INDUCED REPAIR OF GENETIC DAMAGE IN NEUROSPORA

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ABSTRACT

Repair of genetic damage in Neurospora has been studied using a procedure in which one strain is exposed to a potentially lethal dose of UV before being joined in a heterokaryon with an undamaged strain. We have monitored the ability of the second strain to rescue the first. The extent of rescue is greatly enhanced when the rescuing strain has itself received a small, nonlethal dose of UV, thus demonstrating an inducible repair system.—The experiment was modified by substituting X rays or nitrous acid for UV as either the damaging agent or the inducing agent. In every combination, induced rescue was observed.——Three repair-deficient mutants (*uvs-2*, *uvs-3* and *uvs-6*) were substituted for wild type (*uvs*+) as the rescuing component to find out whether any of them lacked the inducible repair system. Both *uvs-2* and *uvs-6* demonstrated inducible repair; *uvs-3* showed none, but gave a high level of repair without induction, suggesting that it is a regulation (derepressed) mutant of an inducible repair system.

THE question of whether enzymatic systems for the repair of genetic damage are induced or constitutive is difficult to study in most organisms, because the experimenter needs to manipulate the treatment that produces genetic damage independently of that which induces the repair activity. The very discovery of dark-repair systems in bacteria owes much to the happy observation of WEIGLE (1953) that such repair was induced in cells that received a nonlethal dose of UV. He separated the damaging treatment from the inducing treatment by using a phage-bacteria system. He observed that phage exposed to a potentially lethal dose of UV before adsorption to bacterial hosts could be rescued if the host cells had themselves received a small dose of UV.

Metabolic systems for genetic repair have been demonstrated in many eukaryotes, including man, mouse, Drosophila, yeast and Neurospora. We noted that an experimental set-up analogous to that used by WEIGLE might be based on a heterokaryon in Neurospora. A heterokaryon is a mycelium (an individual plant) in which two genetically different kinds of haploid nuclei are intermixed in the cytoplasm. Compatible strains of Neurospora form heterokaryons readily when grown in close proximity in liquid cultures or on plates. We decided to ask whether damage inflicted on the nuclei of one of the component strains could be repaired subsequently by the other component after they were brought together in a heterokaryon; and, if so, whether this repair would depend on an inducing treatment given to the second component.

We report here an experiment in which conidia (asexual spores) of a repair-deficient strain were treated with a potentially lethal dose of UV before being allowed to form a heterokaryon with a repair-proficient strain. We found that the extent of rescue was greatly enhanced by exposing the second (rescuing) component to a small dose of UV.

MATERIALS AND METHODS

Strains: Our basic experiment involves the exposure of a vulnerable strain to a potentially lethal dose of UV or another mutagen, followed by the rescue of this damaged strain by a second, undamaged strain in a heterokaryon. For convenience in the tables, we refer to these two components by numbers: the vulnerable component is strain 1; the rescuing component is strain 2. The number is preceded by "S" for a sensitive (repair-deficient mutant) strain, or "R" for a resistant (wild-type repair) strain. S1: *uvs-2 pdx-1 mtr col-4 arg-2 pan-2 a*; R2: *trp-2 col-4 a*. *uvs-2* causes extreme sensitivity to killing by UV (STADLER and SMITH 1968) and is defective in the excision of pyrimidine dimers (WORTHY and EPLER 1973b). *mtr* is a recessive gene for resistance to certain inhibitory analogs of neutral amino acids including *p*-fluoro-phenylalanine (FPA); test media for *mtr* contain 15 μg/ml FPA. *col-4* is a morphological mutant in which the growing hyphae curve and branch excessively, resulting in much slower radial growth of colonies than in wild type. *a* is the mating type allele; two strains must be of the same mating type in order to be compatible for the formation of heterokaryons. *pdx-1*, *arg-2*, *pan-2* and *trp-2* are nutritional mutants for which the respective growth supplements (in μg/ml) are: 5 pyridoxine, 50 L-arginine, 5 pantothenic acid and 50 L-tryptophan (or anthranilic acid). (Preincubation of conidia before heterokaryon formation was carried out in medium containing one-fifth these amounts of supplements.) In the experiments designed to determine which repair system is inducible, the resistant rescuing strain (R2) was replaced by other strains carrying mutations for UV sensitivity: S2–2: *uvs-2 trp-2 col-4 a*; S2–3: *uvs-3 trp-2 a*; S2–6: *uvs-6 trp-2 a*.

Heterokaryon formation: In order to have the greatest chance of detecting induced repair, we felt that it was necessary for the cells of the separate component strains to form heterokaryons quickly on mixing, so that the time between genetic damage and repair would be minimal. Therefore, our first step was to determine whether a pre-incubation of the conidia of the separate cultures would shorten the time required to form a heterokaryon after the mixing of the cultures. Conidia of the two component strains were incubated separately in liquid medium for various lengths of time before mixing. After mixing, the incubation was continued, and samples were withdrawn at intervals to monitor the formation of heterokaryons. Each component strain carried different recessive mutants for nutritional requirements, so that growth on minimal medium signalled the formation of a heterokaryon. 0.2 ml samples of the mixed liquid cultures were suspended in 5 ml minimal sorbose medium (containing 1% agar and kept liquid at 43°) and immediately poured as overlayers on minimal sorbose plates. The numbers of colonies that subsequently appeared on these plates (Table 1) confirmed that pre-incubation shortened the time required for heterokaryon formation after mixing. We found that conidia incubated separately at 33° for 4 hr before mixing were able to form heterokaryons within 1 or 2 hr after mixing.

We do not know the exact time at which the first heterokaryons are formed. We can say only that, when conidia have incubated in separate cultures for 4 hr and then in mixed culture for 1 hr, they have formed a significant number of "persistent pairs" that are not separated in the plating process. Some of these pairs can be separated by sonication (10-sec treatment by a Braunsonic 1510 at 100 w) just before plating. This may mean that a cellular fusion had not yet formed, but it could also indicate newly formed fusion bridges that were still delicate.

As a result of these trials on heterokaryon formation, we chose the following time schedule for the experiments on induced repair of genetic damage: 4 hr of incubation of the separate cultures, followed by UV (or X-ray or nitrous acid) treatment(s) and then immediate mixing, followed by plating 1 hr later (without sonication).

TABLE 1

Pre-incubation of conidia for rapid heterokaryon formation

		Mixed at start	Mixed at 2 hr	Mixed at 4 hr
Plated at:	1 hr	0		
	2 hr	6		
		(5)		
	3 hr	72	38	
		(46)	(10)	
	4 hr	135	110	
		(69)	(56)	
	5 hr	*ca.* 300	*ca.* 300	282
		(*ca.* 200)	(*ca.* 200)	(170)
	6 hr	*ca.* 350	*ca.* 350	*ca.* 350
		(*ca.* 250)	(*ca.* 250)	(*ca.* 250)

Conidia of the two strains (S1 and R2) were incubated separately from "start" in liquid medium in a 34° shaker bath. Both cultures had 2×10^5 cells/ml. The medium was supplemented with pyridoxine, arginine, pantothenate and anthranilic acid. Equal volumes of the suspensions were mixed, and incubation was continued under the same conditions. Aliquots of 0.2 ml (20,000 conidia of each component) were plated on minimal medium; this was a "forcing" medium, so that colonies arising on these plates had to be heterokaryons. Numbers in the table are colonies per plate. Numbers in parentheses represent duplicate samples plated after 10 sec sonication.

Detection of recessive lethal mutations: A sample of the heterokaryons formed in these experiments were tested for recessive lethal mutations in the sensitive component (S1) by growth tests of conidial suspensions on sorbose plate medium supplemented with pyridoxine, arginine and pantothenate and containing the inhibitor FPA. Each conidium contains 1 to 3 (rarely more) haploid nuclei, and thus the conidia produced by a heterokaryon include three genetic types: the two homokaryons and the heterokaryon. Growth on the inhibitor is determined by *mtr*; because this mutant is recessive, the *mtr* homokaryons are the only conidia coming from the heterokaryon that can grow, and the heterokaryotic conidia and the *mtr*+ homokaryons cannot grow. Thus the failure to grow on this medium is the signal that the *mtr* nucleus in the cell that gave rise ot the heterokaryotic colony contained a recessive lethal mutation (STADLER and CRANE 1979).

Samples from a random selection of colonies on the heterokaryon plates were grown in minimal slants, and conidia were then suspended in sterile water for "drop tests" on plates of supplemented sorbose medium containing FPA. A suspension that failed to grow on this medium, while growing on the control plate (minimal), signalled a recessive lethal mutation.

Mutagen treatments: Ultraviolet: 10 ml of a suspension of germinating conidia in growth medium was placed in an open petri dish on a rotator under a germicidal lamp with an output of 1000 ergs/mm²/min.

X ray: the conidial suspension in an open petri dish was placed at such a distance from the 50Kv, 20ma source as to receive 3540 rad/min.

Nitrous acid: The conidial suspension at 33° was brought to pH 4.4 with 0.1M acetate buffer. Freshly dissolved sodium nitrite was added to give a concentration of M/65. Treatment was terminated after two minutes by neutralizing in an equal volume of 0.1M Na_2HPO_4.

RESULTS

The experimental plan was: (1) to start with two heterokaryon-compatible strains of Neurospora, one of which is a repair-deficient mutant (*uvs-2*); (2) to

treat the sensitive strain (S1) with a potentially lethal dose of UV and then put it into a heterokaryon to find out whether it can be rescued by the repair-proficient component (R2); and (3) to find out whether the extent of rescue depends on UV exposure of the repair-proficient component.

Pre-incubated conidia of the sensitive strain were treated with a dose of UV sufficient to kill virtually 100% of them and then mixed with pre-incubated conidia of the resistant strain. Rescue was signalled by the formation of a surviving heterokaryon, which was scored by colony formation on selective (minimal) medium. The results (Table 2) demonstrate UV-induced rescue of cells damaged by UV. The number of rescued nuclei was enhanced several fold by exposure of the resistant conidia to a non-lethal dose of UV. Table 2 gives the results of four experiments out of 12 that all gave similar results. The experimental procedures differed only in the numbers and proportions of conidia of the two strains that were used in the mixtures. There was a high (and unexplained) level of variability in the control (no UV) frequency of heterokaryon formation from one experiment to the next, but this does not diminish the significance of the comparison of the numbers for any single experiment in the last two columns of Table 2. These compare how many cells of the same preparation of UV-killed S1 were rescued with and without an inducing treatment.

We interpret the enhanced number of heterokaryons (in the mixture in which the rescuing component was induced) to result from repair of the genetic damage suffered by the sensitive conidia. An alternative interpretation would hold that the damage was not repaired, but that conidia with recessive lethal mutations had been rescued by becoming part of heterokaryons.

This interpretation can be ruled out on several grounds. It requires the assumption that the inducing dose of UV somehow increases the ability of the resistant conidia to participate in heterokaryon formation. That this is not the case is shown in Table 2 by the comparison of the numbers of heterokaryons formed with no UV to the numbers formed when UV has been administered only to strain R2. A further experiment demonstrated directly that the damaged nuclei had been repaired. A sample of the heterokaryons from the mixture in-

TABLE 2

Induced rescue of UV-damaged nuclei

UV survival†		Cells per plate		Heterokaryons per plate‡			
S1	R2	S1	R2	No UV	UV to R2	UV to S1	UV to both
0/172	182/172	20,000	20,000	*ca.* 500	*ca.* 450	18	112
1/162	85/107	20,000	20,000	*ca.* 600	*ca.* 600	26	134
1/250	172/187	2,500	170,000	*ca.* 475	*ca.* 450	24	90
0/256	94/120	2,500	175,000	74	70	9	31

UV treatments of 2000 ergs/mm² were given just before mixing the separate cultures.

† UV survival was measured on suitably supplemented plates of sorbose medium, each spread with about 200 conidia of the unmixed cultures. Results are shown as fractions, of which the numerators are the numbers of colonies on plates of treated conidia and the denominators are the numbers on the corresponding plates of untreated conidia.

‡ Numbers of heterokaryons are averages of 2 to 4 duplicate plates.

volving induced rescue were isolated and tested for the presence of recessive lethal damage in the S1 component. This strain carries the selectable recessive gene *mtr*, which permits a simple test for the viability of the homokaryotic conidia descending from a heterokaryotic colony (STADLER and CRANE 1979). We isolated and tested 198 rescued heterokaryons from these experiments, and only six were found to carry recessive lethal mutations.

Is the inducible repair system specific to UV damage? Table 3 gives the results of experiments showing that the system is not specific as to either the damaging agent or the inducing agent. Like UV, both X rays and nitrous acid produce damage that can be repaired by the inducible system(s). Induction by either UV or X ray gives repair of damage inflicted by either. We were unable to test induction by nitrous acid, because the acid conditions of this treatment impede subsequent heterokaryon formation, especially when they are applied to the resistant component.

WORTHY and EPLER (1972, 1973a, b) demonstrated that wild-type Neurospora has an efficient system for the excision of pyrimidine dimers from its DNA and that *uvs-2* (the sensitive mutant used in the present study) lacks this repair system. Studies of other sensitive mutants have shown that there is at least one other repair pathway (a "mutation-prone" system), and perhaps more (SCHROEDER 1975). Which repair system is being induced in our heterokaryon experiment? It could be any of the normal repair systems, as they are all intact in our resistant strain, R2.

We have done a series of experiments in which strain R2 was replaced in its rescuing role by strains carrying mutations for UV sensitivity. The three mutants tested (*uvs-2*, *uvs-3* and *uvs-6*) may have lesions in three different repair pathways (SCHROEDER 1975; KÄFER 1980). In these experiments (Table 4a), the inducing UV was a very low dose, causing little kill even of the sensitive cells. Control experiments showed that even these very low doses were sufficient to in-

TABLE 3

Genetic damage and induced rescue involving agents other than UV

Treatment†		Treatment survival‡		Cells per plate		Heterokaryons per plate*		
S1	R2	S1	R2	S1	R2	Neither treated	S1 treated	Both treated
8X	1X	2/187	200/207	1,700	170,000	128	8	29
2NA	2UV	41/184	227/184	20,000	20,000	148	20	73
8X	1X	1/172	77/132	3,000	170,000	103	11	26
8X	2UV	1/172	90/132	3,000	170,000	103	11	35
2UV	1X	3/172	77/132	3,000	170,000	103	8	27
7.5X	1X	1/246	161/151	2,500	220,000	325	31	54
7.5X	2UV	1/246	138/151	2,500	220,000	325	31	80
2UV	1X	3/246	161/151	2,500	220,000	325	32	73

* Numbers of heterokaryons are averages of 2 to 4 duplicate plates.

† Treatments are shown as times (in minutes) of exposure to X rays (X), ultraviolet (UV) or nitrous acid (NA). X ray dose rate was 3540 rad/min; UV dose rate was 1000 ergs/mm^2/min.

‡ See footnote in Table 2.

duce repair activity in resistant strain R2. The results showed that inducible repair was clearly still present in the strain carrying the *uvs-6* lesion.

The experiments involving rescue by *uvs-3* showed no evidence of inducible repair. We conclude that the inducible system requires *uvs-3*+ function. These experiments had another interesting result: the level of *uninduced rescue* (heterokaryons formed when only S1 received UV) was high, somewhat greater than that achieved by the wild-type rescuing strain (R2). This unexpected result was confirmed in an experiment in which these two rescuing strains (R2 and S2-3) were directly compared for their ability to rescue samples from the same population of UV-treated S1 cells, with and without induction (Table 4b, last two columns).

The results with *uvs-2* (the same mutant gene as in strain S1) in the rescuing strain were variable, with two experiments showing a small amount of induced rescue, three others showing none. One possibility was that the inducible repair system was intact in *uvs-2*, but was difficult to demonstrate in this extremely UV-sensitive strain. The *uvs-2* mutant has only an intermediate sensitivity to

TABLE 4a

Induced rescue by UV-sensitive mutants

	Inducing dose (ergs/mm2)	UV survival†		Heterokaryons per plate‡		
		S1	S2 or R2	No UV	UV to S1	UV to both
Control: low-dose induction of *uvs*+ (strain R2):	125	1/129	142/136	300	19	37
	250	1/219	154/136	300	19	55
	2000	1/219	133/136	300	19	74
Rescue by *uvs-6* (strain S2-6):	250	0/205	70/93	150	17	60
	500	0/205	44/93	150	17	61
	250	0/108	90/111	72	16	28
	500	0/108	74/111	72	16	33
Rescue by *uvs-3* (strain S2-3):	167	3/144	38/67	81	31	31
	333	3/144	28/67	81	31	31
	250	0/107	63/90	77	41	28
	500	0/107	28/90	77	41	18
	125	0/131	85/93	128	50	56
	250	0/131	66/93	128	50	45
Rescue by *uvs-2* (strain S2-2):	125	1/172	183/184	78	3.5	4.3
	125	2/150	168/165	81	6	11
	250	2/182	94/195	97	4.2	16.7
	250	0/126	80/93	43	2.5	3
	125	0/126	94/93	43	2.5	2.5

Each heterokaryon plate was inoculated with a mixture of 2500 cells of S1 and 17×10^4 of R2 or S2.

† See footnote in Table 2. Strain S1 received a dose of 2000 ergs/mm² in each experiment.

‡ Numbers of heterokaryons are averages of 2 to 4 duplicate plates.

TABLE 4b

Comparison of rescue by uvs+ *(R2) and by* uvs-3 *(S2-3)*

	Inducing dose (ergs/mm2)	UV survival†		Heterokaryons per plate‡		
		S1	R2 or S2-3	No UV	UV to S1	UV to both
Rescue by R2:	1000	3/244	97/160	500	112	231
Rescue by S2-3:	250	3/244	30/59	430	221	222

† See footnote in Table 2.
‡ See footnote in Table 4a.

TABLE 4c

X-ray induced rescue by uvs-2 *(strain S2-2)*

Treatment*		Treatment survival†		Cells per plate		Heterokaryons per plate‡		
S1	S2-2	S1	S2-2	S1	S2-2	Neither treated	S1 treated	Both treated
2UV	0.5X	4/274	168/161	2000	18×10^4	138	11	26
2UV	1X	4/274	191/161	2000	18×10^4	138	11	33

* See footnote in Table 3.
† See footnote in Table 2.
‡ See footnote in Table 4a.

X rays (SCHROEDER 1975). For this reason we repeated the experiment, using a small dose of X rays for the induction. The result (Table 4c) shows inducible rescue activity is indeed present.

The numbers of heterokaryons reported in Tables 2, 3 and 4 represent colony counts made after two days of incubation (one at 33°, followed by one at 22°). We noted that, after another day (at 22°), more colonies appeared on the plates involving rescue of UV-damaged strain S1, both with and without inducing treatment of R2, but no more appeared on the control (no UV) plates (see Table 5). The significance of these late heterokaryons is unclear, but whether or not they are included, there is clear evidence of induced rescue.

DISCUSSION

Our experiments demonstrate that Neurospora has an inducible system for the repair of genetic damage. Studies of UV-sensitive mutants have led to the

TABLE 5

Delayed rescue

Incubation time	UV survival†		Heterokaryons per plate		
	S1	R2	No UV	UV to S1	UV to both
2 days	0/126	94/121	147	18	61
3 days	0/126	94/121	148	28	86

UV treatments: S1: 2000 ergs/mm^2; R2: 1000 ergs/mm^2.
† See footnote to Table 2.

conclusion that there are several repair systems in Neurospora (SCHROEDER 1975). We have carried out experiments with three of these mutants (*uvs-2*, *uvs-3* and *uvs-6*) in order to find out which lacks the inducible system. *uvs-2* fails to excise dimers from UV-treated DNA; this strain is extremely sensitive to UV. *uvs-6* excises dimers, but may be defective in a later stage of excision repair. *uvs-3* may represent an "error-prone" repair system, as indicated by the failure of strains mutant at this locus to produce UV-induced mutations.

Our experiments show that the *uvs-6* locus is not involved in an inducible repair system: the strain mutant at this locus was still capable of induced rescue of damaged genetic material. The results with *uvs-2* and *uvs-3* merit further consideration.

The experiment in which the rescuing component carried the mutant *uvs-3* had two interesting results: (1) without induction, this strain was much more effective than wild type in rescuing the UV-damaged component, and (2) the amount of rescue by *uvs-3* was not increased by induction. *uvs-3* has been of interest because of the observation that this strain does not show UV-induced mutation (DE SERRES 1980). This property suggested that the mutant was similar to *recA* in *E. coli* (WITKIN 1969) and *rad6* in yeast (LAWRENCE and CHRISTENSEN 1976), in which this behavior led to the conclusion that the strain has a lesion in an error-prone repair system and that this error-prone DNA synthesis is involved in the generation of mutants in wild-type cells following UV treatment. The situation in *uvs-3* is complicated by the further observation (DE SERRES, INOUE and SCHÜPBACH 1980) that this strain has a very high frequency of *spontaneous* mutation, 20-fold higher than that of wild type. This finding, coupled with our observation of rescue without induction, leads us to propose that the *uvs-3* locus is responsible for the normal repression of an inducible, error-prone repair system, and that the *uvs-3* mutant is derepressed (constitutive) for this system. We suggest that wild type fails to produce spontaneous mutants, not for lack of lesions in the DNA, but for lack of the mutation-producing (error-prone) repair system. It seems surprising that UV treatment does not elicit further mutation in strains carrying *uvs-3*; we might expect that a repair system that processes spontaneous lesions to produce mutations could do the same with UV lesions. However, it is possible that the constitutive function of this repair system in the *uvs-3* strain renders it unavailable for effective action in response to UV-induced lesions. This could explain the increased sensitivity to UV in this strain and its failure to convert UV lesions into mutations.

The experiment in which *uvs-2* was the rescuing component was run several times with UV induction (Table 4). In most cases the results were negative, but two experiments gave evidence of a small amount of induction. If the results had been consistently negative, we might have concluded that the *uvs-2* locus was involved in an inducible repair system, perhaps regulated by a repressor from the *uvs-3* locus. However, we wish to propose an alternate interpretation. *uvs-2* is extremely sensitive to UV, far more than any other mutant yet described in Neurospora (SCHROEDER 1975; DE SERRES 1980). Biochemical studies have shown

that this strain fails to excise pyrimidine dimers from UV-treated DNA (WORTHY and EPLER 1973b).

We propose that excision repair is a constitutive system, and the *uvs-2* product plays a role in it. However, mutation at *uvs-2* could lead *indirectly* to the partial failure of an inducible repair system as well. UV-treated *uvs-2* cells might be unable to produce a functional product of their inducible system, because they would have to transcribe or translate a gene or messenger with unexcised dimers in it. This could explain why our experiments on induced rescue by *uvs-2* gave mostly negative results. It could also explain why the S1 component in our heterokaryons (*uvs-2*) was unable to provide its own induced repair. In this regard it may be noted that the ideal strain for the S1 component in our basic experiment would have been one that lacked *all* DNA repair systems; that would have insured that any inducible system in the rescuing component would have been revealed in the result. Such a totally defenseless strain might be constructed by crosses to produce the appropriate multiple mutant with at least one genetic lesion in each repair system, as has been done in yeast (Cox and GAME 1974). Since we did not have such a strain in Neurospora, we chose *uvs-2*, as it shows a much greater sensitivity to UV than any of the other mutants. In retrospect, we see that *uvs-2* was especially suitable for this role; even though it has only a single genetic lesion, it may fail in other repair functions after UV treatment.

The conclusion that induced repair is intact in the *uvs-2* strain is strengthened by the observation that repair activity can be induced by X-ray treatment (Table 4c). This finding also suggests another interpretation of our failure to induce *uvs-2* with UV. Perhaps the specific inducer of the inducible repair system is DNA containing single-strand gaps, as has been proposed in *E. coli* (BALUCH, CHASE and SUSSMAN 1980). In wild type, this inducer would be produced after UV treatment by the action of the dimer excision system. *uvs-2*, lacking dimer excision, could not produce the inducer after being treated with UV. However, X rays might produce these inducing lesions even without repair activity.

The possibility that the *uvs-2* gene produces a constitutive product is supported by a different kind of heterokaryon experiment performed much earlier (STADLER and SMITH 1968). The conidia produced by a heterokaryon are a mixture of three genetic classes: the two homokaryons and the heterokaryon. A heterokaryon was constructed between a *uvs-2* strain and a *uvs*$^+$ strain. It was observed that all three classes of conidia had wild-type resistance to killing by UV, even the homokaryons that were *genetically* sensitive. This meant that a *uvs-2*$^+$ gene product had been distributed to all the condia during their formation, even though there had been no inducing treatment at that time.

Our observation that X rays and nitrous acid are cross-reactive with UV with regard to induced repair suggests that a single repair system can work on lesions produced by these different mutagens. This is consistent with the observations of SCHROEDER (1970, 1974) that *uvs-2* and *uvs-3* are sensitive to both UV and X rays. *uvs-2* is very sensitive to nitrous acid (D. STADLER and E. CRANE, unpublished). *uvs-3* has not been tested for nitrous acid sensitivity.

In our basic experiment (Table 2), the mixture without induction produced only about one-fourth as many rescued heterokaryons as the mixture in which the rescuing component had received an inducing treatment. This suggested that the majority of the rescuing action came from induced (as opposed to constitutive) repair. This is rather surprising. The extreme UV sensitivity of the mutant strain lacking excision repair (*uvs-2*) might lead to the conclusion that this is the major repair pathway in Neurospora. If, as we suggest, this is a constitutive system, its poor showing in our experiment must be explained. We propose that the timing is responsible. The heterokaryons do not form quickly enough for the rescuing strain to reach the damaged genomes in time for effective excision repair. Induced repair, on the other hand, is still effective, and this may mean that it is a post-replication system, like induced repair in bacteria (WITKIN 1976).

ATWOOD (1954) and NORMAN (1954) suggested long ago that products of repair genes from one nucleus in a Neurospora heterokaryon might correct damages in another nucleus. They proposed such interaction to account for the survival patterns of heterokaryotic conidia after UV treatment. More recently STADLER and SMITH (1968) and SHELBY, STINE and DE SERRES (1975) showed that conidia of *uvs* mutants of Neurospora displayed increased resistance to UV if they had been formed by a heterokaryon that contained the wild-type allele in the other component. SHELBY, STINE and DE SERRES (1975) demonstrated this same kind of transfer of gene products between nuclei for repair following ionizing radiation.

In mammalian cells, there is evidence for inducible genetic repair from experiments analagous to those of WEIGLE (1953). Both survival and mutation in Herpes virus are enhanced if the host cells have received an inducing dose of UV (DASGUPTA and SUMMERS 1978).

There is indirect evidence of induced repair in lower eukaryotes and in mammalian cells from "split-dose" experiments. A small dose of UV, some hours prior to the main exposure, is found to give enhanced survival or other evidence of repair activity. Experiments with UV in yeast showed that dose splitting gave enhanced mutation frequency and resistance to killing (ECKHARDT, MOUSTACCHI and HAYNES 1978). AUERBACH and RAMSAY (1972) observed that the spectrum of mutation (relative frequencies of reversion at two different loci) produced by UV in Neurospora could be drastically altered by pre- or post-treatment with any of several chemical mutagens. They pointed out that this effect might be mediated by the action of the chemical mutagens on a repair process.

D'AMBROSIO and SETLOW (1976) monitored post-replication repair in hamster cells by the rate at which the small molecules of DNA synthesized after UV treatment were converted to large molecules. They concluded from split-dose experiments and from cycloheximide treatments that this process was induced and that it required protein synthesis. This experiment was repeated by PAINTER (1978), who confirmed the result, but suggested that the large labeled molecules seen after split-dose treatment were *not* the product of post-replication repair.

The repair of double-strand breaks produced by ionizing radiation was monitored in Ustilago by LEAPER, RESNICK and HOLLIDAY (1980). They found that

cycloheximide treatment immediately after irradiation impeded this repair, and it also lowered the survival frequency. They concluded that this was a case of induced repair that required protein synthesis.

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