Dose Dependence of the Excision of Ultraviolet-Induced Pyrimidine Dimers from Nuclear Deoxyribonucleic Acids of Haploid and Diploid Saccharomyces cerevisiae

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The yield of ultraviolet-induced dimers is similar for a fixed dose in both haploid and diploid Saccharomyces cerevisiae. The excision of these photoproducts from the nuclear deoxyribonucleic acids of cells of both ploidies after ultraviolet incident doses of $2 \times 10^4$ to $4 \times 10^4$ ergs/mm$^2$ decreased with the corresponding increasing dose. Postirradiation incubation in saline followed by a further incubation in nutrient medium increases the excision as compared to that seen in either nutrient medium or saline alone. Previous data regarding both pyrimidine dimer removal and the survival of haploid and diploid cells after ultraviolet irradiation and either immediate or delayed plating are discussed.

To date, various authors have presented results concerning the removal of pyrimidine dimers (pyr [ ] pyr) (4) from the deoxyribonucleic acid (DNA) of ultraviolet (UV)-irradiated Saccharomyces cerevisiae (9, 12, 14). After a relatively high incident UV dose (6,600 ergs/mm$^2$), Unrau et al. found a 70% excision of pyr [ ] pyr from the total cell DNA of stationary phase haploid cells after a postirradiation incubation in nutrient medium (12), whereas we have recently reported a much lower value of 5 to 8% excision of pyr [ ] pyr from the nuclear DNA of similarly treated haploid cells (14). Resnick and Setlow determined the pyr [ ] pyr content of exponential phase haploid cells via an indirect method (9) and indicated that 90% of the pyr [ ] pyr induced by a dose of 1,500 ergs/mm$^2$ was removed on postirradiation incubation in nutrient medium.

To clarify the above differences, we have investigated the removal of UV-induced pyr [ ] pyr from the nuclear DNA of both haploid and diploid S. cerevisiae after UV incident doses of $2 \times 10^4$, $3 \times 10^4$, and $4 \times 10^4$ ergs/mm$^2$. This has been monitored after postirradiation incubation in nutrient medium, saline, or saline followed by nutrient medium. These conditions were used in an attempt to simulate those leading to the liquid holding recovery (8) seen on the delayed plating of UV-irradiated stationary phase S. cerevisiae.

Survivals were estimated by immediate and delayed plating after each dose used.

MATERIALS AND METHODS

Strains and media. The haploid strain used was N123 a his$^{-}$, and the diploid strain used was N123 x 10018 (a/a his$^{+}$/+ +/adj). Both have survival curves typical of Rad and Rad/Rad strains, respectively. Precultures were grown from an inoculum of $2 \times 10^4$ to $4 \times 10^4$ cells/ml for 24 h ($4 \times 10^4$ to $5 \times 10^4$ cells/ml, less than 5% buds) in liquid complete medium aerated by shaking.

Labeling and incubation. Labeling and incubation were carried out as previously described (14). Regardless of ploidy, cells were harvested for irradiation when cultures were at an early stationary phase (i.e., when the percentage of buds dropped below 8% and cell concentration was $3 \times 10^4$ or $5 \times 10^4$ cells/ml for diploid or haploid cells, respectively).

UV irradiation and post-UV treatments. UV irradiation and post-UV treatments were performed as previously described (14). Incident doses were measured with a Latarjet dosimeter. They correspond to an absorbed dose equal to 87%, as determined from the percentage of transmission at a wavelength of 254 nm of a suspension equal in concentration and thickness to our irradiated samples.

DNA extraction. The isolation and purification of DNA was performed via isopycnic CsCl gradients according to Williamson et al. (17). The characteristic features of such gradients have been reported (15).

Hydrolysis of DNA, chromatography, and liquid scintillation counting. Chromatography, liquid scintillation countings, and hydrolysis of DNA were carried out as previously described (14).

Estimation of dimer content. The amount of pyr [ ] pyr found in the dimer region of chromatograms is expressed as the percentage of counts per minute in pyrimidine dimer peaks/counts per minute in total
RESULTS

Induction of pyr [ ] pyr in the nuclear DNA of haploid and diploid S. cerevisiae. As the uracil-uracil dimers (resulting from the deamination of cytosine-cytosine dimers) (10) constitute such a small percentage of the total pyr [ ] pyr induced, and its retardation factor is relatively low with the chromatographic system utilized, the ensuing data will not include figures for this photoproduct. Figure 1 shows the induction of pyr [ ] pyr (thy [ ] thy + ura [ ] thy), thymine-thymine dimers (thy [ ] thy), and uracil-thymine dimers (ura [ ] thy) after the UV irradiation of both haploid and diploid early stationary phase cells with $2 \times 10^3$, $3 \times 10^3$, and $4 \times 10^3$ ergs/mm$^2$ of incident UV. The induction is seen to be approximately linear, with twice as many thy [ ] thy as ura [ ] thy being induced per dose and very little difference occurring between haploid and diploid cells.

The removal of pyr [ ] pyr from the nuclear DNA of haploid and diploid S. cerevisiae. Both haploid and diploid early stationary phase cells irradiated with the above incident doses of $2 \times 10^3$, $3 \times 10^3$, and $4 \times 10^3$ ergs/mm$^2$ were incubated in nutrient medium for 4 h, in saline for 72 h, or in saline for 72 h followed by 4 h in nutrient medium; these conditions did not result in postirradiation growth. The amount of excision of pyr [ ] pyr observed after such treatments is presented in Fig. 2; the actual number of pyr [ ] pyr excised per $10^7$ nucleotides as a function of the original number of pyr [ ] pyr induced per $10^7$ nucleotides is given in Table 1.

Approximately the same percentage of excision following a given dose is seen, regardless of whether cells were incubated in nutrient medium for 4 h or saline for 72 h. However, the percentage of removal obtained by incubation in saline alone is enhanced by a further 4 h of incubation in nutrient medium of such dark-liquid held cells. Excision was seen to be at a maximum after 72 h of incubation in saline or 4 h in nutrient medium. In all cases, both thy [ ] thy and ura [ ] thy seemed to be excised to an equal percentage. The amount of pyr [ ] pyr
excised plotted against the original amount of pyr [ ] pyr induced exhibits a zero order kinetic in the range of 0.48 to 0.7% induced dimers. Thus the excision system appears to be saturated. Estimations of pyr [ ] pyr removal after cell multiplication, which occurs after 5 and 7 h, respectively, for diploid and haploid cells, showed only a slight increase.

Figure 3 shows the percentage of survival obtained by immediate plating and delayed plating after liquid holding for 72 h in saline of both haploid and diploid cells. It can be seen that survival follows the usual trend, in that diploid cells are more resistant than haploid cells and the efficiency of the increase in survival seen on delayed plating is higher for diploid cells.

DISCUSSION

The induction of pyr [ ] pyr in the nuclear DNA of S. cerevisiae by UV incident doses of 2
x 10^3, 3 x 10^3 and 4 x 10^3 ergs/mm^2 does not differ greatly from that reported by Unrau et al. (12) for total cell yeast DNA.

It is important to note that the percentage of pyr [ ] pyr induced by a given dose of UV was approximately the same in both the haploid and diploid strains used. Consequently, as it was reported that twice the number of pyr [ ] pyr are induced in vitro as compared to in vivo (13), any shielding of the DNA by the cytoplasm would appear to be much the same in haploid and diploid cells, in spite of the high amount of ribonucleic acid present in these cells.

The percentage of pyr [ ] pyr removed from the nuclear DNAs of haploid or diploid cells on postirradiation incubation in medium or saline alone is correspondingly diminished from 45 to 20% after the induction of 0.48 to 0.9% pyr [ ] pyr by UV incident doses of 2 x 10^3 to 4 x 10^3 ergs/mm^2. These values are in accord with those earlier reported by us: a 5 to 8% excision of pyr [ ] pyr after a UV incident dose of 5 x 10^3 ergs/mm^2 (14). UV doses low enough to determine the dose at which the zero order kinetic began for the plot of the percentage of dimers excised as a function of the percentage induced could not be used, owing to limitation of the chromatographic technique employed. However, as the number excised per 10^7 nucleotides after both 2 x 10^3 and 3 x 10^3 ergs/mm^2 incident doses is the same (1.1 x 10^4), this is likely to represent the number of pyr [ ] pyr maximally excisable. Further support for this figure is afforded by the 56% excision of pyr [ ] pyr after 1.5 x 10^3 ergs/mm^2 observed by A. Nasim, who used an electrophoretic technique.

**Fig. 3.** Survival of haploid and diploid S. cerevisiae following immediate plating after UV irradiation (■, haploid; ○, diploid); or after 72 h of liquid holding in saline prior to plating (□, haploid; ●, diploid).
The general yield induced by ergs/mm² under was not used, dose ergs/mm² (8). Other organisms. However, comparison authors (0.67%) be mm² found that ergs/mm² is the maximally excisable amount (Fig. 2), excision would be saturated at approximately 1.1 x 10⁴ pyr per 10⁷ nucleotides. This corresponds to an absorbed dose of approximately 765 ergs/mm². Table 2 compares this figure with previous estimates of the saturation of excision in other organisms.

The estimate for S. cerevisiae is seen to be higher than that for both the procaryotes and identical to that for human fibroblasts. Regarding the resemblance with human fibroblasts, we have assumed that the excision is saturated by 300 ergs/mm² (3), whereas more recent evidence has indicated it may be as low as 65 to 125 ergs/mm² (8).

The 70% excision following 6.6 x 10⁴ ergs/mm² found by Unrau et al. (12) is not in accord with our finding. In spite of the high incident dose used, the yield of pyr [ ] pyr given by these authors (0.67%) is more consistent with the yield induced by a dose of about 3 x 10⁴ ergs/mm² at our conditions. As the survival was not measured by these authors, it renders comparison difficult. However, we feel that the differences in the percentages of excision reported are not as large as originally thought. The general trend of inhibition of excision with increase in dose is in accord with that found by R. Wheatcroft (personal communication), but in our experiments less excision per UV dose is found. This discrepancy may well be related, among other physiological factors, to differences in media compositions (for instance, 4% glucose is used by R. Wheatcroft as opposed to our 2%). A. Nasim (Mol. Gen. Genet., in press), using media similar to ours, has verified our results in that 56% removal of pyr [ ] pyr occurred after an incident dose of 1,500 ergs/mm² to Rad cells (rad₁), shown by us not to remove pyr [ ] pyr after high UV doses; again does not excise more than 6% pyr [ ] pyr after 1,500 ergs/mm². Resnick and Setlow (9) indicated that 90% of UV-induced pyr [ ] pyr was removed from the DNA of exponential phase haploid Rad cells after 1.5 x 10⁴ ergs/mm², this removal being absent in the rad₁ UV-sensitive mutant. Although this removal would seem higher than predicted by our results, these workers measured the pyr [ ] pyr content via the competition of pyr [ ] pyr with UV-irradiated transforming DNA photoreactivating enzyme. This different approach to estimating dimers may account for the differences observed. Also, these workers used cells of a different age, and this may well have influenced the removal observed, as they saw no excision in very old stationary phase Rad cells. The removal of pyr [ ] pyr as a function of the time of incubation in nutrient medium is in accord with previously published data (9, 12, 14).

We have confirmed our earlier observations that the incubation in nutrient medium of UV-treated cells (liquid held in saline for 72 h) increases the amount of pyr [ ] pyr excised as compared to that seen on incubation in nutrient medium or saline alone (14). This feature was apparent at all doses for both haploid and diploid cells, and may be well one phenomenon responsible for the increase in survival seen following the liquid holding of UV-irradiated stationary phase cells (8). The postirradiation treatments applied resulted in approximately the same percentage of removal of pyr [ ] pyr after a specific dose, regardless of ploidy. Hence, on considering the survival curves in Fig. 3, the differences seen in survival by ploidy are not attributable to (i) less pyr [ ] pyr being induced per UV dose in the diploid or (ii) a more efficient excision of pyr [ ] pyr occurring in diploid as compared to haploid cells.

As suggested by various authors (6, 15), the increased resistance of diploid cultures may thus be due to a DNA repair mechanism other than that of excision resynthesis.

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