ Enzyme-treated UV-DNA	Light[c]	
+	−	−	−	−	0
−	+	−	−	−	0
+	+	−	−	−	1.4×10^3
+	+	−	−	+	1.1×10^3
+	−	Wild type	+	−	1.3×10^3
+	−	Wild type	+	+	4.6×10^3
+	−	Wild type	+[d]	+	1.1×10^3
+	−	*upr-1*	+	−	1.2×10^3
+	−	*upr-1*	+	+	3.8×10^3
+	−	*upr-1*	+[d]	+	1.3×10^3
+	−	*uvs-3*	+	−	1.0×10^3
+	−	*uvs-3*	+	+	5.2×10^3
+	−	*uvs-3*	+[d]	+	1.0×10^3
+	−	*upr-1, uvs-3*	+	−	1.0×10^3
+	−	*upr-1, uvs-3*	+	+	4.0×10^3
+	−	*upr-1, uvs-3*	+[d]	+	1.2×10^3

[a] UV dose = 1.68×10^3 ergs/mm^2 = 4.5% survival of str transforming activity. DNA, deoxyribonucleic acid.

[b] Viable competent cells = 4.9×10^8. It should be noted that the transforming DNA concentration used is below saturation (saturation = 0.1γ DNA/transformation tube or greater)

[c] A 0.2-ml amount of crude *N. crassa* enzyme extract (1 mg of protein per ml) was mixed with 0.2 ml of UV-treated DNA (0.15γ/ml). The mixture was exposed to photo-reactivation light (10) for 15 min at 37 C or held in the dark at 37 C.

[d] The crude *N. crassa* enzyme preparation was boiled for 5 min before exposure to UV-DNA.

original mutants.

The lines presented in Fig. 2 represent the relative efficiency of PR in wild-type and *upr-1* strains (13). The letters and numbers (S and S3) represent points derived from UV-inactivation curves presented by Schroeder (7). Although our systems for inducing PR differ, Schroeder's results for wild type are in substantial agreement with our results. In addition, Schroeder's results (confirmed by our experiments, Fig. 2) indicate that the *uvs-3* mutant is about as impaired in vivo for PR as is *upr-1* mutant. Since both the *upr-1* and *uvs-3* mutants are impaired for in vivo PR, it was expected that the double mutant would be similarly impaired (Fig. 2).

The results presented in Table 1 indicate that all the strains tested produce an enzyme equivalent to that found in wild type which is capable of reactivating UV-inactivated transforming DNA and confirm our previous observations with wild-type and the *upr-1* mutant (11, 12). One is forced to conclude that the impaired in vivo PR observed in the two single mutants (*upr-1* or *uvs-3*) or the double mutant (*upr-1*, *uvs-3*) must be an indirect effect not involving the formation of a functional PR enzyme.

This work was supported by Public Health Service grant no. FR-07030 from the Division of Research Facilities and Resources.

LITERATURE CITED

1. Chang, L. T., and R. W. Tuveson. 1967. Ultraviolet-sensitive mutants in *Neurospora crassa*. Genetics **56**:801–810.
2. Ganesan, Ann K., and K. C. Smith. 1969. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. II. Effect of *uvr* genes on liquid holding recovery. J. Bacteriol. **97**:1129–1133.
3. Howard-Flanders, P., L. Theriot, and J. B. Stedeford. 1969. Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J. Bacteriol. **97**:1134–1141.
4. Miura, A., and J. Tomizawa. 1968. Studies on radiation-sensitive mutants of *E. coli* III. Participation of the REC system in the induction of mutation by ultraviolet irradiation. Mol. Gen. Genet. **103**:1–9.
5. Monk, M., and J. Kinross. 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **109**:971–978.
6. Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of *Neurospora* I. Genetic basis and effect on recombination. Mol. Gen. Genet. **107**:291–304.
7. Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of *Neurospora* II. Radiation Studies. Mol. Gen. Genet. **107**:305–320.
8. Stadler, D. R., and D. A. Smith. 1968. A new mutation in *Neurospora* for sensitivity to ultraviolet. Can. J. Genet. Cytol. **10**:916–919.
9. Terry, C. E., and Jane K. Setlow. 1967. Photoreactivating enzyme from *Neurospora crassa*. Photochem. Photobiol. **6**:799–803.
10. Tuveson, R. W., and J. Mangan. 1970. A UV-sensitive mutant of *Neurospora* defective for photoreactivation. Mutat. Res. **9**:455–466.
11. Tuveson, R. W. 1972. Comparison of two transformation systems for the assay of *Neurospora* photoreactivating enzyme. Genet. Res. *In press*.
12. Tuveson, R. W. 1972. Genetic and enzymatic analysis of a gene controlling UV sensitivity in *Neurospora crassa*. Mutat. Res. **15**:411–424.