ULTRAVIOLET-SENSITIVE MUTANTS IN *NEUROSPORA CRASSA*[1]

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RADIATION-SENSITIVE mutants in bacteria have been reported repeatedly in recent years (see SETLOW 1966). Several loci concerned with sensitivity have been mapped. Some of these mutants not only lacked the ability to repair damaged DNA but also failed to undergo genetic recombination (CLARK and MARGULIES 1965; HOWARD-FLANDERS and THERIOT 1966; VAN DE PUTTE *et al.* 1966). Among the eucaryotic organisms, mutants sensitive to ultraviolet light (UV) have been obtained in *Ustilago maydis* (HOLLIDAY 1965), *Aspergillus rugulosus* (LENNOX and TUVESON 1967), *Aspergillus nidulans* (LANIER and TUVESON 1965), and *Schizosaccharomyces pombe* (HAEFNER and HOWREY 1967). In *A. rugulosus* and *A. nidulans*, the UV-sensitive mutants were found to be sensitive to sodium nitrite ($NaNO_2$) as well.

This paper presents data on two mutants of *Neurospora crassa* which are sensitive to both UV-irradiation and $NaNO_2$. The sensitivity of one of the mutants is clearly under the control of a single nuclear gene, whereas that of the other seems to be non-nuclear in origin. Evidence is also presented concerning the effects of the nuclear gene mutation controlling UV sensitivity on the recombination of linked genes.

MATERIALS AND METHODS

The strain (FGSC No. 331) from which UV-sensitive mutants and specific auxotrophs were derived produces predominantly uninucleate microconidia and carries the following markers: crisp (*cr*; B123), ragged (*rg*; B53); peach (*pe*; Y8743m), fluffy (*fl*; L). The numbers (or the letter L) in parenthesis refer to the allele of each marker. This and a second strain used in the crosses, FGSC No. 338 (*cr*; *pe fl*), were obtained from the Fungal Genetics Stock Center, Dartmouth College, Hanover, N.H.

The microconidial strains of Neurospora are known to exhibit exceptionally low conidial viability, particularly when such cultures are incubated for extended periods in dry environments (BARRATT 1964). In order to maintain high conidial viability in suspension, 0.066 M phosphate buffer (Na-Na) at pH 7.0 was used for the preparation of suspensions and serial dilutions. By suspending in 0.066 M phosphate buffer from 6- to 8-day old cultures, conidial viability of over 60% was obtained based on haemocytometer counts, and viability was maintained for at least three successive days at 4°C.

Techniques for preparing conidial suspensions, isolating UV-sensitive mutants, and obtaining UV survival curves for Neurospora were essentially the same as those described for Aspergillus (LENNOX and TUVESON 1967). Briefly, UV-sensitive mutants were obtained by irradiating replicas of colonies which were derived from previously irradiated conidia, and isolating colonies whose replicas showed limited growth after incubation. All the scoring of UV sensitivity was based on survival curves for conidia in suspension. The UV dose used for inducing mutants and

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obtaining survival curves was approximately 11 ergs/mm^2/sec at the level of conidial suspension. UV irradiation and plating were performed in the absence of visible light other than a red fluorescent safe light to avoid photoreactivation (PR). Control experiments had shown that the safe light did not allow PR. All the platings were on Neurospora complex medium (medium N [VOGEL 1964] + 0.5% yeast extract [Difco] + 0.5% Casamino acids [Difco]). After plating, all plates were incubated at 32°C for three days.

For photoreactivation of irradiated cells, a light fixture with two General Electric 90 watt fluorescent lamps was placed horizontally on a table. A 10.0 × 1.2 cm tube containing 1.0 ml of irradiated suspension was placed vertically in the 12.0 cm gap between the two lamps. In order to minimize the effect of heat dissipated from the lamps, an electric fan was positioned to blow in the direction of the illuminated tube. Using this apparatus, maximum PR was attained within 90 min. Illumination of unirradiated cells before plating was not found to affect viability. Conidial suspensions irradiated with various doses of UV were divided into two aliquots; one was subjected to illumination for 90 min immediately after irradiation, the other was kept in the dark for 90 min at room temperature (25 to 28°C) before plating. The amount of PR was measured as an increase in the surviving fraction after this treatment.

Sodium nitrite survival curves for conidia were obtained following a modification of techniques described by SIDDIQI (1962). Conidia were suspended in 0.1 M citrate buffer at pH 4.4 and treated with 0.01 M $NaNO_2$ (nitrous acid) for various times. Treatment was terminated by diluting 1:10 into 0.1 M phosphate buffer at pH 7.0 and the conidia were plated immediately on complex medium. No significant loss of viability was observed for the controls in citrate buffer. Since it was observed that the killing effect of $NaNO_2$ treatment could be reversed by subsequent exposure to visible light, all the operations were performed under a safe light and any delay in plating was avoided.

Crosses were made at 25°C on synthetic crossing medium (WESTERGAARD and MITCHELL 1947) with appropriate supplements.

The scoring of mating type was accomplished by spermatizing both strains *pe fl*; *a* (FGSC No. 568) and *pe fl*; *A* (FGSC No. 569) on synthetic crossing medium. The induction of perithecia in one strain but not on the other after incubation at 25°C for five days was used as the criterion for determining mating type.

Heterokaryons were synthesized from mixed conidial suspensions spotted on VOGEL's (1964) minimal N medium plates. The conidia of the component strains in a suspension were adjusted to an approximate 1:1 ratio (based on haemocytometer counts). Mycelial outgrowths which emerged after two days of incubation at 32°C were carefully transferred to new minimal medium plates for colony development. Single hyphal tips were then isolated from the colonies which developed and maintained as individual cultures on minimal medium. The heterokaryotic nature of such hyphal tip cultures was verified by the observation that the great majority of the uninucleate microconidia (95%) resolved from them failed to grow on minimal medium. The recovery of both of the homokaryotic strains from a heterokaryon also excluded the possibility of reversion in either of the component strains.

RESULTS

Two UV-sensitive mutants (UVS) were obtained by screening approximately 7.5 × 10^3 colonies. The UV survival curves of conidia from the two parental strains (UVS$^+$), and the two UV-sensitive strains are presented in Figure 1. UVS-1 was derived from strain 331m (methionine-requiring), a UV induced auxotrophic mutant of strain 331. UVS-2 was derived directly from strain 331. It is clear that the survival curves for conidia of the parental strains are characterized by a shoulder followed by a region in which survival declines exponentially. The conidia of the mutant strain UVS-1, on the other hand, exhibit strictly exponential kinetics of inactivation. The survival curve for conidia of UVS-2

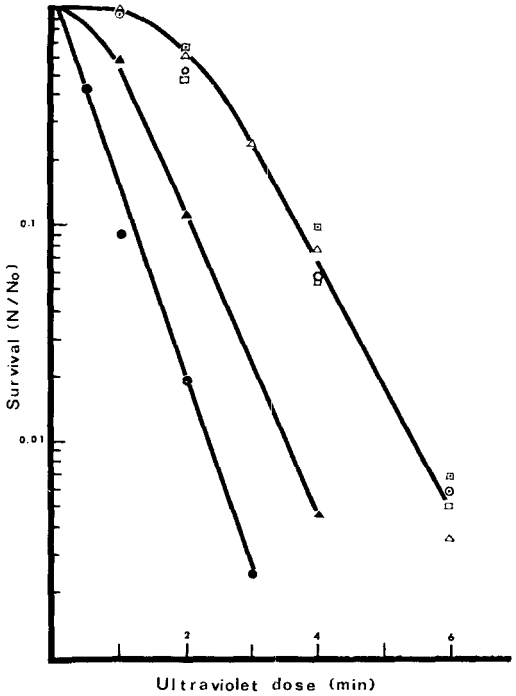

FIGURE 1.—Ultraviolet survival curves of four UVS+ microconidial strains and two UVS mutants. ●—USV-1 (*cr rg; pe fl; me*), ▲—USV-2 (*cr rg; pe fl*), △—331 (*cr rg; pe fl*), ○—331m (*cr rg; pe fl; me*), "Square with dot" —331i (*cr rg; pe fl; inos*), □—338 (*cr; pe fl*).

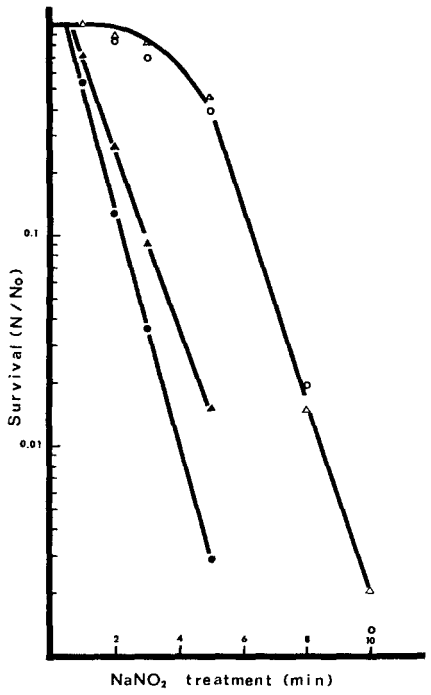

FIGURE 2.—Sodium nitrite (0.01 M $NaNO_2$) survival curves of the two UVS mutants and two UVS+ parental strains. ●—UVS-1, ▲—UVS-2, ○—331m (UVS+), △—331 (UVS+).

exhibits a short shoulder followed by region in which the kinetics of inactivation is exponential. It appears that the slope of the survival curve for conidia of UVS-1 is steeper than the final slope of the survival curve for conidia of the strain from which it was derived (331m). In contrast, UVS-2 yielded a survival curve for conidia with a final slope approximately parallel to that of the strain from which it was isolated (331). Based on the dosage of UV resulting in 37% survival (D_{37}), 331m appears to be approximately five times more resistant to UV than UVS-1, and 331 about two times more resistant than UVS-2.

Cross-sensitivity to chemicals: The survival curves of conidia from various strains following exposure to 0.01 M $NaNO_2$ are presented in Figure 2. Both of the UVS mutants are more sensitive to $NaNO_2$ than are the two parental strains, and UVS-1 is more sensitive than UVS-2. In an experiment using N-methyl-N'-nitro-N-nitrosoguanidine (NG), UVS-1 was also more sensitive to NG than the parential strain 331m (Table 1). It thus appears in the case of UVS-1 that a mutation which leads to a sensitivity to UV also results in a sensitivity to $NaNO_2$ and NG.

Photoreactivation: Since it is known that *Neurospora crassa* is capable of repair-

TABLE 1

Sensitivity of two microconidial strains to N-methyl-N'-nitro-N-nitrosoguanidine (50 μg/ml)

Strains	Surviving fraction after 30 min treatment*	Surviving fraction after 60 min treatment*
UVS–1 (*cr rg; pe fl; me*)	.035	.0028
UVS+ (*cr rg; pe fl; me*)	.21	.035

* Treatment was performed in 0.1 M phosphate buffer at pH 6.0 and terminated by diluting 1:10 into 0.1 M phosphate buffer at pH 7.0.

ing radiation damage upon exposure to visible light (photoreactivation) (GOODGAL 1950), it was necessary to determine whether the damage caused by UV in both the sensitive strains and in the parental strains was photoreactivable. The results of such experiments with UVS-1 and the strain from which it was derived (331m) are given in Figure 3. It is clear that UVS-1 is photoreactivable such that the survival curve for conidia of the mutant subsequent to PR interesects with survival curves for the UVS+ parental strains without PR. The survival curve for UVS-1 after PR maintains its exponential character. Following PR, the survival curve for conidia of the mutant does not coincide or intersect with that of the UVS+ parental strain. The results of PR experiments with UVS-2 were qualitatively equivalent to those obtained for UVS-1.

Genetic basis: The genetic basis of UV sensitivity in one of the mutant strains, UVS-1, was investigated by a heterokaryon test and by tetrad analysis. A heterokaryon was synthesized from UVS-1 (methionine-requiring) and strain 331i

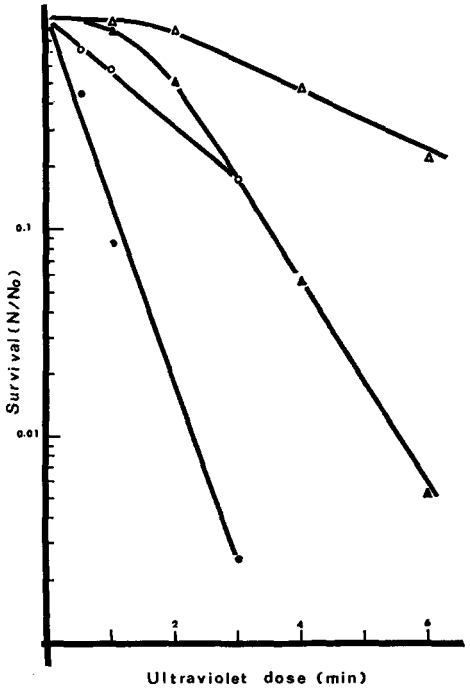

FIGURE 3.—The effect of photoreactivation on the survival of UVS-1 and the UVS+ strain from which it was derived. ●—survival of UVS-1 before PR, ○—survival of UVS-1 after PR, ▲—survival of 331m (UVS+) before PR, △—survival of 331m (UVS+) after PR.

(inositol-requiring), a UV induced auxotrophic derivative of strain 331. From a colony derived from a hyphal tip growing on minimal medium, conidia were harvested and plated on complex medium. Among the eight colonies randomly selected, five were methionine-requiring and three inositol-requiring. The five methionine-requiring colonies exhibited exponential survival curves characteristic of UVS-1 when tested, whereas the three inositol-requiring colonies exhibited shouldered survival curves indistinguishable from that of strain 331i (UVS$^+$). Since none of the isolates recovered from the heterokaryon showed sensitivity intermediate between that of the UVS mutant and the UVS$^+$ parental strain, it was concluded that cytoplasmic factors do not play a role in the mutation leading to UV sensitivity in UVS-1.

The noncytoplasmic nature of the UVS-1 mutation was confirmed by breeding data. UVS-1 was crossed with strain FGSC No. 338 (UVS$^+$; *cr*; *pe fl*). The survival curve for conidia of strain 338 was that typical of UVS$^+$ microconidial strains 331 and 331m (Figure 1). The results from four ordered asci are consistent with 1:1 segregation of a single nuclear gene responsible for UV-sensitivity. No segregants were found that had intermediate sensitivity. (Of the four asci, one had all four products represented among the germinants [Figure 4],

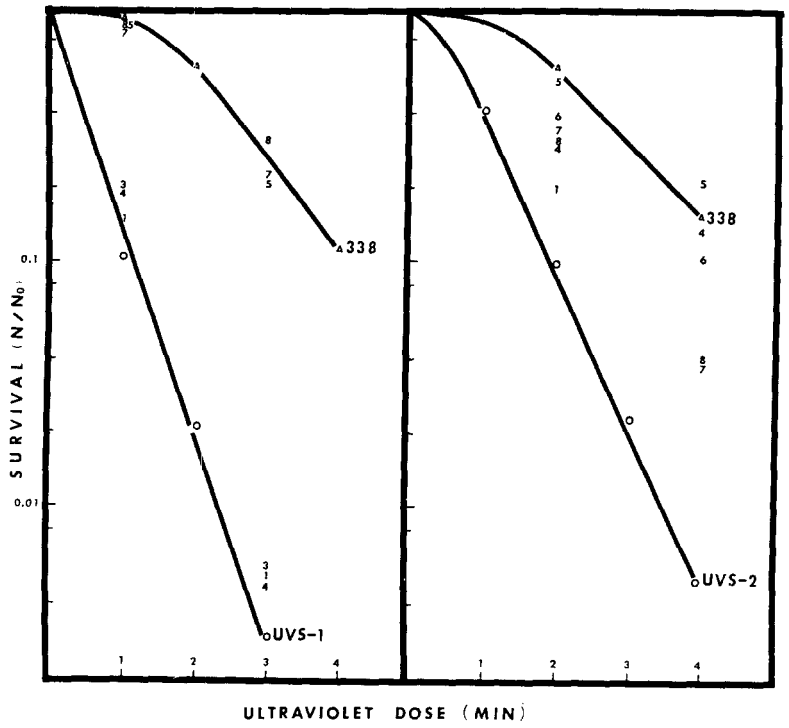

FIGURE 4.—Ultraviolet sensitivity of segregants derived from single asci in the crosses of UVS-1 and UVS-2 with strain 338 (UVS$^+$) as compared to the parental strains (338 served as the protoperithecial strain for both crosses). The order of the segregant in the ascus and its conidial survival at a particular dose are indicated by the numbers.

two had three products, and one had only two products.) These asci indicate that *uvs-1*, *rg*, and *me* are not closely linked.

In contrast to UVS-1, the sensitivity of UVS-2 does not follow Mendelian segregation in a cross between UVS-2 and strain 338 (UVS$^+$). Among the three ordered asci tested, there was a range of UV sensitivity among the segregants derived from a single ascus. A typical segregation pattern for UV sensitivity in a single ascus is represented in Figure 4. It is apparent that none of the segregants exhibited sensitivity characteristic of the original mutant, but rather tended towards normal sensitivity in sharp contrast to the situation involving UVS-1. The protoperithecial parent for this cross was UVS$^+$. Unfortunately, the UVS-2 strain did not produce protoperithecia and the reciprocal cross could not be done. It is significant that conidia from colonies derived from sister ascospores were not identical in their sensitivity to UV.

Effect of uvs-1 *on crossing over:* In an attempt to test the effect of the UVS nuclear gene on recombination, crosses homozygous for *uvs-1* were made with the following combination of marker genes: *pe fl*; *cr a* × *pe fl*; *cr rg A*. This cross led to a high degree of ascus and ascospore abortion which was not observed in equivalent crosses heterozygous or homozygous for *uvs-1*$^+$. Results of tetrads scored for *rg* morphology in the three possible crosses are given in Table 2. It is clear that there is no significant difference in the numbers of tetrads showing second division segregation for *rg* between crosses homozygous and heterozygous for *uvs-1*. However, of the 20 half-tetrads obtained from the cross homozygous for *uvs-1* only one clearly represents second division segregation for *rg*. In this cross either none, one, or two pairs of ascospores in the dissected asci were germinable, the other inviable spore pairs usually being light-colored (99 total asci were dissected). Because of the inviability of spore pairs, in type II half-tetrads,

TABLE 2

Effect of the gene uvs-1 on the segregation of rg *in tetrads*

Crosses*	Types of tetrads						Frequency
rg$^+$ *a*; *uvs-1* × *rg A*; *uvs-1*	I.	*rg*	*rg*	—	—		8
	or	*rg*$^+$	*rg*$^+$	—	—		
	II.	*rg*	—	*rg*$^+$	—		11
	or	—	*rg*	*rg*$^+$	—		
	or	*rg*	—	—	*rg*$^+$		
	III.	*rg*	*rg*$^+$	—	—		1
						Total	20
rg$^+$ *a*; *uvs-1*$^+$ × *rg A*; *uvs-1*		*rg*	*rg*	*rg*$^+$	*rg*$^+$		7
		rg	*rg*$^+$	*rg*	*rg*$^+$		4
						Total	11
rg$^+$ *a*; *uvs-1*$^+$ × *rg A*; *uvs-1*$^+$		*rg*	*rg*	*rg*$^+$	*rg*$^+$		8
		rg	*rg*$^+$	*rg*	*rg*$^+$		4
						Total	12

* All strains carried *pe fl*; *cr*.

TABLE 3

Effect of the gene uvs-1 *on crossing over between* rg *and mating type*

	Crosses*			
	rg⁺ a; uvs-1 × *rg A; uvs-1*	*rg⁺ a; uvs-1⁺* × *rg A; uvs-1*	*rg⁺ a; uvs-1⁺* × *rg A; uvs-1⁺*	Total
Parentals (*rg A* or *rg⁺ a*)				
Observed	14	17	12	43
Expected	15.2	15.9	11.9	
Recombinants (*rg a* or *rg⁺ A*)				
Observed	9	7	6	22
Expected	7.8	8.1	6.1	
Total	23	24	18	65
	$\chi^2 = .510$, df = 2, $.70 < P < .80$			

* All strains carried *pe fl; cr*.

it was not possible to infer whether these tetrads represented first division or second division segregation for *rg*.

Since *rg* and the mating type locus (*mt* or *A/a*) are linked on linkage group I (*rg*: IR; *mt*: IL, FINCHAM and DAY 1965), it was possible to test whether normal crossing over takes place in a cross homozygous for *uvs-1*. The results of a random ascospore analysis from such a cross are given in Table 3, together with crosses homozygous and heterozygous for *uvs-1⁺*. The proportions of recombinant spores in the three experiments did not differ significantly.

DISCUSSION

The shoulders present in the survival curves for conidia of macroconidial strains of *Neurospora crassa* have been attributed to the presence of more than one nucleus in each conidium. By extrapolating the survival curves to the ordinate, the length of the shoulder has been correlated with the number of nuclei in the cells (NORMAN 1954). For microconidial strains which produce predominantly uninucleate conidia, all the UV survival curves for conidia thus far reported exhibit strictly exponential kinetics of inactivation (NORMAN 1954; GILES 1951). GILES (1951) reported that the shape of the survival curve for conidia of a microconidial strain at survival percentages higher than about 60 to 70 exhibited a nonlinear relationship with dose.

We have shown in this study that with the UV dose rate employed one can obtain shouldered survival curves in a microconidial strain (Figure 1). Moreover, our results indicate that the shoulder present in UVS⁺ microconidial strains can be eliminated through mutation to UV sensitivity (UVS-1). Since there are no obvious morphological differences between the UVS mutant strain and the UVS⁺ parental strain, the sensitivity to UV is probably not attributable to structural alterations or changes in pigment systems (LENNOX and TUVESON 1967). Shouldered survival curves imply that UV inactivation of microconidial strains

cannot solely be explained by the "target theory" in which one assumes that a single "hit" in the target (nucleus) inactivates. Recent work with bacteria indicates that there exist repair mechanisms which can be altered or eliminated through mutation to UV sensitivity (see SETLOW 1966). Attempts have been made to demonstrate dark repair by liquid holding (CASTELLANI *et al.* 1964) or by the use of inhibitors (e.g., acriflavine or caffeine; WITKIN 1961) in both UVS and UVS$^+$ strains of Neurospora, in a manner equivalent to that with bacteria. Although all attempts have failed to establish a relationship between UV sensitivity and repair, the possibility that both UVS-1 and 2 represent defects in a dark repair system cannot be excluded.

The cross-sensitivity of UVS-1 and 2 to $NaNO_2$ (Figure 2) and of UVS-1 to N-methyl-N′-nitro-N-nitrosoguanidine (Table 1) seems to indicate the cells are sensitized to a range of inactivating agents by these mutations. Cross-sensitivity to $NaNO_2$ has also been reported by LENNOX and TUVESON (1967) in *Aspergillus rugulosus*, and by LANIER and TUVESON in *Aspergillus nidulans* (1965). Thus the results with Neurospora parallel those with Aspergillus. (Cross sensitivity, of course, has long been known in bacteria).

If one plots the ratio of survivors of UV irradiation in a suspension subsequent to PR over survivors in an unilluminated suspension (N_{PR}/N), against the surviving fraction in the unilluminated suspension (N/N_0), one finds a linear relationship which appears to be identical for the UVS$^+$ and UVS strains (Figure 5). It is concluded not only that the amount of PR is inversely proportional to the initial surviving fraction in both UVS$^+$ and UVS strains, but also that PR is equally efficient in the UVS$^+$ as in the UVS strains. Thus mutation to UV sensitivity is independent of the ability to photoreactivate.

The data from the heterokaryon test with UVS-1 (methionine-requiring) and 331i (inositol-requiring) and from the cross of UVS-1 with strain 338 (*cr*; *pe fl*) support the hypothesis that UV sensitivity in UVS-1 resulted from the alteration

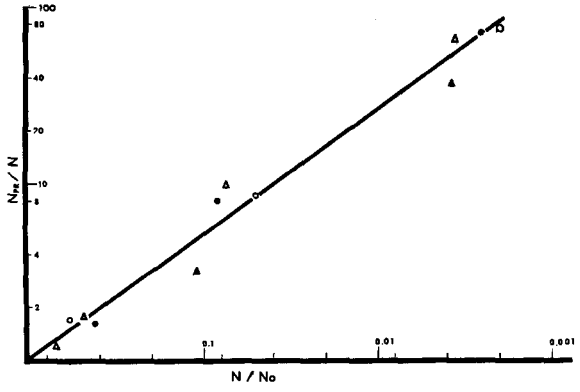

FIGURE 5.—Photoreactivation in two UVS mutants and two UVS$^+$ parental strains at equivalent survival levels. The efficiency of PR at a specific survival level is expressed as the ratio of survival subsequent to PR over survival without PR (N_{PR}/N). ●—UVS-1, ▲—UVS-2, ○—331m (UVS$^+$), △—331 (UVS$^+$).

of a single nuclear gene, *uvs-1* (Figure 4). (The tetrad data also show that the *uvs-1* is not tightly linked to *rg* or *me*.) No information is available as to the possible location of the *uvs-1* and *me* genes. In contrast, UVS-2 probably does not result from the alteration of a single nuclear gene. The cross of UVS-2 with strain 338 yielded progeny which exhibited varying degrees of UV sensitivity tending towards normal sensitivity (UVS^+) (Figure 4). This is of interest since the protoperithecial parent was UVS^+. The possibility that UV sensitivity of UVS-2 was determined by several nuclear genes (multigenic) was ruled out by the observation that sister spores in an ascus may differ in sensitivity (Figure 4). Despite the lack of data from an appropriate reciprocal cross and a heterokaryon test, these data strongly suggest the involvement of non-nuclear factors in determining the sensitivity of UVS-2 (a heterokaryon test was not possible since UVS-2 was derived from a prototrophic strain [331] and heterokaryosis could not be forced). Survival curves for spore cultures from single tetrads isolated in crosses involving mutants UVS-11 and UVS-32 of *Schizosaccharomyces pombe* are consistent with UV sensitivity for these two mutants being non-nuclear in origin (HAEFNER and HOWREY 1967). The data reported for these two mutants are analogous to those reported here for the UVS-2 mutant of Neurospora, except that sister ascospores cannot be tested for UV sensitivity in *S. pombe*.

Crosses homozygous for *uvs-1* were not normal with respect to ascus and ascospore development, compared to crosses homozygous or heterozygous for *uvs-1*$^+$. The inviability of ascospores in asci homozygous for *uvs-1* made it impossible to determine with certainty the effect of *uvs-1* on the segregation of *rg* in tetrads (Table 2). However, random ascospore analyses showed that in crosses homozygous for *uvs-1*, recombination between the linked genes *rg* and *mt* was not significantly affected. It is concluded that *uvs-1* in the homozygous condition affects the development of asci, but that it has no detectable effect on the events leading to crossing over.

SUMMARY

Two ultraviolet-sensitive mutants, UVS-1 and UVS-2, have been isolated from a microconidial strain. In contrast to the shouldered survival curves for conidia of parental microconidial strains, UVS-1 exhibits strictly exponential kinetics of UV inactivation, whereas UVS-2 retains a short shoulder in its survival curve. Both mutants are cross-sensitive to sodium nitrite ($NaNO_2$). UVS-1 was also tested against N-methyl-N′-nitro-N-nitrosoguanidine and is more sensitive than the strain from which it was derived. A heterokaryon test and tetrad analysis clearly indicate that the sensitivity of UVS-1 resulted from the alteration of a single nuclear gene. In contrast, the sensitivity of UVS-2 seems to be non-nuclear in origin. Crosses homozygous for *uvs-1* had no effect on crossing over between the linked genes *rg* and *mt*, although the development of asci and ascospores was affected.

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