Reparability of Lethal Lesions Produced by Phosphorus Photoabsorption in Yeast Cells

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The characteristics of DNA lesions produced by the photoabsorption of phosphorus in yeast cells were studied using monochromatized soft X-rays tuned to the absorption peak of the phosphorus K-edge (2153 eV) and below the peak energy (2147 eV). The repaired fractions of DNA double-strand breaks (dsb) were measured relatively by using both a mutant, rad 54-3, which shows the temperature-sensitive dsb repair-deficient phenotype, and a wild-type strain. The repaired fraction of lesion in rad 54-3, which corresponds to the relative yield of dsb reparable by the RAD 54 pathway, was not affected by the phosphorus photoabsorption. Repair of the produced lesions in the wild-type cells was also measured by comparing the surviving fraction of the immediately plated cells to that of those cells plated after holding in a non-nutrient medium for 80 hrs. The recovery of the surviving fraction after the holding treatment was dependent upon the irradiated X-ray energy. These results suggest that irreparable lesions are produced by the inner-shell photoabsorption of phosphorus in DNA, although its yield is small.

INTRODUCTION

Photoabsorption in biologically functional molecules is an important primary process concerning the biological action of photons. If photoabsorption occurs at an inner-shell orbital of the constituent atoms in biomolecules, a photoelectron and several Auger electrons are ejected, and hence several ionization events after one photoabsorption event might occur in the vicinity of the absorption site. Moreover, since the atom, itself, is multiply ionized, drastic molecular changes can be expected.

Experimentally, iodine or bromine atoms artificially incorporated into DNA as haloge-
nated pyrimidine have been frequently used as a target of photoabsorption\textsuperscript{1–3}). In our previous work, the photoabsorption of bromine in yeast chromosomal DNA was induced by monochromatized synchrotron radiation, and biological enhancement caused by bromine photoabsorption and the following Auger process was observed\textsuperscript{4,5}).

Studies focussing on the biological effects of photoabsorption at naturally constituent elements have been quite few because the binding energies of the K-shell of these elements lie in the soft X-ray region, and hence monochromatic X-rays corresponding to these energies have been difficult to obtain with practical intensity. Using synchrotron radiation as a light source with a continuous spectrum from visible light to X-ray, the fine structures of an absorption spectrum of DNA around the K-shell absorption edge of phosphorus\textsuperscript{6)}, and around K-shell edges of nitrogen and oxygen\textsuperscript{7)} were observed. It was successfully shown that monochromatized soft X-rays at the absorption peak of the K-shell absorption edge of phosphorus in DNA effectively induce killing and gene conversion in yeast cells\textsuperscript{6)}.

The enhancement by phosphorus photoabsorption could be explained by two hypotheses. One is that the number of lesions increased due to an increase in the locally deposited energy by phosphorus photoabsorption. The other is that biologically more important; in other words, irreparable lesions are produced through specific processes associated with Auger decay of the excited molecules. The latter hypothesis might be supported by the observation of base substitution spectra specific to the phosphorus photoabsorption in the induced mutants in \textit{B. subtilis}\textsuperscript{8}). The purpose of this study was to test the latter hypothesis. Although inner-shell photoabsorption can induce various types of DNA damage, the most conceivable lesion produced by the Auger processes of phosphorus is considered to be DNA double-strand breaks (abbreviated as dsb hereafter), because phosphorus atoms form phosphodiester bonds in DNA strands. We tried in this work to find some evidence that irreparable DNA lesions are actually produced by phosphorus photoabsorption followed by Auger decay. We used two types of yeast, \textit{Saccharomyces cerevisiae}, a dsb repair deficient mutant strain, and a wild-type strain, in view of the radiation sensitivity. This mutant carrying the \textit{rad 54-3} gene showed a temperature sensitive phenotype; at 23°C dsbs are partly repaired, while at 36°C they are not repaired at all\textsuperscript{9}). Therefore, switching the incubation temperature can control the activity of the dsb repair in this strain. Using this strain, Bud and Mortimer reported on the relationship between DNA dsb and lethality, suggesting that cell lethality can be ascribed to unrepaired dsb. We assume in this paper that the quantity of dsb can be discussed based on the survival curves obtained under various conditions. The relative yield of the dsb reparable by the \textit{RAD 54} pathway was obtained by comparing the surviving fraction of the cells incubated at a permissive temperature (23°C) and at a restrictive temperature (36°C). As another approach to find the difference in the produced lesions, the repair rate of dsb by the \textit{RAD 54} repair pathway was examined by switching the incubation temperature.

The recovery of the surviving fraction during a delayed plating treatment in the wild-type cells was also measured for a comparison. Frankenberger at al\textsuperscript{10)} reported that the recovery after this treatment can be ascribed to the repair of dsb. The recovery of the surviving fractions can be considered to indicate the relative yield of the lesions repaired by the repair activity possessed by the wild-type cell during a liquid holding treatment. Susceptibility to repair is
a biologically important property of lesions, since easily reparable lesions usually do not lead to cell killing. Based on these experimental results, we discuss the characteristics of the lesions produced by phosphorus photoabsorption.

**MATERIALS AND METHODS**

**Used Strain and Sample Preparation**

Two strains of diploid yeast, *Saccharomyces cerevisiae*, were used. One was a dsb repair-deficient mutant (*rad54-3*), X7546D1B strain obtained from Prof. J. Kiefer (Justus-Liebig-Universität, Giessen, Germany); the other was a wild-type, XS1972 strain obtained from Dr. T. Saeki (National Institute of Radiological Sciences, Japan). The former strain shows a temperature-sensitive phenotype; at 23°C dsbs are partly repaired, while at 36°C they are not repaired at all\(^9\). Therefore, the repaired fraction of dsb can be controlled by changing the incubation temperature after irradiation. Genotypes of these strains are as follows:

**X7546D1B**

\[ a \text{ rad54-3 } \text{ tup7-1 } \text{ his1-1 } \text{ trp2-1 } + \text{ ade4-501} \\
\alpha \text{ rad54-3 } \text{ tup7-1 } \text{ his1-1 } \text{ trp2-1 } \text{ ask1-7 } \text{ ade4-501} \]

**XS1972**

\[ a \text{ RAD } \text{ ade6 } \text{ leu1-12 } + \text{ cyh2 } \text{ met13 } \text{ lys5} \\
\alpha \text{ RAD } + \text{ leu1-1 } \text{ try5 } + + + \]

The cells were cultured in a YPD liquid medium (1% yeast extract, 2% pepton and 2% dextrose) for 2 days to attain the stationary phase, washed twice with sterilized distilled water to remove the medium, and resuspended in 67 mM phosphate buffer (pH 6.8). Then, \(3 \times 10^5\) cells were placed in an area of \(2 \text{ mm} \times 5 \text{ mm}\) on membrane filters (Millipore HA type). From a calculation using the size of the cell, the cells were considered to be a monolayer in the area. The attenuation of soft X-rays within the sample layer was negligible and the secondary electron equilibrium is considered to hold.

**Monochromatized soft X-ray irradiation**

Monochromatized soft X-rays were obtained with synchrotron radiation from a 2.5 GeV electron/positron storage ring at the Photon Factory, National Laboratory for High Energy Physics (Tsukuba, Japan). Irradiation was performed at beamline 1B1 for the *rad 54-3* strain and at beamline 11B for the wild strain. The latter has a focusing beam optics, and hence the intensity of the soft X-rays is about 10 – 100 times higher than that in beamline 1B1. The synchrotron radiation was monochromatized with a monochromator equipped with an indium antimonite (InSb) (111) channel cut crystal (in the beamline 1B1) or a double crystal (in 11B). Contamination of higher harmonics at a photon energy of around 2.15 keV was less than 0.1% at both beamlines.
The photon energy was adjusted to the absorption peak of the K-shell edge of phosphorus (2153 eV) in the absorption spectrum of DNA, since the phosphorus atom in DNA absorbs soft X-ray photons most effectively at this energy. This peak was assigned to the transition to a defined excited level, designated as \( t_2^* \), of phosphorus. For reference experiments, a photon energy of 2147 eV was used, which is slightly (6 eV) lower than the absorption peak, and not absorbed by K-shell electrons of phosphorus at all.

All irradiation experiments were performed under atmospheric condition using a monochromatized soft X-ray irradiation system for biological samples. The details of the system were described previously. The exposure rates were measured using a specially designed ionization chamber before and after irradiation. The exposure rates at the sample position were about 0.3 C/kg per min at beamline 1B. In the scanning irradiation mode at beamline 11B, where a smaller, but more intense, beam was available, the effective exposure rate was 6.5 C/kg per min. The beam intensity during irradiation was monitored by measuring the photoionization current generated at an aluminum-coated Mylar film (thickness; 5 \( \mu \)m) inserted in the beam path.

The sample cells on the membrane filter were put on a sample holder made by copper, which was cooled by water during irradiation to prevent DNA repair during irradiation. The samples were irradiated with monochromatized X-rays on a cooled holder attached to the scanning stage. At beamline 11B, the samples were scanned vertically in order to compensate for the vertical distribution of the beam intensity. In beamline 1B, where a wider beam was available, the samples were irradiated while being fixed at the beam center.

**Correction to obtain exposure at the sample position**

Since soft X-rays are strongly absorbed by air, an X-ray beam led out into air is immediately attenuated. In our experiments, the sample was positioned very close (5 mm) to the beam exit so as to obtain a higher intensity at the sample. We thus needed to know the attenuation of the beam by the air between the sample position and the center of the ionization chamber, which was set downstream of the sample position. Moreover, we should note that the beam was also attenuated exponentially within the ionization volume defined by the length of the collecting electrodes of the ionization chamber (30 mm). The exposure at the sample position was obtained from the exposure measured with the ionization chamber by the following procedure.

The exposure at the geometrical center of the ionization chamber is different from the exposure measured by a 30 mm electrode (integrated value over 30 mm electrode). The exposure at the geometrical center \( (D_c) \) is

\[
D_c = \frac{1}{30} \int_0^{30} e^{-\mu x} dx \approx 0.9104 D_m,
\]

where \( D_m \) is the exposure measured by the ionization chamber and \( \mu \) is the linear attenuation coefficient at 2.15 keV, which was measured experimentally to be 0.0502 mm\(^{-1}\). Therefore, if
the sample is placed at $d$ mm upstream from the center of the ionization chamber, the actual exposure at the sample position ($D$) is

$$D = e^{0.0502d} \times D_c = e^{0.0502d} \times 0.9104 \times D_m.$$ 

For all irradiation experiments, the values for exposure were corrected using the above equation.

**Post-irradiation treatment for the rad 54-3 strain**

While the *rad 54-3* strain cannot repair dsbs at 36°C, it shows partial repair activity at 23°C. This temperature-sensitive character can be seen both under nongrowth conditions and under growth conditions. Therefore, the lesions repairable in the nongrowth condition and those repairable in the growth condition can be distinguished. Using this advantage, the relative yields of the dsb repairable by the *RAD 54* pathway, which were produced with the irradiation of either the K-shell photoabsorption peak X-rays or below the peak X-rays, were examined. The details of the procedure are as follows:

1. Post-irradiation treatment of *rad 54* cells in a growth condition: The irradiated cells were plated on synthetic complete agar plates immediately after irradiation. Half of the plates were incubated at 23°C (permissive temperature), and the remainder at 36°C (restrictive temperature). After 7 day-incubation, colonies were counted and the surviving fractions were calculated.

2. Post-irradiation treatment of *rad 54* cells in a nongrowth condition: The irradiated cells were resuspended in a 67 mM phosphate buffer. Then, part of the suspension was held at 23°C and the rest was held at 36°C for 80 hours. After holding, the cells were plated on synthetic complete agar plates. The plates were incubated at 36°C to prevent repair under the growth condition.

3. Time course measurement of recovery by the *RAD 54* pathway in growth and nongrowth conditions: During 7 days of incubation on a nutrient plate, or 80 hrs holding in the buffer, the temperature was shifted from permissive to restrictive at various times. During the period only in the permissive temperature, the *RAD 54* pathway worked and some of the dsb were repaired.

**Post-irradiation treatment for wild strain**

The irradiated wild-type yeast cells were resuspended in a 67 mM phosphate buffer. Part of the suspension was plated on synthetic complete agar medium plates immediately after irradiation (immediate plating, IP). The rest was incubated at 30°C for 80 hrs before plating (delayed plating, DP) for liquid holding recovery. The recovery of colony-forming activity saturated after 72 hrs and the surviving fraction did not change thereafter up to 120 hrs. After incubation at 30°C for 7 days, the colonies formed on the plates were counted and the surviving fractions were calculated.
RESULTS AND DISCUSSIONS

Enhancement of biological effects by phosphorus photoabsorption

Survival curves of the yeast rad 54-3 mutant cells irradiated with the monochromatized soft X-rays on and off the K-shell absorption peak of phosphorus under the various post-irradiation treatments are shown in Fig. 1 (repair under the growth condition) and Fig. 2 (repair under the nongrowth condition) as a function of the exposure. The values of 37%-survival exposure ($D_{37}$), 10%-survival exposure ($D_{10}$), and the extrapolation number ($m$) of these survival curves are tabulated in Table 1.

The 2153 eV soft X-rays were more effective for cell killing than the 2147 eV X-rays, and the ratios of the effectiveness between the two X-ray energies were almost the same irrespective of the nutrient condition. The observed enhancements with irradiation of 2153 eV X-rays indicate that the photoabsorption of phosphorus induces cell killing. The monochromatized soft X-rays at the absorption peak of phosphorus induced cell killing more effec-

![Fig. 1. Survival curves of the yeast rad 54-3 strain irradiated with monochromatized X-rays at the K-shell absorption peak of phosphorus (2153 eV; circles) and below the peak (2147 eV; squares). After irradiation, the repair activity was controlled by the incubation temperature under the growth condition (open symbols, incubated at 23°C; closed symbols, at 36°C).](https://academic.oup.com/jrr/article-abstract/42/3/317/914707)
Fig. 2. Survival curves of the yeast rad 54-3 strain irradiated with monochromatized X-rays at the K-shell absorption peak of phosphorus (2153 eV; circles) and below the peak (2147 eV; squares). After irradiation, the repair activity under non-growth condition was controlled by the incubation temperature (open symbols, incubated at 23°C; closed symbols, at 36°C).

| Temperature | D_{37} (C/kg) | D_{10} (C/kg) | m     | D_{37} (C/kg) | D_{10} (C/kg) | m     |
|-------------|---------------|---------------|-------|---------------|---------------|-------|
| 36°C        | 2153 eV       | 0.59          | 1.33  | 1             | 0.67          | 1.52  |
|             | 2147 eV       | 0.86          | 1.96  | 1             | 0.98          | 2.28  |
| 23°C        | 2153 eV       | 1.20          | 2.31  | 1.49          | 1.45          | 2.65  | 1.79  |
|             | 2147 eV       | 1.78          | 3.41  | 1.54          | 2.14          | 4.02  | 1.61  |

Table 1. The values of D_{37}, D_{10} and extrapolation number (m) of the survival curves of rad 54 presented in Figs. 1 and 2. The ratios of 2153 eV to 2147 eV or 23°C to 36°C of these parameters are also shown.
Relatively (about 1.5-times more effective, as seen in the values of $D_{10}$ and $D_{37}$ in both strains) than the X-rays below the peak. A rough estimation of the absorbed energy within the yeast nuclei at 2153 and 2147 eV was performed, assuming the relative mass abundance of phosphorus in the nuclei to be 0.4%\(^5\). The ratio of the $f$-factor (conversion factor from exposure to absorbed dose) at 2153 and 2147 eV was calculated to be 1.15. If the enhancement was caused only by the increase in the absorbed dose, the value of the enhancement should be 1.15. This means that the observed enhancement cannot be explained solely by the increase in the absorbed dose within the nucleus in the yeast. Localized energy deposition by the Auger electrons or multiple-charged phosphorus might exhibit biological enhancement. The amount of energy locally deposited around the phosphorus atoms in the DNA, however, cannot be expressed exactly as an average of the deposited energy of the whole nucleus. Further microscopic discussions on the dosimetry are required in analyzing the irradiation effect of low-energy X-rays.

**Yield of the dsbs reparable by the RAD 54 pathway in cells irradiated with monochromatized X-rays at the phosphorus K-shell edge**

Another possible explanation of the biological enhancement by phosphorus photoabsorption is that inner-shell excitation followed by the Auger process may produce lesions which can be repaired less efficiently by the repair activity of cells. In order to test this possibility, we used the rad54-3 strain, since the repair activity of this strain can be switched on and off merely by changing the incubation temperature. As can be seen in Figs. 1 & 2, the surviving fractions under post-irradiation incubation at 23°C were larger than those at 36°C with both irradiation energies. These results confirm that the RAD 54 repair pathway was operative at 23°C, but not at 36°C, as expected.

Since the number of lesions is considered to be produced proportional to the dose, we could quantitatively compare the number of lesions produced by radiation at doses which give the same biological effect. Therefore, the difference in the dose which gave the same surviving fraction between 23°C and 36°C indicates the amount of dsbs repaired by the RAD 54 pathway. It could be considered that the ratios of the doses, $D_{37}$ and $D_{10}$, at 23°C to those at 36°C correspond to the relative yield of the dsbs reparable by the RAD 54 pathway. The ratios of $D_{37}$ were 2.04 (2153 eV) and 2.08 (2147 eV) under the growth condition, and 2.18 (2153 eV), 2.18 (2147 eV) under the non-growth condition as can be seen in Table 1. This ratio did not show any dependence on the photon energy. We could not detect any difference in the reparability by the RAD 54 pathway between the lesions by 2153 eV and those by 2147 eV. These results indicate either that dsb produced by inner-shell phosphorus photoabsorption is the same as other dsb from the viewpoint of the reparability by RAD 54, or that the number of dsb less reparable by RAD 54 is not large enough to be observable.

According to a report by Budd and Mortimer\(^9\), the values of $D_{37}$ of this strain were 150 Gy at 23°C and 25 Gy at 36°C when irradiated with white X-rays from an X-ray tube (60 kVp). The ratio between them becomes 6, which means more than 80% of the lethal lesions were repaired by the RAD 54 repair activity. However, the ratio that we presented in this report is about 2, indicating that half of the lesions were repaired. This discrepancy could be
attributed to the difference in the X-ray photon energy (2.15 keV, in this work and the peak energy around 30 keV, Budd and Mortimer). Soft X-rays around 2 keV might produce irreparable lesions more efficiently than the commonly used white X-rays from X-ray tubes.

**Repair kinetics of the RAD 54 pathway in cells irradiated with monochromatized X-rays at the phosphorus K-shell edge**

The time course of recovery in *rad 54-3* cells irradiated with monochromatized soft X-rays around the K-shell absorption edge of phosphorus was studied in order to detect any difference in the repair rate of lesions by the *RAD54* pathway. The results are shown in Fig. 3. The repair under growth condition proceeded fast, and was almost completed in 20 hrs. The repair under the nongrowth condition proceeded relatively more slowly, and continued for about 70 hrs. When compared after 100 hrs, the survival fraction recovered under the nongrowth condition more than under the growth condition. To obtain time constants of *RAD 54* repair (*τ*), which is defined as the time when the remaining fraction of reparable lesions by the *RAD 54* repair pathway decreased to 1/e, the remaining fraction of reparable

![Fig. 3. Repair kinetics of the RAD 54 pathway of yeast cells irradiated with monochromatized soft X-rays at the K-shell absorption peak of phosphorus (2153 eV; open symbols) and below the edge (2147 eV; closed symbols). Square and circle symbols stand for the surviving fractions of cells post-treated in the buffer or in YPD medium, respectively.](image-url)
lesions was calculated using an equation proposed by Johnson and Haynes\textsuperscript{15),}

\[ f(t) = \frac{\ln S(\infty) - \ln S(0)}{\ln S(\infty) - \ln S(t)} = e^{\frac{t}{\tau}}, \]

where \( t \) is the time when the incubation temperature is shifted from 23\(^\circ\)C to 36\(^\circ\)C. \( S(t) \) is the surviving fraction of the sample, which was transferred from 23\(^\circ\)C to 36\(^\circ\)C at time \( t \), and \( S(\infty) \) and \( S(0) \) are the surviving fractions of the sample treated throughout the period at 23\(^\circ\)C and 36\(^\circ\)C, respectively. The calculated values of \( f(t) \) are plotted against time \( t \) in Fig. 4. The slopes of the curves represent the reciprocal of the rate constants (\( \tau \)). The time constants were \( \tau = 11.9 \) hrs under the growth condition and 28.7 hrs under the nongrowth condition. These values were independent of the irradiated photon energy. We could not find any evidence that the inner-shell photoabsorption may produce some specific lesions.

Fig. 4. Remaining fraction of removable lethal hits replotted using the equation in the text from data in Fig. 3. The symbols are the same as in the Fig. 3.

\textit{Repair of lesions produced by phosphorus photoabsorption in wild-type yeast cells}

It is well known that wild-type yeast cells can recover with a liquid holding (delayed plating) treatment after irradiation. During the treatment the cells are considered to repair dsb.
The survival curves of the wild yeast cells irradiated with monochromatized soft X-rays around the K-shell absorption edge of phosphorus were examined both for immediate plating (IP) and for delayed plating (DP) to see the relative yield of dsb, which were repaired during the treatment. Since all of the repair activities possessed by the wild-type cells, including the RAD54 pathway, are expressed during the delayed plating treatment, the difference in the survival curves between the IP and DP treatments corresponds to the number of dsb repaired by the repair activity in the wild-type cells.

The obtained survival curves are shown in Fig. 5. The values of the parameters obtained from the survival curves are tabulated in Table 2. It can be clearly seen that the soft X-rays at 2153 eV were more effective for cell killing than the 2147 eV X-rays, and that with post irradiation incubation (DP), the surviving fraction increased at both energies. However, the extent of recovery with the DP treatment was clearly dependent upon the irradiation energy; the cells irradiated at the peak absorption energy recovered less than those irradiated at below the peak energy. This difference is seen in the ratio of $D_{10}$ or $D_{37}$ between the DP and IP treatments. The ratio of $D_{10}$ for 2153 eV was 2.1, while that for 2147 eV was 2.3. We can consider from these results that part of the dsb produced with the irradiation of 2153 eV X-rays is less repairable than those by 2147 eV. The difference in the mode of the X-ray action between 2153 eV and 2147 eV is that 2153 eV can be absorbed by the phosphorus K shell, but 2147 eV can not. Therefore, we can conclude that the lesions produced by the K-shell photoabsorption of phosphorus are less repairable than those produced by other processes. In other words, irreparable

![Fig. 5. Survival curves of wild-type yeast cells irradiated with monochromatized X-rays at the absorption peak of phosphorus (2153 eV) (a) and below the peak (2147 eV) (b) with a treatment of immediate plating (open symbols) and delayed plating (closed symbols).](image-url)
Table 2. The value of $D_{37}$, $D_{10}$ and extrapolation number (m) of the survival curves of the wild strain presented in Fig. 5. The ratios of 2153 eV to 2147 eV or IP to DP of these parameters are also shown.

|       | $D_{37}$ (C/kg) | $D_{10}$ (C/kg) | m  |
|-------|----------------|-----------------|----|
| IP    | 2153 eV        | 6.81            | 12.4|1.80|
|       | 2147 eV        | 9.87            | 17.6|1.97|
| DP    | 2153 eV        | 19.3            | 26.0|15.3|
|       | 2147 eV        | 31.4            | 40.7|29.5|
|       | 2153eV/2147eV V | IP              | 1.45 |1.42|1.09|
|       | DP             | 1.63            | 1.57|1.93|
|       | 2153eV/2147eV V | DP              | 2.83 |2.10|8.50|
|       |                | 2147eV          | 3.18 |2.31|15.0|

lesions were produced more efficiently by inner-shell photoabsorption followed by Auger processes at phosphorus atoms in DNA within the cells.

Yield of the irreparable lesion by phosphorus photoabsorption

In this study we tried to find any difference between lesions by phosphorus photoabsorption and lesions produced by other processes, from the viewpoint of reparability using two types of cells. The results were negative with the rad54-3 strain, and positive in the wild strain. In order to explain these experimental results, we quantitatively analyzed the obtained parameters given in Tables 1 and 2. The differences in the sensitivity to monochromatized soft X-rays of the two strains under various conditions are illustrated in Fig. 6. The 37% survival exposure ($D_{37}$) of each condition is normalized to that of the most sensitive condition (rad 54-3 in restrictive condition) and shown relatively. It is clear that the rad 54-3 strain was quite sensitive to the soft X-rays compared to the wild strain, which means that the repair activity of RAD54 in this strain is rather limited, even at a permissive temperature. Quantitatively, the wild strain in DP is 16.5 (=33/2.0) or 17.6 (=37/2.1) fold more resistant than rad 54 incubated in 23°C, indicating that 15.5 out of 16.5 or 16.6 out of 17.6 of the lesions that can not be repaired in rad 54 strain, are repaired in the DP-treated wild cells.

Our explanation is schematically illustrated in Fig. 7, taking into account the observed large difference in the sensitivity between the rad 54-3 strain and the wild strain. There exist two types of dsbs, one of which can be repaired, and the other can not be repaired; the latter are produced much less than the former. The latter are more efficiently produced by phosphorus photoabsorption than by the other types of energy deposition events. In the figure, four types of cells are illustrated; cells without lesions (O), cells with reparable lesions (x), cells with irreparable lesions (∆) and cells with repaired lesions (⊗). In rad 54 cells treated in the restrictive temperature (shown in panel (a)), the fraction of lethal cells caused by irreparable lesion in the total lethal cells is very small (2/20). When rad 54 cells were treated at 23°C (panel (b)), some of the lesions are repaired but the fraction of lethal cells caused by irreparable lesion remains still small (2/17), since the repair activity of rad 54 cells on the reparable
Fig. 6. Comparison of the survival curves of the wild and rad 54 yeast cells under various repair conditions irradiated with 2153 eV X-rays (left panel) and with 2147 eV (right panel). In both panels, the surviving curves are for rad 54 cells treated in the restrictive temperature, rad 54 cells in the permissive temperature, wild cells with IP and wild cells with DP, respectively. The numerals attached to each survival curve in the panels are the relative values of \(D_{37}\) normalized with the most sensitive case (rad 54, post-treated in the restrictive temperature, 1’s are omitted).

Fig. 7. Schematic illustration to explain the difference in the survival curves shown in Fig. 6. In panel (a), the fraction of lethal cells caused by irreparable lesion in the total lethal cells is very small (2/20). When rad 54 cells were treated at 23°C (panel (b)), the fraction increases a little but still small (2/17). In wild type cell which has more repair ability, the fraction becomes larger (2/11) in panel (c). In panel (d) where full repair ability is exhibited, the fraction showed the highest (2/4). See the text for more detail.

lesion is much less than that of the wild strain (Fig. 6). In wild type cell which has more repair ability than rad 54 cell, the fraction becomes larger (2/11 in panel (c)) due to the increase of repaired lesion. In panel (d) where full repair ability is exhibited with DP treatment, the fraction showed the highest (2/4). Therefore, we could not observe the effect of irreparable lesions due to phosphorus photoabsorption in the survival study or a repair kinetic study in
rad54-3 experiments, irrespective of the growth condition, as shown in Figs. 1, 2 and 3 and summarized in Tables 1. In contrast, in the wild-type cells, in which the repair system is fully active, most of the reparable lesions are repaired by the repair system of the wild strain, especially in the DP treatment. Hence, the fraction of lethal cells killed by the irreparable dsb became evident in the lethal cells.

Using the values of relative lethal dose ($D_{37}$) shown in Fig. 6, the fraction of irreparable lesions could be estimated, if we assume that remaining lesions in the DP-treated wild strain are irreparable. When irradiated with 2147 eV X-rays, the irreparable fraction could be estimated as $1/37$, while the fraction with 2153 eV, $1/33$. Difference between these values could be considered as the increase of irreparable lesion due to the phosphorus photoabsorption in the total lesion produced by the irradiation. The difference calculated become 0.4%. In other words, irreparable lesions increased about 12% ($=(1/33)/(1/37)–1$) with the phosphorus photoabsorption. We could say that the irreparable lesions were concentrated by the repair activity in the wild cells. Consequently, the effect of an irreparable lesion produced by phosphorus photoabsorption, becomes observable in the survival curve of DP-treated wild-type cells, when compared with IP-treated cells.

In conclusion, we successfully observed a difference in the repairability in wild-type yeast cells, depending upon the irradiated X-ray energy. This could only be explained by the hypothesis that irreparable DNA lesions are produced more efficiently by inner-shell phosphorus photoabsorption followed by the Auger process, presumably irreparable dsb. Due to the small fraction in the total DNA damage, irreparable lesions by phosphorus photoabsorption could not be observed in a repair-deficient cell system.

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