Repair-induced Changes in Yeast Radiosensitivity

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Potentially lethal X-ray or ultraviolet damage in the diploid yeast, *Saccharomyces cerevisiae*, can be reversed if the irradiated cells are incubated in distilled water or buffer for a number of hours prior to plating. This phenomenon is called liquid-holding recovery. We found that the liquid-holding procedure served not only to restore the viability of the irradiated cells, but also to alter their sensitivity to further doses of radiation. Specifically, the ultraviolet sensitivity of cells which had undergone liquid-holding recovery was markedly decreased, whereas their X-ray sensitivity appeared to be slightly increased. These sensitivity changes were qualitatively the same irrespective of whether the initial radiation exposure was to X rays or ultraviolet light. (In contrast, the radiation sensitivity of cells which had undergone maximal photoreactivation was essentially the same as that of untreated controls.) It is suggested that these changes in radiosensitivity are the result of structural alterations induced in the cell's deoxyribonucleic acid by the execution of at least the initial steps of a deoxyribonucleic acid repair process during the liquid-holding period.

The viability of X- or ultraviolet (UV)-irradiated diploid yeast may be enhanced by incubation in distilled water prior to plating (1, 10, 11). This effect is variously described as "delayed plating," "storage," or "liquid-holding" recovery; it is not known whether it is related to a similar phenomenon in *Escherichia coli* B (6). Recent work (3, 5, 12) suggests that both photo- and dark reactivation in bacteria are based on the enzymatic repair of potentially lethal structural defects in deoxyribonucleic acid (DNA); previous studies of liquid-holding recovery in yeast are consistent with the view that this organism also possesses enzymatic repair mechanisms, although some evidence suggests that the mode of repair might be different from that in bacteria (8). Irradiated yeast are metabolically active during the storage period (9, 11); however, it is uncertain whether any of the DNA structural changes relevant to repair occur during this time. As one approach to this problem, we measured the radiosensitivity of the population at the end of the period of storage in distilled water. We found that these cells become more resistant to UV, yet slightly more sensitive to X rays, than those which did not undergo the distilled-water incubation. These sensitivity changes are the same whether the population was exposed initially to X rays or to UV. The results lead us to suspect that at least some of the macromolecular steps involved in DNA repair occur during the storage period.

MATERIALS AND METHODS

Interdivisional cells of the diploid strain SC-6 of the yeast *Saccharomyces cerevisiae* were harvested from 72-hr malt-agar slants, washed twice, and suspended in distilled water for irradiation either with 145 kv of X rays (dose rate, 4 krads/min; cells fully oxygenated) or 2,537 Å of UV (incident dose rate: 17 ergs per mm² per sec). A description of the irradiation and calibration techniques for the X-ray, UV, and visible light source for photoreactivation has been published (11).

The basic experimental design is sketched in Fig. 1. Two 10-ml suspensions (2×10^6 cells/ml) were irradiated, and the number of survivors was determined by immediately plating small samples of each (survival levels i_1 and i_2). The irradiated suspensions were then stored in the dark for 4 days at 30°C, at which time samples were again plated, giving the enhanced survival levels r_1 and r_2 . Both the UV and X-ray sensitivities of these surviving cells were obtained by determining the full survival curves of the r_1 and r_2 populations as indicated in the right-hand half of Fig. 1. The resulting curves are to be compared either with the survival curves for freshly prepared suspensions, or with unirradiated suspensions which had been stored for 4 or more days under similar conditions. (Storage alone has no effect on radiosensitivity.) The initial radiation doses were chosen so that the survival levels i_1 and i_2 would be in the shoulder and exponential regions, respectively. In all cases, survival was determined by the ability of the cells to form visible colonies after 3 days of incubation on YEPD plates, containing: 1% yeast extract, 2% peptone, 2% dextrose, and 2% agar (Difco) in dis-

FIG. 1. Experimental design used to determine the radiosensitivity of cell populations that have been subject to a preliminary radiation treatment and then exposure to liquid-holding recovery conditions. We refer to the "radiosensitivity after liquid-holding recovery" as given by the apparent limiting slope of curves such as those designated r_1 or r_2 in the right-hand panel.

tilled water. All results reported were based on at least two separate experimental trials.

RESULTS

Increased UV resistance after storage. Following the procedure described above, we found a marked increase in the UV resistance of cells which had undergone liquid-holding recovery. This increased resistance occurred for both X- and UV-irradiated cells (Fig. 2). Control experiments showed that, by itself, distilled water incubation has no effect on either X-ray or UV sensitivity. [However, in *E. coli* B/r a protective effect of preirradiation incubation has been reported (4).] On the other hand, photoreactivated suspensions displayed the same UV sensitivity (as measured in terms of the limiting slope) as did control populations (Fig. 3).

These observed changes in UV resistance did not persist in cells which were allowed to grow after liquid-holding recovery; that is, the UV response for suspensions of macrocolonies derived from irradiated or unirradiated cells, which were either plated immediately or subjected to liquid holding prior to plating, was the same in all cases. We feel, therefore, that the resistance increases shown in Fig. 2 most likely reflect intracellular events stimulated by the initial irradiation, events which occur during the liquid-holding treatment.

Low doses of UV exerted a reactivating effect on heavily X-irradiated cells immediately after

exposure to the latter (Fig. 2a); after 24 hr, this phenomenon was no longer observed. No such effect was apparent at lower X-ray doses (Fig. 2a). Although it is premature to speculate on the mechanism of this effect, it is not unreasonable to assume at present that it is in some way related to the above-described increase in UV resistance of cells which have undergone liquid-holding recovery. Such a reactivation of lethal X-ray damage by post-UV treatment has previously been observed for budding populations of haploid and diploid yeast (2); similar phenomena have also been reported for X ray-induced chromosome breaks in *Tradescantia* pollen tubes (13) and *Drosophila* sperm (7).

X-ray sensitivity after storage. Liquid-held cells, initially exposed to either X rays or UV light, exhibited no increase in X-ray resistance; if anything, they became more sensitive to X rays. Careful measurement of the curves shown in Fig. 4 suggests a slight increase in the apparent limiting slope as well as a reduction in the shoulder of the X-ray survival curves for the irradiated and stored r_1 and r_2 populations. However, it is difficult to determine the significance of the slight increases observed; in fact, it might be argued that the X-ray sensitivity of the r_1 and r_2 populations is in fact the same as that of the corresponding control populations. For, if the origin of the former is displaced to the levels i_1 and i_2 , respectively, it would seem that the two curves are almost superimposable. In any case, the recovered populations showed a measurable increase in sensitivity over that expected at the r_1 and r_2 survival levels. It should also be noted, in agreement with observations reported by Uretz (14), that the X-ray survival curve of cells determined immediately after an initial dose of UV (no liquid holding) is characterized by a reduced shoulder unaccompanied by any change in limiting slope.

These results differ from those recently reported by Becchetti and Mauro (1), who found that diploid yeast reirradiated up to 180 min after an initial X-ray dose gave survival curves with the same slope and extrapolation number as did a population that was not preirradiated. However, these investigators employed different holding conditions; furthermore, we have previously observed that, for storage times of 3 hr or less, only a small recovery of viability occurs. It is then possible that the increase in X-ray sensitivity is observed only after the relatively long incubation time necessary for maximal recovery of viability.

In all of these experiments, even after the second irradiation, the cells were still capable of recovery upon delayed plating, and the dose-modifying factor for recovery after the second exposure was approximately the same as that

FIG. 2. UV survival curves of diploid yeast after liquid-holding recovery. (a) Preirradiation with X-ray doses of 50 and 100 krad (surviving fraction: $i_1 = 0.10$; $i_2 = 0.0010$). In this experiment the UV survival curve was also measured immediately after the preliminary X-ray treatment (●) as well as at the end of the liquid holding period (○). The decrease in UV sensitivity of the r_1 and r_2 populations over that of the control, given in terms of the ratio of the apparent limiting slopes, is by factors of 0.52 ($= r_1/\text{control}$) and 0.47 ($= r_2/\text{control}$), respectively. (b) Preirradiation with UV doses of 1,600 and 2,900 ergs/mm² (surviving fraction: $i_1 = 0.20$; $i_2 = 0.0016$). The decrease in UV sensitivity is: $r_1/c = 0.53$ and $r_2/c = 0.35$.

FIG. 3. Comparison of the UV survival curve obtained after dark, liquid-holding recovery (DR, UV) with that obtained after maximal photoreactivation (PR, UV). In each case, the preliminary UV dose was 1,530 ergs/mm².

observed after the first. This suggests that the recovery mechanism is not impaired by repeated exposure at these dose levels.

It is worth noting the r_1 and r_2 curves (Fig. 2) show no tail or inflection points, so that there is no obvious indication of population heterogeneity under these circumstances. It may therefore be inappropriate to regard the population as being conceptually divisible into two categories such as initially surviving cells and those which survive as a result of the distilled-water incubation. Rather, it would seem that both lethal and "sub-lethal" damage are modified in the same way, yielding a population that is uniformly more sensitive to X rays.

DISCUSSION

The principal results of these experiments can be summarized as follows. First, an increase in UV resistance is observed in diploid yeast after liquid-holding recovery; little or no change occurs after photoreactivation, which is consistent with our understanding of this process as a single

FIG. 4. X-ray survival curves of diploid yeast after liquid-holding recovery. (a) Preirradiation with X-ray doses of 50 and 175 krad (surviving fraction: $i_1 = 0.36$; $i_2 = 0.00020$). The increase in X-ray sensitivity of the r_1 and r_2 populations over that of the control is by factors of 1.25 and 1.50, respectively, as measured by the ratio of the corresponding slopes: $r_1/\text{control}$ and $r_2/\text{control}$. (b) Preirradiation with UV doses of 1,600 and 2,900 ergs/mm² (surviving fraction: $i_1 = 0.20$; $i_2 = 0.00050$). The increase in X-ray sensitivity of the

enzymatic reaction. Second, this change in UV resistance is the same irrespective of the nature of the initial irradiation treatment. Third, no such increased resistance is observed for X rays after liquid-holding recovery. (If anything, the cells become somewhat more sensitive to X rays.)

Although the biochemical mechanism of this recovery process in yeast is unknown, these observations enable us to draw a number of general conclusions regarding the nature of the presumable multistep DNA repair process that underlies the observed phenomenon. To the extent that the radiosensitivity depends on the physical state of the DNA, the mere finding of sensitivity changes rules out the suggestion that no steps relevant to DNA repair occur during the liquid-holding period. However, it would seem that not all the steps of the repair process are completed during the holding period, since further changes in radiosensitivity occur once the cells are returned to a growth medium. We therefore conclude that one or more of the initial steps in repair occur during the liquid-holding period; a similar conclusion has been drawn for liquid-holding recovery in *E. coli* (4). Since the change in radiosensitivity after liquid holding is the same for both pre-UV and pre-X irradiation, we further conclude that those initial steps of repair that occur during the holding period leave the DNA in essentially the same physical state in each case. Although this "state" is unknown, it is one such that the cells are rendered simultaneously more resistant to UV and slightly more sensitive to X rays. At present, this observation cannot be interpreted in terms of any of the current models for dark repair in bacteria (3); nonetheless, it does provide a rather stringent requirement that any proposed mechanism of DNA repair in yeast must satisfy.

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r_1 and r_2 populations over that of the control is: $r_1/\text{control} = 1.24$ and $r_2/\text{control} = 1.54$, respectively. The difference in X-ray sensitivity of the two control curves arises from the fact that the two experiments shown here were separated by several months, during which time the cells had been subcultured a few times. However, this does not affect our interpretations, which depend only on comparisons within each experiment.

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