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The effects of dark holding and photoreactivation on ultraviolet light-induced mitotic recombination and survival in yeast

By JAMES M. PARRY* AND B. S. COX
Botany School, South Parks Road, Oxford

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1. INTRODUCTION

Dark recovery from ultraviolet light in bacteria has been postulated as an enzyme-mediated excision and repair process involving the removal of radiation induced lesions of the DNA (Boyce & Howard-Flanders, 1964; Petijohn & Hanawalt, 1964; Setlow & Carrier, 1964). UV-induced pyrimidine dimers may also be removed by the action of photoreactivating light (Rupert, 1964; Setlow, Boling & Bollom, 1965).

A dark recovery mechanism in yeast has been demonstrated by Patrick, Haynes & Uretz (1964). They showed that incubation for periods of up to 4 days in distilled water or a phosphate buffer in the dark after UV treatment resulted in an increase in cell viability and a reduction in the frequency of petite colonies present among the survivors.

In a previous publication (Parry & Cox, 1965) we demonstrated that treatment with photoreactivating light decreased UV light-induced cell death and intragenic recombination but had no influence on intergenic recombination in *Saccharomyces cerevisiae*. This paper describes experiments investigating the effect of dark holding on survival, intragenic and intergenic recombination in UV-treated cultures of the same organism. The UV-treated cultures were also exposed to photoreactivating light before and after periods of dark holding so that the interaction of the two mechanisms of repair could be studied.

2. METHODS AND MATERIALS

Strains. The diploid yeast used in the experiments had the following genotype:

$$\text{J20–10} \times \text{126W59} = \frac{a}{\alpha}\dagger\frac{ad_{2,1} \; + \; hi_8}{ad_{2,c} \; se_1 \; +} \; \frac{+}{me_2} \; \frac{tr_1}{tr_1}.$$

The markers ad_2, se_1 and hi_8 are linked on chromosome XIII (Mortimer & Hawthorne, 1963). The diploid was derived from strains originally provided by Dr D. C. Hawthorne. It forms red colonies on complete medium and requires adenine and tryptophan for growth.

* Present address: Department of Genetics, University College of Swansea, Singleton Park, Swansea.

† Abbreviations used are: *a*/*α*, mating-type; *ad*, a gene determining a requirement for adenine (adenineless); *hi*, histidineless; *me*, methionineless; *se*, serineless; *tr*, tryptophanless; UV, ultraviolet light from the lamp described.

A mixed sample of white adenine-independent revertants derived from this diploid was used in a reconstruction experiment.

Media. The complete medium used (YC) was described by Cox & Bevan (1962). It is a yeast extract and peptone medium with 4% (w/v) glucose, pH 6·7. The minimal medium (YNB) was Difco Yeast Nitrogen Base without amino-acids, solidified with Oxoid ionagar and supplemented with growth factors as necessary.

UV treatment. The source of ultraviolet light was a Hanovia model 11 mercury discharge tube generating almost monochromatic radiation at 2537 Å. The dose rate was determined by the technique of Hatchard & Parker (1956) using a chemical actinometer. Cultures were grown for three days on solid YC medium, washed off and suspended in saline at a titre of approximately 10^7 cells/ml. These cultures are at the end of log phase and contain less than 5% of budded cells. 10 ml. portions were exposed to UV in an open Petri dish. All manipulations were carried out in red light.

Dark holding. UV-treated suspensions were stored in saline in light-proof bottles at 28 °C.

Visible light treatment. Suspensions of cells in saline were exposed to a 500 W Philips photoflood bulb at a distance of 5 cm. The sample bottles were immersed during the treatment in 5% (w/v) copper sulphate solution held at 20 ± 2 °C.

Incubation and scoring. All plated cultures were incubated at 28° in the dark and scored after 5 day's growth. Survival was scored by counting colony-forming units on YC medium. Intragenic recombination was scored by counting adenine independent colonies on adenineless YNB. Intergenic recombination was scored by testing colonies growing on the YC plates for auxotrophy due to the formation of recessive homozygotes of the hi_8, se_1 and me_2 genes by mitotic crossing-over. Colonies were subcultured and replicated to YNB omission media.

Experiments. In the first series of experiments the cultures were exposed to various UV doses at a dose rate of 43 ergs/mm²/sec. Suitable dilutions of the treated cultures were plated on appropriate media immediately after UV exposure and after 1, 5 and 8 days of dark holding. Portions of each sample were also treated with 5 min of visible light immediately before plating. Thus the treatments given before plating were as follows:

(1) UV alone;
(2) UV + 5′ visible light;
(3) UV + dark holding;
(4) UV + dark holding + 5′ visible light.

In the second series of experiments, the cultures were exposed to various doses of UV at a dose rate of 43 ergs/mm²/sec and then divided into three samples. These were respectively untreated or treated with 5 or 30 min of visible light. Portions of each were diluted and plated immediately and then all three were stored in the dark. Samples were taken for dilution and plating after 2 and 5 day's dark holding. The treatments given were thus:

(1) UV alone;
(2) UV + dark holding;

(3) UV + 5′ visible light;
(4) UV + 5′ visible light + dark holding;
(5) UV + 30′ visible light;
(6) UV + 30′ visible light + dark holding.

A reconstruction experiment was also performed comparing the effects of UV exposure and dark holding in a culture of J20–10 × 126W59 and a mixed sample of adenine independent revertants spontaneously arising in it.

All experiments were performed at least twice. The results of the first series of experiments reported here are combined from two experiments. The results of the second series reported are those of one of a number of replicates giving quantitatively similar results.

3. RESULTS

The results of dark storage and 5 min visible light on a control culture not previously exposed to UV light are shown in Table 1. They demonstrate that neither treatment had significant effect on cell viability, the frequency of adenine-independent revertants or recessive homozygosis.

Table 1. *The effect of dark holding and of 5 min. visible light on cultures not exposed to UV. The values are shown with their 95 % confidence limits*

Event	Light treatment (min)	Days of dark storage 0	1	5	8
Survival	None	100 ± 11·9	116 ± 14·3	98·5 ± 12·3	105·8 ± 13
	5	99·4 ± 12·4	105·4 ± 9·5	102·7 ± 12·9	108 ± 14·2
AD$^+$ Prototrophs in 10^5 survivors	None	13·4 ± 1·4	12·4 ± 1·3	13·2 ± 1·5	12·9 ± 1·3
	5	13·5 ± 1·2	13·7 ± 1·5	12·8 ± 1·6	13·1 ± 1·8
Homozygotes (%)	None	0	0·2	0	0·2
	5	0·2	0·2	0·2	0

The effect of dark holding and light treatment on the survival of UV-treated cells are shown in Fig. 1. In this experiment the light treatment was given just before plating. The figure shows that either dark holding or immediate light treatment increase the survival of the treated cells, but that light treatment has no further effect after the period of dark-holding. Eight days dark holding appears to be more effective than photoreactivation alone in causing the recovery of UV-treated cells.

The increase in the frequency of adenine-independent revertants produced by UV exposure with immediate plating and after dark holding for 8 days is shown in Fig. 2. The results demonstrate that at all the UV doses, a significant increase in reversion frequency is produced by dark holding. This increase in reversion frequency is also shown in Fig. 3 A–D. Here the UV-induced yields of revertants after varying periods of dark holding are shown. At each stage, portions of each suspension were plated either directly or with five minutes visible light treatment just

before plating. The results demonstrate that reversion frequency increases with dark holding. At all stages the yield of revertants can be reduced by photoreactivation before plating, more so during the initial period of dark storage than after 8 days.

The effect of dark holding and 5 min visible light treatment after UV treatment on the yield of recessive homozygotes is shown in Fig. 4. The yield is significantly

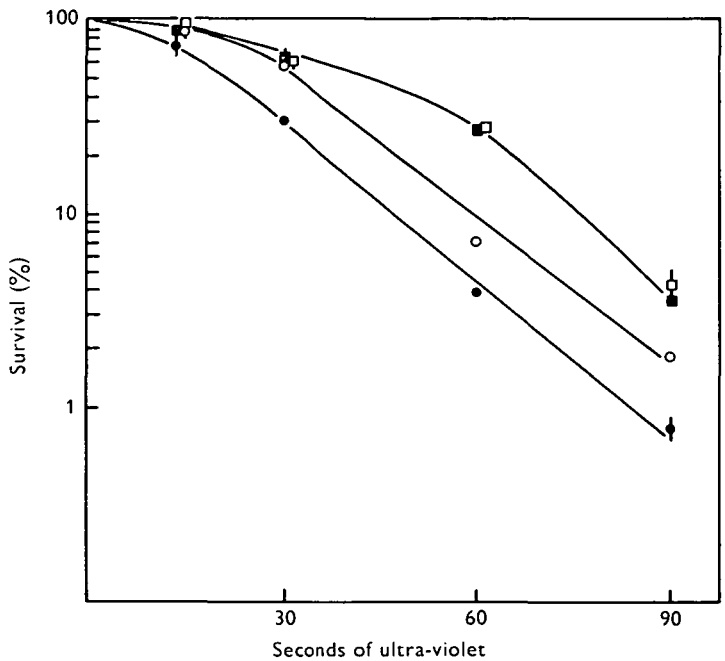

Fig. 1. The effects on the survival of UV-treated cells of post-treatment with visible light, dark holding or dark holding followed by visible light. Dose rate = 43 ergs/mm²/sec. ●——● UV alone; ○——○ UV + 5 min visible light; ■——■ UV + 8 day's dark holding; □——□ UV + 8 day's dark holding + 5 min visible light.

reduced by dark holding before plating at all the UV doses tried. The results also show that 5 min of visible light treatment had no significant effect on the yield of homozygotes at any stage.

Results from the second series of experiments in which UV-treated cultures were treated with 5 or 30 min of visible light *before* dark storage are given in Tables 2–4. The effects on cell survival, intragenic recombination and homozygosis are shown.

Table 2 shows the effect of dark holding after visible light treatment on cell survival. The results demonstrate that dark storage of UV-treated cultures after visible light treatment produces a further increase in survival above the increase produced by visible light or dark holding alone. This increase is significant only at the higher UV doses given when the effects of the two post-treatments appear to be synergistic, while at the low dose they are competitive, with some indication of additivity after dark holding.

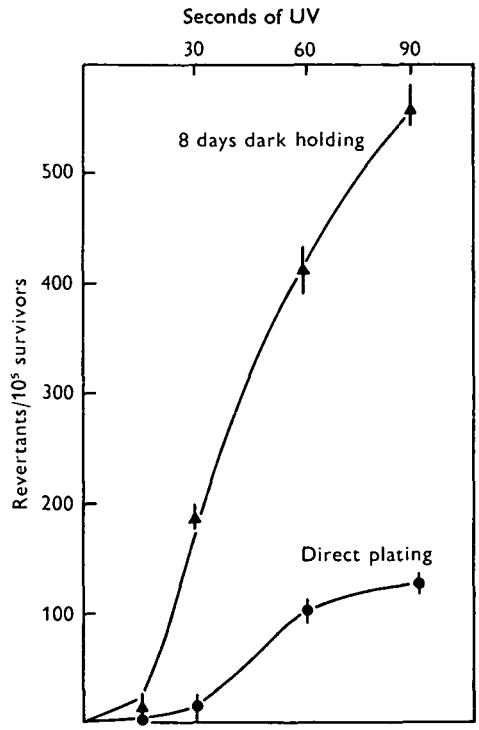

Fig. 2. The frequency of adenine-independent revertants after UV treatment, with or without 8 day's dark holding. ●——●, UV, directly plated; ▲——▲, UV, followed by 8 day's dark holding. Dose rate = 43 ergs/mm²/sec.

Table 2. *The percentage survival of UV-treated cells after a visible light treatment followed by dark holding. The* 95% *confidence limits are given. With each value is given the UV dose-equivalent in seconds represented by that survival (DE), and the dose modification factor (DMF) compared to the sample given the same light treatment, but not stored in the dark. UV dose rate* = 43 *ergs/mm²/sec*

		Light treatment								
		None			5 min			30 min		
UV dose (sec)	Days of dark holding	Survival	DE (sec)	DMF	Survival	DE (sec)	DMF	Survival	DE (sec)	DMF
30	0	15·6 ± 3·4	—	—	76·6 ± 7·7	6	—	87·6 ± 8·2	4	—
	2	78·2 ± 7·7	5	5·5	83·6 ± 8·0	5	1·2	91·8 ± 8·4	3	1·7
	5	74·0 ± 7·5	6	4·6	87·2 ± 8·2	4	1·5	97·0 ± 8·6	2	1·6
60	0	1·4 ± 1·1	—	—	4·4 ± 1·9	48	—	7·1 ± 2·3	42	—
	2	5·3 ± 2·0	46	1·3	25·6 ± 4·4	22	2·2	33·8 ± 5·0	18	2·3
	5	7·1 ± 2·3	42	1·5	29·6 ± 5·7	20	2·4	43·7 ± 5·8	14	3·0
90	0	0·3 ± 0·5	—	—	2·0 ± 1·3	60	—	3·3 ± 1·6	53	—
	2	2·2 ± 1·3	59	1·5	14·4 ± 3·3	31	1·9	32·9 ± 5·0	19	2·8
	5	6·6 ± 2·1	43	2·1	25·3 ± 4·4	23	2·6	33·6 ± 5·1	18	2·9

Table 3 shows the effects on the frequency of UV-induced intragenic recombination, of dark holding following light treatment. It demonstrates that the dark holding after UV exposure increases the yield of adenine independent revertants even in cultures which have been treated with visible light before dark storage.

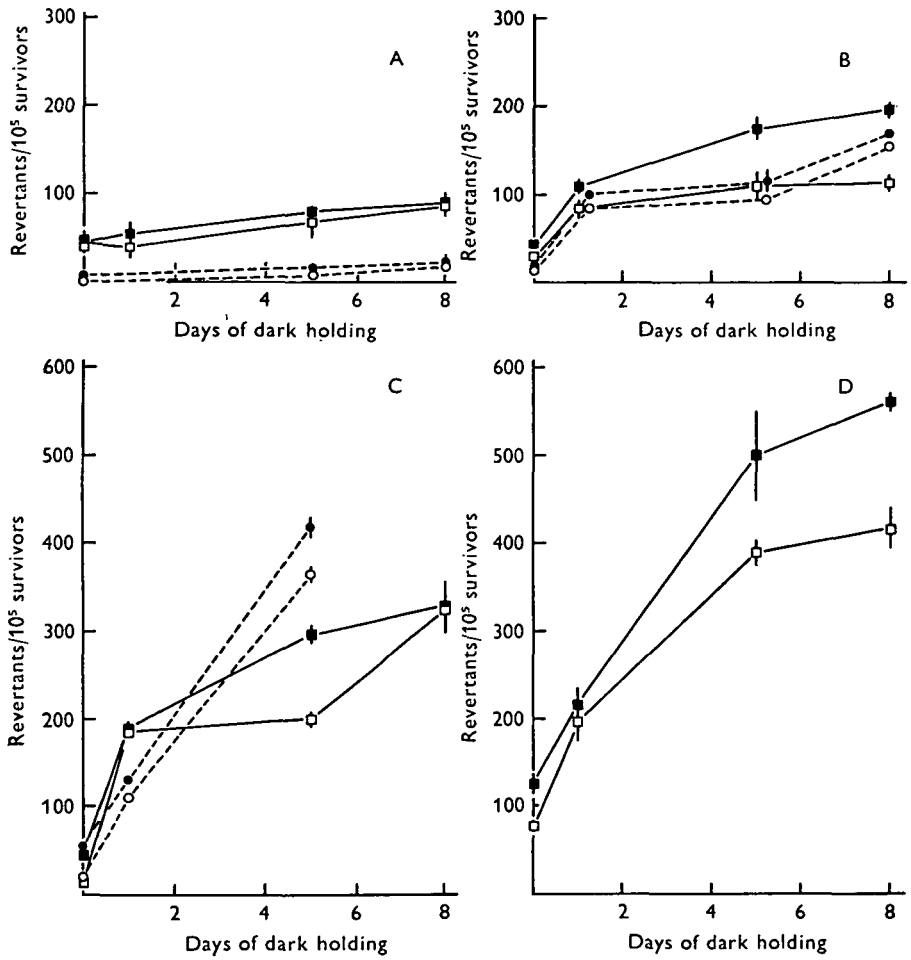

Fig. 3. The frequency of UV-induced adenine-independent revertants after various periods of dark holding, with and without photoreactivation before plating. UV dose = A, 15 sec; B, 30 sec; C, 60 sec; D, 90 sec. ● Expt. 1, dark; ○ expt. 1, light treated; ■, □ expt. 2. Dose rate = 43 ergs/mm²/sec.

Measurements of the frequency of homozygosis shown in Table 4 demonstrate again that dark holding reduces the frequency in all cultures, including those treated with visible light before dark holding.

In a reconstruction experiment, a comparison has been made of the effect of UV exposure and dark storage on viability in the original strain, J20–10 × 126W59 and in a mixed sample of adenine-independent cells spontaneously derived from it. The results of this experiment are shown in Fig. 5, and they demonstrate that no

significant difference in viability could be observed between the adenine requiring and adenine independent cultures with or without 2 or 5 days dark storage following UV exposure.

Cultures which had been exposed to UV and stored in the dark for 8 days were examined under the microscope. No asci were observed in them, indicating that they do not sporulate during the period of dark storage in these conditions.

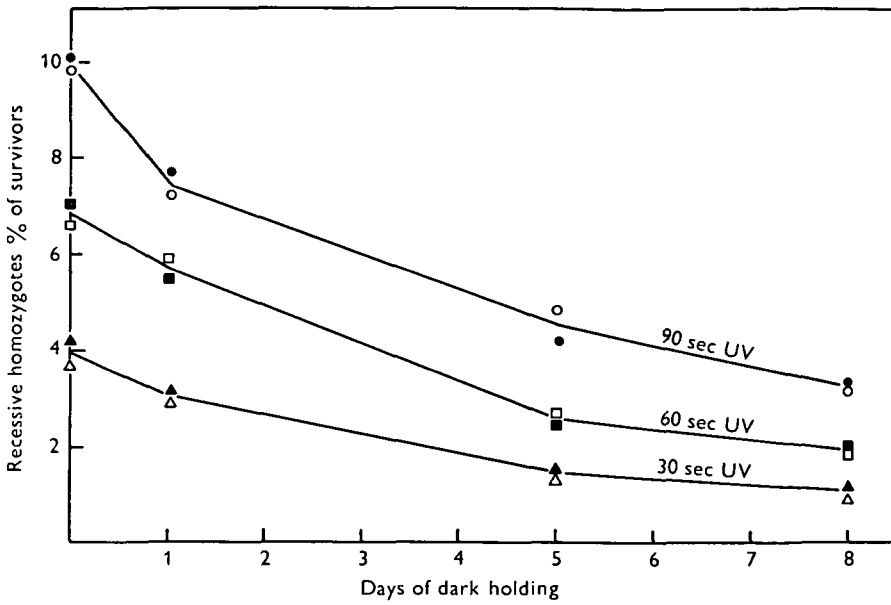

Fig. 4. The frequency of UV-induced homozygotes after various periods of dark holding, with and without photoreactivation just before plating. Solid symbols: dark only; open symbols, light treated. Each point shown is based on tests of at least 500 colonies. Dose rate = 43 ergs/mm²/sec.

Table 3. *The frequency of adenine-independent revertants in* 10^5 *survivors of UV-treated cells followed by light treatment and then by dark holding. UV dose = 43 ergs/mm²/sec. The 95% confidence limits are given*

UV dose (sec)	Days of dark holding	Light treatment: None	5 min.	30 min
30	0	35·3 ± 5·2	11·1 ± 2·9	4·2 ± 1·8
	2	59·8 ± 6·8	46·7 ± 5·2	10·2 ± 2·8
	5	75·4 ± 8·1	58·8 ± 6·7	53·0 ± 6·2
60	0	60·3 ± 6·8	10·3 ± 2·8	10·4 ± 2·8
	2	162·0 ± 11·2	93·6 ± 8·4	84·5 ± 8·2
	5	405·0 ± 17·7	238·0 ± 13·5	232 ± 13·3
90	0	148·0 ± 10·7	88·8 ± 8·3	81·0 ± 7·9
	2	278·0 ± 14·6	236·8 ± 13·5	195·5 ± 12·3
	5	1198·0 ± 30·0	404·0 ± 17·6	299 ± 15·1

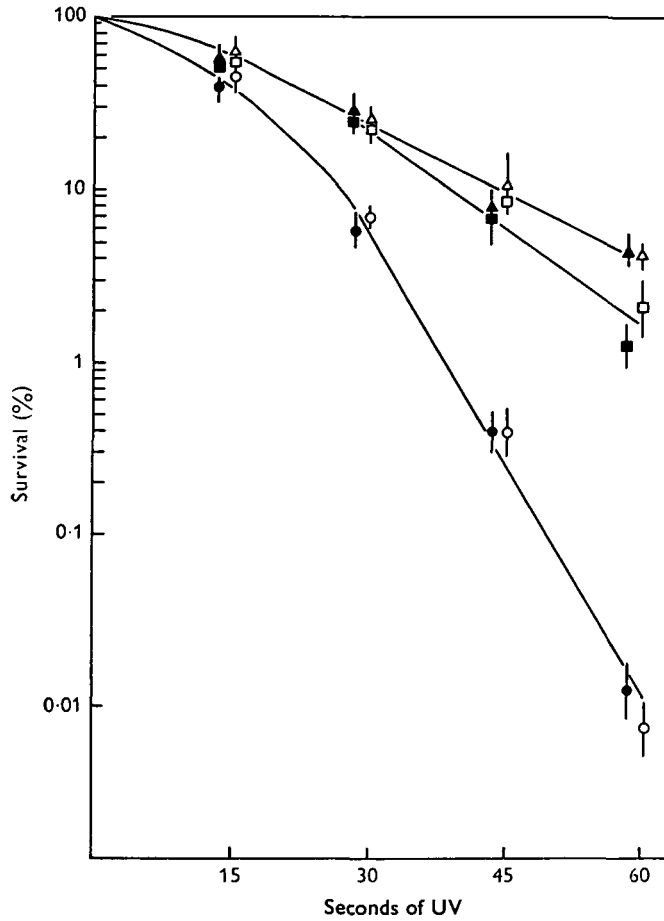

Fig. 5. The survival of UV-treated adenine-requiring and adenine-independent cultures of J20–10 × 126W59 with and without dark holding. The 95% confidence limits are shown ● *ad*⁻, ○ *ad*⁺, directly plated; ■ *ad*⁻, □ *ad*⁺ after 2 day's holding; ▲ *ad*⁻, △ *ad*⁺ after 5 day's dark holding. Dose rate = 100 ergs/mm²/sec.

4. DISCUSSION

The results of the experiments may be summarised as follows:

(1) Survival after UV is increased both by visible light treatment and by a period of dark holding before plating. After the maximum period of dark storage, no further increase in viability is produced by visible light treatment. The effects of light treatment and dark holding when the light treatment is given first are competitive following a low dose of UV, but synergistic when they follow higher doses.

(2) The yield of UV-induced adenine-independent revertants increases with the period of dark holding. This increase could be observed even in cultures where the yield of adenine revertants had been maximally reduced by pretreatment with visible light.

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(3) The frequency of recessive homozygotes was reduced by a period of dark storage. In contrast, visible light treatment had no effect on homozygosis, whether or not the cultures were held in dark conditions.

Table 4. *The homozygotes among the survivors of UV treatment followed by light treatment and then by dark holding. UV dose = 43 ergs/mm²/sec*

		Light treatment					
		None		5 min		30 min	
UV dose (sec)	Days of dark holding	Colonies	Homo-zygous (%)	Colonies	Homo-zygous (%)	Colonies	Homo-zygous (%)
30	0	634	3·46	404	3·21	464	3·88
	2	534	1·87	705	1·86	558	1·08
	5	710	1·41	790	1·27	626	1·05
60	0	828	5·93	382	6·27	540	5·94
	2	736	3·80	710	3·38	600	2·00
	5	969	2·48	550	3·63	856	1·75
90	0	558	6·82	475	6·73	710	6·22
	2	650	6·62	510	4·90	670	5·38
	5	700	2·85	1069	2·72	590	2·08

(4) No significant differences in viability could be observed between adenine-requiring and adenine-independent cultures derived from the same diploid strain after UV exposure, before or after dark storage. No asci were seen in dark stored cultures and neither dark storage nor visible light had any effect on cultures which had not been exposed by UV.

The results suggest that a fraction of the UV-induced events leading to inviability and intergenic recombination are reduced by a period of dark holding. This indicates that the events are sensitive to 'dark-repair' by an intracellular enzyme system. The reduction in the events leading to cell death and intergenic recombination may be the result of extending the period between UV treatment and plating, which enables the dark-repair system to operate before DNA replication.

The lesions leading to death of the cells are also sensitive to photoreactivation, which repairs pyrimidine dimers (Setlow, 1966). Most of these dimers seem to be removed during the dark holding period, since after eight day's holding, no further photoreactivation of survival is observable. When the photoreactivating light is given before dark holding, however, an interesting observation is made. As Patrick, Haynes & Uretz (1964) report, when the treatments are given in the reverse order, their effects are additive, since the dose modification factor (DMF) of dark holding after photoreactivation is similar to the DMF without light. In our experiments, there is evidence of a synergistic effect at the two higher doses, since the DMF of dark holding is invariably larger after photoreactivation than without it. This additive, possibly synergistic effect, plainly means that the preceeding light treatment, in addition to removing pyrimidine dimers is releasing a large amount of some other kind of UV-induced damage to a form which is still potentially

lethal, but is removable by dark-repair. The relative inefficiency of recovery after dark holding alone also suggests that this kind of damage may be otherwise inaccessible to dark-repair systems.

The rather variable DMF of photoreactivation alone in this experiment indicates that the photoreactivation of survival does not reach a maximum after only 5 or even 30 min of light treatment (cf. Fig. 1). For this reason our comments have been confined to comparing the effects of one dark incubation treatment with another.

Intergenic recombination, although reduced by dark holding, is not influenced by treatment with photoreactivating light. A possible explanation for the lack of effect of the photoreactivating light on intergenic recombination may be that the lesions leading to this event are not pyrimidine dimers. There are conditions in which dimers may lead to intergenic recombination. In starved cultures of yeast (Parry, 1966), as in *Ustilago maydis* (Holliday, 1962), intergenic recombination is photoreactivable. So it is when UV treatment is given during the S period of a cell cycle (Parry & Cox, 1968). We must conclude, since dimers certainly were induced in these experiments, either that they are not normally repaired through a process which leads to intergenic recombination, or that if they are, the first stage of this process is completed before photoreactivation can take place. We suggest that what starved cultures and cells in S phase have in common is an inability to carry out a dark repair of dimers and that in these circumstances an emergency repair via a recombination process may take place on the lines suggested by Howard-Flanders & Boyce (1966).

The presence of some other lesion is suggested by the fact that complete recovery of neither cell viability, after higher doses of UV, nor of UV-induced gene conversion or mutation (Parry & Cox, 1965) can be achieved by photoreactivation, which indicates that pyrimidine dimers may not be the only lesion of importance induced by UV exposure.

The interpretation of the response of intragenic recombination to these treatments is more difficult. The salient observations are:

(1) The increase in intragenic recombination after dark holding cannot be due to selection, since in neither the reconstruction experiment nor in the unirradiated control could any increase be detected.

(2) Some intragenic recombination is caused by dimers, since it is partly photoreactivable.

(3) Some but by no means all of it must take place after plating, since even after dark holding, it can still be reduced by photoreactivation just before plating.

(4) Some intragenic recombination may be caused by lesions other than dimers, because even in conditions where photoreactivation has produced its maximum effects, as judged by the reduction of adenine revertants (Table 3), a large increase is observable after dark holding.

(5) Regardless of the causes, UV-induced intragenic recombination is increased by dark holding. It is clear to us that the dark-repair processes which repair the UV-induced lesions causing inviability carry out *en route* this recombination pro-

cess. Probably most of the lesions involved, the dimers, are identical. If the processes were independent, then the dark repair of lesions would reduce the amount of intragenic recombination observed, as it does mutation in bacteria (Witkin, 1966) and in yeast (Parry, 1966). Little is known about the nature of mitotic intragenic recombination in yeast except that it is non-reciprocal, both when it occurs spontaneously and after UV (Roman, 1956; Roman & Jacob, 1958). Two schemes have been proposed which account for the non-reciprocality of much intragenic recombination in meiosis in fungi (Whitehouse & Hastings, 1965; Holliday, 1964). In both, gene conversion results from the repair processes which correct errors in base-repairing following the formation of heteroduplex DNA. It may be that UV-induced intragenic recombination involves the repair of this kind of DNA lesion. However, a pre-requisite is the formation of hybrid DNA and it follows that this would also have to be carried out during the dark-repair of lesions induced by UV. It may be problematical whether it is a process of DNA, hybridization which increases during the dark holding period after UV treatment, or whether it is the error-correction. It is worth pointing out, though, that in mitosis, non-reciprocal recombination need not involve the correction of base-pairing errors in heteroduplex DNA, but only its formation. Segregation of hybrid DNA during mitosis can produce the effect of non-reciprocality, even if the original hybridizations were reciprocal.

SUMMARY

UV induces lesions in DNA which lead to the death of cells, mutation and, in yeast, intragenic and intergenic mitotic recombination. We have investigated the interaction of two post-treatments, dark holding and photoreactivation, on the frequencies of these events. It was found that dark holding reduces cell death and intergenic recombinants, but causes an increase in intragenic recombination frequency. Photoreactivation reduces cell death and intragenic recombination, but has no effect on intergenic recombination. After dark holding, photoreactivation has no further effect on cell survival or intergenic recombination, but may reduce the frequency of intragenic recombinants. After photoreactivation, dark holding still causes an increase in cell survival and the frequency of intragenic recombination, and reduces the frequency of intergenic recombination.

Part of this work was carried out while one of us (J. M. P.) held a Science Research Council Research Studentship award.

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