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ANOTHER TWO GENES CONTROLLING MITOTIC INTRAGENIC RECOMBINATION AND RECOVERY FROM UV DAMAGE IN *ASPERGILLUS NIDULANS*

III. PHOTOREACTIVATION OF UV DAMAGE IN *uvsD* AND *uvsE* MUTANTS

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SUMMARY

In this paper photoreactivation of UV damage in growing *uvs*$^+$, *uvsD53* and *uvsE82* conidia of *Aspergillus nidulans* is examined. The results indicate that *uvs*$^+$ and *uvsE82* conidia immediately start to lose photoreactivable lesions following incubation in the dark, but that *uvsD53* conidia retain these lesions for at least several hours. The observations are interpreted as indicating that *uvs*$^+$ and *uvsE82* conidia are excision-efficient but that *uvsD53* is defective in excision repair.

INTRODUCTION

It has been shown for bacteria that a major part of the injury caused by UV irradiation consists of pyrimidine dimers in the DNA[13,16]. These dimers can be cleared away in several ways. One way is excision of a stretch of single-stranded DNA containing the dimer, followed by repair replication to fill the resulting gap (excision repair[14]). Enzymatic photoreactivation (PR) is the process whereby the pyrimidine dimers are enzymatically split under the influence of near-UV light[4,5,13,16]. It has recently been found that near-UV light (with wavelengths of about 313 nm) can probably also photochemically revert pyrimidine dimers[8,18]. Recombination repair, as a fourth means to recovery from UV damage, may indirectly repair UV damage: replication of DNA containing dimers results in gaps in the new strands, and the gaps are closed by a process involving recombination with a sister DNA strand[6].

The extent of the reactivation of UV-damaged cells can be modified by several treatments. From bacterial investigations liquid holding recovery and photoprotection are known[16]. Liquid holding recovery is the phenomenon that UV-irradiated cells which are held in a liquid medium not supporting growth show an enhanced survival, probably as a result of the prolonged time available for excision repair. Photo-

Abbreviations: PR, photoreactivation; SGM, minimal medium supplemented with required growth substances and containing 30 mg/l G3300; SLSGM, semi-liquid SGM.

protection is the phenomenon that the survival of UV-irradiated cells is enhanced by illumination with PR light preceding UV irradiation. Photoprotection is probably due to a light-induced delay of cellular processes, which may favour the repair of UV-induced lesions in the dark.

The fact that liquid holding recovery has been found in UV-irradiated mutants of *Aspergillus nidulans*[20] suggests that excision repair may occur in *A. nidulans*. Neither excision repair nor pyrimidine dimers as such have, however, ever been demonstrated directly in *A. nidulans*. The reactivating effects of light have as yet never been examined either, though PR of UV-induced mitotic recombination in *A. nidulans* has been reported[9]. Enzymatic PR has been found in the related fungus Neurospora, from which organism an enzyme has been extracted that is able to split pyrimidine dimers[17].

If repair under the influence of near-UV light and excision repair affect the same lesions, they may act competitively. It may then be expected that repair of UV damage by excision results in a loss of photoreactivability and also that the photoreactivability is retained if the excision mechanism is defective. This has been examined by HARM[3] who found in *Escherichia coli* that a strain that was UV-sensitive because of an excision defect, in contrast to a UV-resistant strain, retained its photoreactivability when kept in buffer.

The persistence of photoreactivability in a UV-sensitive strain may therefore point to an excision defect. This idea has been taken as the basis for several studies with yeast[12,19], and served also as the starting point for the present study. I examined the photoreversal of UV damage in the UV sensitive strains *uvsD53* and *uvsE82* and in the non sensitive strain *uvs*$^+$ of *Aspergillus nidulans*. From earlier studies the view had evolved that the *uvsD* mutant is defective in excision repair and that the *uvsE* mutant is deficient in recombination repair[1,2].

MATERIALS AND METHODS

Strains

The strains used were UT 437 (*uvs*$^+$), UT 517 (*uvsD53*) and UT 531 (*uvsE82*). The full genotype of these strains is given in ref. 1. The strains required proline, *p*-aminobenzoic acid, biotin and pyridoxine hydrochloride for their growth.

Media

Media have been described in previous papers[1,2].

PR of UV damage

Conidial suspensions were prepared as described earlier (ref. 2, section on X-ray irradiation). About 10^7 resting conidia were UV-irradiated for 240 sec in 10 ml SLSGM in an open petri dish (diameter 9 cm) under the conditions described[1]. The irradiated conidia were plated in layers of 5 ml SGM on top of several series of plates of the same medium. The plates were then incubated in the dark. After various periods of incubation in the dark (0–6 h), plates of a series were illuminated with PR light for 1 h under the conditions described below. The illuminated plates were further incubated in the dark until, after about 3 days, colonies could be counted. One pair of plates of the series was not illuminated with PR light and served as a control.

To find whether the PR mechanism of the plated conidia remained active during incubation, the conidia on another series of plates were given a second dose of UV light (30 sec for *uvsD53*, 60 sec for *uvsE82* and *uvs*$^+$) after various periods of incubation in the dark (0–6 h) immediately before being illuminated with PR light for 1 h. After that they were further incubated in the dark. Control plates were incubated in the dark after the second UV irradiation without having been illuminated.

To establish whether illumination with PR light could play any indirect part in the reactivation of the UV-irradiated conidia, the photoprotecting effect of illumination with PR light was determined. Plates containing non-UV-irradiated conidia were illuminated with PR light for 1 h, then UV-irradiated for 240 sec, and subsequently incubated in the dark. Control plates were incubated for 1 h in the dark before being UV-irradiated.

In separate experiments the effect of PR light only was determined. For that purpose conidia that had not been UV-irradiated were treated with 1-h doses of PR light during subsequent periods of their growth.

All incubations were at 37°. Sets of two plates were used for each treatment. Manipulations were carried out in dim yellow light. All experiments were repeated several times.

Illumination with PR light

Plates were exposed to PR light for 1 h at 37° at a distance of 8 cm from two 40-W OSRAM-L fluorescent tubes (maximal output at a wavelength of 365–370 nm). A dose of 1 h of PR light at 37° is sufficient to induce maximal PR in *uvs*$^+$. The intensity of illumination, as measured by potassium ferrioxalate actinometry[7], was about 280 erg/mm^2/sec at the level of the plates.

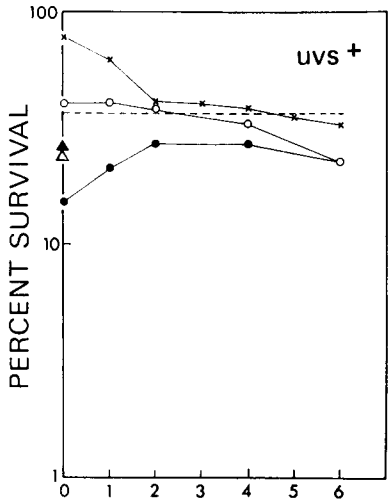
RESULTS AND DISCUSSION

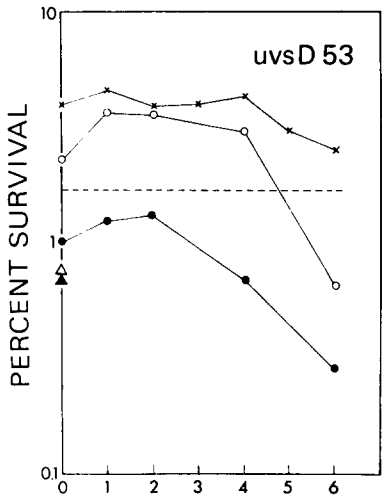
The experiments to be discussed were designed to study PR of UV-damaged *uvs*$^+$, *uvsD53* and *uvsE82* conidia during incubation in the dark. Dormant conidia were successively UV-irradiated, incubated in the dark at 37° for various periods, illuminated with PR light for 1 h at 37°, and further incubated in the dark. The survival measured as the colony-forming ability was determined. The results were reproducible. Figs. 1–3 give the results of single experiments.

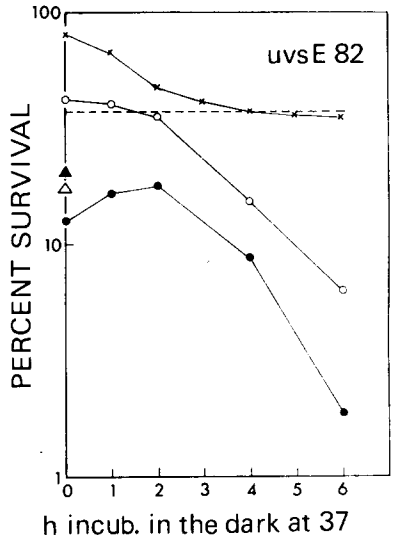
The figures show that the survival of UV-irradiated dormant conidia is enhanced by 1 h of PR light immediately after UV irradiation. The factor by which the survival is enhanced is about equal for the three strains. This would imply, since dormant *uvsD53* conidia are more UV-sensitive than are dormant *uvs*$^+$ or *uvsE82* conidia, that the dose-modifying influence of PR light treatment is smaller for UV-irradiated *uvsD53* than for UV-irradiated *uvs*$^+$ or *uvsE82* conidia. But maybe the anomaly is due to *uvsD53* not being maximally photoreactivated after 1 h of PR light while *uvs*$^+$ and *uvsE82* are.

The important thing shown by the experiments is that the photoreactivability of UV-irradiated *uvs*$^+$ and *uvsE82* conidia instantly declines following incubation in the dark, but that the photoreactivability of *uvsD53* conidia remains unchanged for about 4 h before decreasing.

The reactivating effect of PR light was probably due to direct PR. If the effect







Figs. 1–3. PR of UV damage in *uvs*$^+$, *uvsD53* and *uvsE82*. The survival measured as the colony-forming ability of conidia was determined. The treatments were: ---, dormant conidia were successively UV-irradiated for 240 sec, plated on SGM and incubated in the dark at 37° until colonies could be counted; ×——×, conidia were treated as indicated for ---, and in addition illuminated for 1 h at 37° with PR light at various times after the onset of incubation in the dark; ●——●, conidia were treated as indicated for ---, and in addition irradiated with a second dose of UV light (30 sec for *uvsD53*, 60 sec for *uvs*$^+$ and *uvsE82*) at various times after the onset of incubation in the dark; ○——○, conidia were treated as indicated for ●——●, and in addition illuminated with PR light for 1 h at 37° immediately after the second UV dose; ▲, dormant conidia were plated in SGM, incubated for 1 h at 37° in the dark, UV-irradiated for 240 sec and further incubated in the dark until the colonies could be counted; △, conidia were treated as indicated for ▲, except that during the first hour after plating they were illuminated with PR light before UV irradiation.

of PR light had been due to a modification of cellular conditions, then illumination of conidia with PR light before UV irradiation could have been expected to have a reactivating effect too. But the figures show that conidia that have been irradiated for 1 h with PR light before being UV-irradiated, have about the same survival as conidia that were incubated for 1 h in the dark before UV irradiation. This shows that photoprotection did not occur and that PR light may exert no indirect reactivating effect.

The decline of the photoreactivability of UV-irradiated *uvs*$^+$ and *uvsE82* conidia during incubation in the dark may be due to a reduction in the number of photoreactivable lesions in these conidia. In the first place, the diminished photoreactivability is not likely to result from a harmful effect of PR light on the conidia because in separate experiments it was found that irradiation with PR light did not affect the viability of non-UV-irradiated *uvs*$^+$ and *uvsE82* conidia which had been incubated in the dark for various periods before illumination.

Secondly, in all probability the loss of photoreactivability of *uvs*$^+$ and *uvsE82* conidia cannot be attributed to a reduction in the activity of the PR enzyme. As can be seen in the figures, illumination of conidia that had been UV-irradiated and had been kept in the dark, led at any time during incubation to an enhancement of survival, if at least the PR-light treatment had immediately been preceded by the administration of another UV dose to the conidia. *uvs*$^+$ conidia formed an exception in that they showed PR ability until only 4–5 h of incubation in the dark. This could suggest that the PR system is inactivated in the wild-type strain during germination, but it is more probable that it indicates that the PR processes compete with other kinds of repair processes (excision and/or recombination repair) in *uvs*$^+$.

Our results suggest that photoreactivable lesions are excised from the DNA in UV-irradiated *uvs*$^+$ and *uvsE82* conidia during incubation in the dark and that excision of photoreactivable lesions is absent or delayed in *uvsD53* conidia. This interpretation is supported by observations made by HARM[3] that an excision-deficient mutant of *Escherichia coli*, when UV-irradiated and kept in buffer in the dark, remained photoreactivable, whereas an excision-efficient strain lost its photoreactivability under these conditions. This suggested that the loss of PR ability of UV damage in the bacteria was due to excision of photoreactivable lesions from the DNA. Others[11], however, have found that an excision-defective strain of *Escherichia coli* also lost its PR ability, though much slower than did the excision-efficient strain. In this case the bacteria were grown in a growth-supporting medium, and the loss of PR ability in the excision-deficient strain has been demonstrated to be dependent upon DNA replication. The same explanation may hold for the eventual decrease of PR in UV-irradiated *uvsD53* conidia in a growth-supporting medium as it has been shown that in Aspergillus conidia replication starts only after about 4 h of growth[15]. There are no indications that *uvsD53* conidia grow slower than do *uvs*$^+$ or *uvsE82* conidia, so the delay of the decrease of PR in *uvsD53* conidia is probably not due to a delayed growth. The conclusion may therefore be that the delayed loss of PR of UV damage in *uvsD53* conidia indicates that the *uvsD* mutant of *Aspergillus nidulans* is excision-deficient. This supports the view expressed in earlier papers[1,2].

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