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Different Oxygen Enhancement Ratios for Induced and Unrejoined DNA Double-Strand Breaks in Eukaryotic Cells

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DNA double-strand breaks (DSBs) are 2.9 times more frequently induced in yeast cells exposed to sparsely ionizing 30-MeV electrons under oxic compared to anoxic conditions. The rejoining of DSBs induced under anoxic conditions was investigated under conditions allowing repair of potentially lethal damage and compared to the rejoining of DSBs induced in oxic cells. In contrast to the biphasic rejoining kinetics of DSBs induced in oxic cells, the rejoining kinetics of DSBs induced in anoxic cells is complicated by the formation of secondary DSBs. These arise during postirradiation incubation of cells, presumably as a consequence of repair processes acting on radiation-induced lesions other than DSBs. These secondary DSBs may at least partially explain the finding that a greater fraction of unrejoinable DSBs is present in cells irradiated under anoxic compared to oxic conditions. As a consequence, the oxygen enhancement ratio of the yield of the remaining DSBs is decreasing in the course of DSB rejoining.

INTRODUCTION

In a previous study the oxygen enhancement ratio (OER)[1] for the induction of double-strand breaks (DSBs) in yeast cells by 30-MeV electrons was reported to be higher than that found for cell killing (*1*). In contrast to induced DSBs which were measured immediately after irradiation, the latter end point comprises repair of DSBs, since growth functions required for the macrocolony assay usually cannot be separated from repair functions. In view of the increasing evidence that DSBs are involved in radiation-induced cell killing, one could expect a decrease of the OER due to a more efficient rejoining of DSBs induced under oxic conditions, as suggested previously (*1*). To test this idea, rejoining of DSBs in yeast cells irradiated under oxic and anoxic conditions was investigated during incubation of cells under conditions suitable for repair of potentially lethal damage (PLD). Furthermore, the OER for the yield of induced and unrejoined DSBs was determined (preliminary results were given in (*2*)).

Parts of the data on the rejoining of DSBs induced under oxic conditions have been published previously (*3, 4*) but are included in this paper, together with new data, for the purpose of comparison with the results obtained for DSBs induced under anoxic conditions.

[1] The following abbreviations are used: DSBs, double-strand breaks; SSBs, single-strand breaks; OER, oxygen enhancement ratio; PLD, potentially lethal damage; $t_{1/2}$, half-life time.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions

*Strain 211*B.* For DSB measurements the diploid strain 211*B of *Saccharomyces cerevisiae* (ATCC 42607) was used. This strain is auxotrophic for 2′-deoxythymidine-5′-monophosphate (dTMP) and lacks mitochondrial DNA, i.e., is respiratory-deficient. Its nuclear DNA can be specifically labeled by [^{3}H]dTMP (*5*).

An overnight culture of cells was grown in a double-concentrated medium (4% glucose, 1.3% nitrogen base without amino acids, 0.4% casamino acids, vitamin free) supplemented with 7 mg/ml 5′-dTMP. Cells were diluted into fresh medium and methyl[^{3}H]dTMP (Amersham) was added to give 148 kBq/ml. The culture was rotated at 30°C until cells had proceeded to the stationary phase. After irradiation cells were resuspended in nongrowth medium (67 mmol phosphate buffer (KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$), pH 5.0, supplemented with 100 mmol glucose and 7 mg/ml 5′-dTMP) at a concentration not higher than about 5×10^6 cells/ml and kept at 30°C in rolling tubes for up to 72 h. The addition of glucose to the nongrowth medium was required to provide an energy source for repair processes in this respiratory-deficient strain. These were the optimum conditions for strain 211*B to perform PLD repair as well as to rejoin DSBs. Repair of PLD in strain 211*B could be followed up to only 24 h. Upon further incubation these cells failed to grow to macrocolonies, although DSB rejoining continued up to 72 h. This failure may be related to the altered membrane properties of this strain, allowing the uptake of dTMP (for further details see (*3, 6*)).

Strain 211. For studying the repair of PLD the diploid wild-type yeast strain 211, a parent strain of 211*B was used. In general, repair of PLD is observed when irradiated cells are incubated in nongrowth medium (such as buffer, distilled water, and "exhausted" or "conditioned" growth medium) before plating on nutrient agar for the macrocolony assay. This treatment is also known as "delayed plating." Cells of strain 211 were grown on nutrient agar plates to stationary phase, washed twice in a double-concentrated phosphate buffer (134 mmol KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$), pH 6.0, and kept after irradiation in this buffer at 30°C

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for 72 h before plating on nutrient agar for the macrocolony assay. This buffer was found to give optimum conditions for PLD repair for strain 211 (*7*).

The reason for using this strain instead of strain 211*B for PLD repair studies was that, although the "immediate plating" curves for both strains were identical (data not shown), maximum PLD repair after 48 to 72 h of incubation in nongrowth medium could be observed only in strain 211.

Thus DSB rejoining within a period of 72 h could be measured only in strain 211*B, while PLD repair up to 72 h could be investigated in strain 211, but was restricted in strain 211*B to 24 h.

Irradiation conditions. The conditions of irradiation of cells with 30-MeV electrons in the presence of oxygen or nitrogen have already been described (*1*). Briefly, labeled stationary-phase cells suspended in phosphate buffer were cooled (4°C) and gassed 20 min with oxygen or nitrogen before irradiation at 4°C with 30-MeV electrons (dose rate: 100 Gy/min) in the presence of oxygen or nitrogen. Samples were taken for DSB measurements either immediately after irradiation or after incubation of cells under PLD repair conditions.

Measurements of double-strand breaks. Spheroplasts were prepared and lysed on top of a neutral sucrose gradient (5–20%) as described elsewhere (*1*). To avoid a speed effect the released DNA was sedimented for 21 h at about 9000 rpm in a Beckman ultracentrifuge using the rotor SW 40 Ti. Gradients were fractionated onto glass fiber filters (Schleicher and Schüll GF 32). High-molecular mass DNA was precipitated with ice-cold 6% TCA. Filters were washed with ethanol and dried and the radioactivity of each filter was determined. The percentage of total radioactivity in the DNA of each fraction as a function of the sedimented volume yielded DNA profiles which were used to determine the number of radiation-induced DSBs. The average number of DSBs per average molar mass of DNA was calculated by computer simulation of random breakage as applied to the DNA of unirradiated cells and by fitting these curves to the DNA profiles obtained from irradiated cells (for details see (*1*)). The calculation was performed on the basis of the relationship between the sedimentation coefficient and the molar mass of DNA as described by Frankenberg *et al.* (*8*).

RESULTS

Evidence Pointing to Differences in the Repairability of Double-Strand Breaks Induced under Oxic and Anoxic Conditions

Cells irradiated under oxic conditions were more radiation-sensitive than cells irradiated under anoxic conditions as shown in Fig. 1 for yeast strain 211 after delayed plating. Since DSBs are considered critical lesions involved in radiation-induced cell killing, we have determined the frequency of DSBs induced in cells under these irradiation conditions (see Figs. 3 and 4). For DSBs induced in anoxic or oxic yeast cells a linear regression curve was fitted. The frequency of DSBs induced in anoxic cells (Fig. 3) was

$$N_{\mathrm{DSB,ind.}} \cdot m_r^{-1} = (1.55 \pm 0.06) \times 10^{-12} \cdot D \cdot \mathrm{Gy}^{-1} \quad \text{(anoxic irradiation).} \quad (1)$$

The frequency of DSBs (including newer data) induced in oxic cells (Fig. 4) was

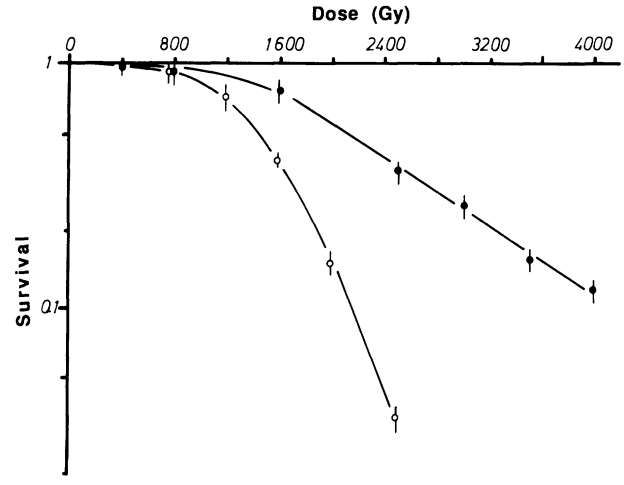

FIG. 1. Survival curves obtained after potentially lethal damage repair (or after delayed plating) of diploid wild-type yeast 211 irradiated in stationary phase under oxic (○) or anoxic (●) conditions and then incubated for 72 h in nongrowth medium before plating on nutrient agar.

$$N_{\mathrm{DSB,ind.}} \cdot m_r^{-1} = (4.49 \pm 0.11) \times 10^{-12} \cdot D \cdot \mathrm{Gy}^{-1} \quad \text{(oxic irradiation).} \quad (2)$$

In Fig. 2 we have plotted on the abscissa the number of DSBs induced per cell by a given dose, using the DSB induction frequencies obtained for anoxic (Eq. (1)) and oxic (Eq. (2)) irradiation conditions. It can be seen that when the data are plotted in this manner the shoulder of the survival curve of cells exposed in anoxic conditions is smaller than under oxic conditions, the opposite of that seen in Fig. 1. Based on the evidence that about one unrepaired radiation-induced DSB kills a yeast cell (see (*9*)) one may conclude that at dose levels corresponding, for example, to 50 induced DSBs per cell, virtually all of these DSBs were repaired if these were induced in oxic yeast cells (i.e., surviving fraction is nearly 1), but less efficient repair occurred if these were induced in anoxic cells (i.e., surviving fraction is 0.7). This seems to indicate that DSBs induced under oxic conditions have a higher probability of being repaired than DSBs induced under anoxic conditions.

For comparison, Fig. 2 also depicts the regression curves for the inactivation of the diploid yeast mutant rad54-3 after irradiation under oxic and anoxic conditions, for which DSB rejoining is undetectable at 36°C (survival data presented in (*10*)). For this mutant about 1 DSB per cell corresponds to a lethal event (*10*). The exponential survival curves of rad54-3 cells (at 36°C) were different when plotted versus dose (D_0 = 18.7 Gy (O_2) and D_0 = 34.3 Gy (N_2) (*10*); however, they were identical when plotted versus the number of DSBs induced per cell (Fig. 2), suggesting that a DSB induced under either condition is equally lethal, if it remains unrejoined.

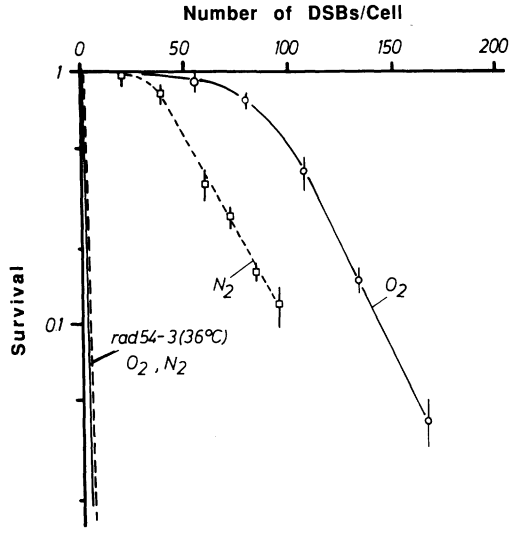

FIG. 2. Survival data shown in Fig. 1 are plotted versus the average number of DSBs induced by a given dose per cell (N_{DSB}) irradiated under oxic (○) or anoxic (□) conditions. Also shown for comparison are the regression lines (which are identical when plotted in this way) of the exponential survival curves obtained after oxic or anoxic irradiation of the diploid yeast mutant rad54-3 (survival data taken from (*10*)) which—at 36°C—is deficient in the rejoining of DSBs (for explanation see text). For the calculation of N_{DSB} the DSB induction frequency for anoxic (Eq. (1)) and oxic (Eq. (2)) irradiation conditions were used and the value of $(1.8 \pm 0.4) \times 10^{10}$ g mol^{-1} was taken as the molar mass of DNA of DNA of diploid yeast cells (*31*).

Formation of Secondary Double-Strand Breaks in Cells Irradiated under Anoxic Conditions

To investigate the possibility that DSBs induced by radiation under oxic conditions may have a higher chance to be repaired compared to DSBs induced under anoxic conditions, we studied the kinetics of rejoining of DSBs, induced in anoxic or oxic yeast cells, during postirradiation incubation of cells under PLD repair conditions, i.e., in nongrowth medium at 30°C. For cells irradiated under anoxic conditions the results are shown in Fig. 3, where the dose relationship for the number of DSBs measured after an incubation period of 0, 1, 3, 6, 16, 24, and 72 h is depicted. The data shown are the mean values. For the sake of clarity, the standard deviation is shown only once for each symbol. The curves were fitted by eye with the exception of the 0-h curve, where the best-fitting regression curve was taken (Eq. (1)).

For cells irradiated under oxic conditions, the corresponding results are shown in Fig. 4. For 0, 3, 24, 48, and 72 h of incubation in nongrowth medium only the best-fitting regression curves are shown, since the experimental data have already been published (*3*). In addition are shown experimental points for 6 and 16 h, together with their best-fitting regression curves.

Figure 3 shows that, at high doses and short periods of PLD repair (1, 3, and 6 h), more DSBs were measured than were originally present immediately after irradiation of anoxic cells. This "overshoot" of secondary DSBs was not detectable in cells irradiated under oxia (Fig. 4). No further rejoining of DSBs induced under anoxic conditions was observed after 24 h of incubation for PLD repair (Fig. 3). Continuing the incubation of these cells for 2 more days yielded a slight increase in the number of DSBs, presumably due to a few incisions during this long period. Since the minimum number of DSBs induced under anoxic conditions was observed after a 24-h repair incubation, these data were used for further evaluation of unrejoined DSBs. In contrast to these findings for DSBs induced under anoxic conditions, the rejoining of DSBs induced under oxic conditions was slower and continued up to at least 48 h (Fig. 4).

Cells Irradiated under Anoxic Conditions Exhibit a Complex Rejoining Kinetics of Double-Strand Breaks

The kinetics of rejoining of DSBs during incubation of yeast cells in nongrowth medium after irradiation under anoxic conditions is shown in a semilogarithmic plot in Fig.

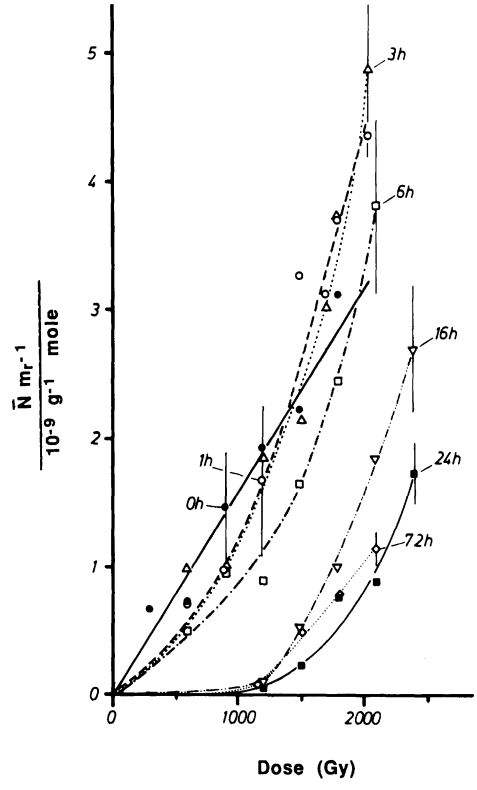

FIG. 3. Induction of DNA DSBs in anoxically irradiated yeast and their rejoining during postirradiation incubation under conditions allowing PLD repair, i.e., oxic incubation in nongrowth medium for 0, 1, 3, 6, 16, 24, and 72 h as indicated in the figure. The data shown are the mean values. For the sake of clarity the mean standard deviation is shown only once for each symbol. The curves were fitted by eye with the exception of the 0-h data, where the curve was fitted by regression analysis (see Eq. (1)). $\bar{N} \cdot m_r^{-1}$: average number ($\bar{N}$) of DSBs per relative molar mass of DNA, m_r.

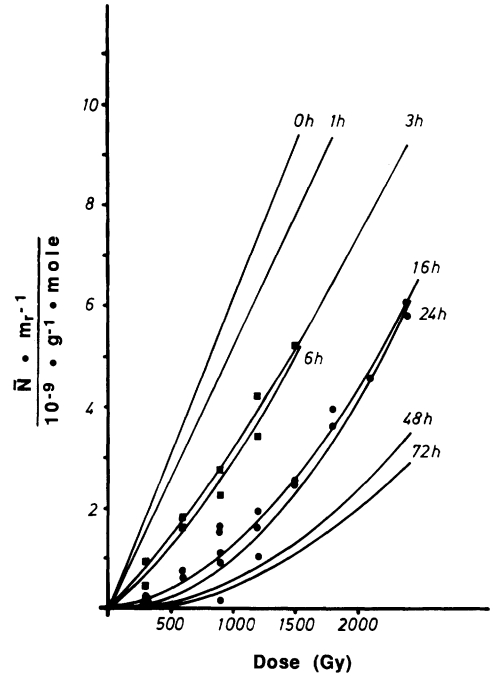

FIG. 4. Induction of DNA DSBs in yeast irradiated under oxic conditions and their rejoining during postirradiation incubation under PLD repair conditions for 0, 1, 3, 6, 16, 24, 48, and 72 h depicted by the corresponding regression curves fitting best to the experimental data. Data points are given only for 6 (■) and 16 h (●) incubation periods. All other data were published previously (*3*). $\bar{N} \cdot m_r^{-1}$: average number ($\bar{N}$) of DSBs per relative molar mass of DNA, m_r.

5 for doses of 250, 500, 750, 1200, 1500, and 2000 Gy. These doses correspond to cell surviving fractions after PLD repair (72 h) of ≈1.0, ≈1.0, ≈1.0, ≈0.98, 0.84, and 0.57 for cells of strain 211 (see Fig. 1). The values for DSBs were taken from the fitted curves presented in Fig. 3. The error bars are mean values of the standard deviations determined experimentally at various doses for each incubation period. The kinetics appears very complex. At high doses (1500 and 2000 Gy) more DSBs could be detected within the first hour than were initially induced. At low doses, however, considerably fewer DSBs were detected after the first hour of incubation (despite the possible production of secondary DSBs within this time), pointing to a rapid initial component of rejoining of DSBs induced under anoxic conditions with a half-life value of about 60 min or less. At incubation times longer than 1 h a slight increase in DSBs was observed even at the low doses applied. After the appearance of secondary DSBs, the rejoining process continued during further incubation in a dose-dependent manner with a $t_{1/2}$ of about 9 h for 2000 Gy, 6 h for 1500 Gy, and 3 h for 1200 Gy.

The OER for Unrejoined Double-Strand Breaks is Lower than That for Induced Double-Strand Breaks

From Eqs. (1) and (2) a dose-independent OER of 2.9 ± 0.1 for induced DSBs was calculated. For anoxic and oxic conditions a direct comparison between induced and unrejoined DSBs (i.e., the minimum level of DSBs induced under anoxic conditions (after 24 h) as well as of DSBs induced under oxic conditions (after 72 h)) is presented in Fig. 6, using the corresponding fitted curves shown in Figs. 3 and 4. The curve for the remaining DSBs induced under anoxic conditions would approach the corresponding curve for DSBs induced under oxic conditions at somewhat lower doses, if the number of DSBs observed after a 72-h incubation for PLD repair (instead of 24 h) were chosen. In principle, however, the same results would be derived. Based on the *same number* of radiation-induced DSBs, for example, $\bar{N} \cdot m_r^{-1}$: = 2×10^{-9} g^{-1}mol or more, the extent of DSB rejoining in cells irradiated under anoxic conditions is less than that observed in cells irradiated under oxic conditions (Fig. 6). Furthermore, while the OER for induced DSBs was dose-independent, the OER for the yield of unrejoined DSBs was decreased in a dose-dependent manner as illustrated in Fig. 7. Because of the difficulty in measuring unre-

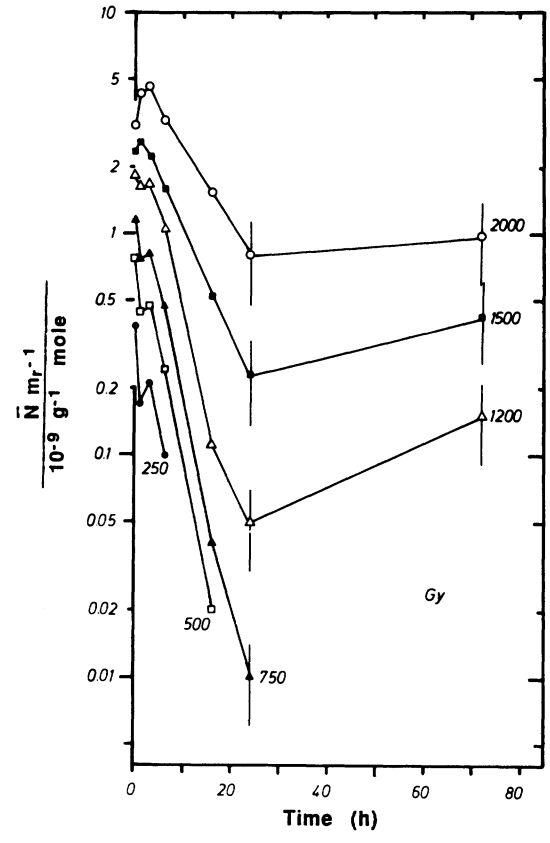

FIG. 5. Kinetics of rejoining of anoxically induced DSBs under PLD repair conditions after irradiation of yeast cells with six different doses, which correspond at the survival level to surviving fractions of ≈1.0, ≈1.0, ≈1.0, ≈0.98, 0.94, and 0.57, respectively. The data points at the various doses are not directly measured but are taken from the curves (Fig. 3) fitted for each incubation period. For the sake of clarity, mean standard deviations, calculated for the data obtained for each incubation period, are presented only for the longer incubation periods. $\bar{N} \cdot m_r^{-1}$: average number ($\bar{N}$) of DSBs per relative molar mass of DNA, m_r.

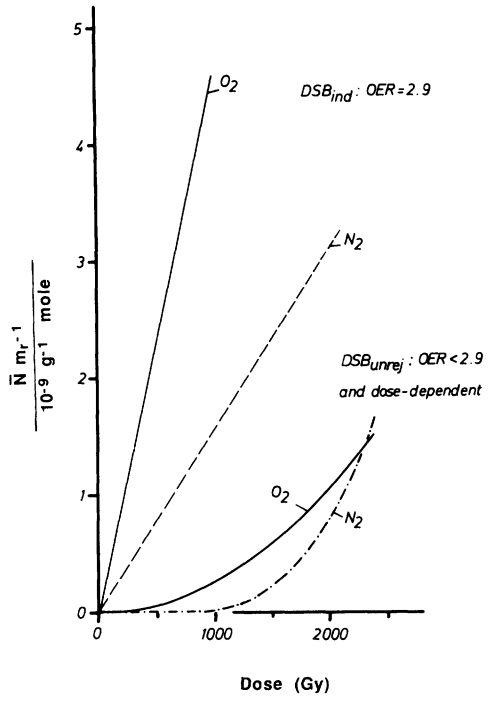

FIG. 6. Comparison of the dose relationship for induced and unrejoined DSBs observed in yeast cells irradiated under oxic (solid lines) or anoxic (dashed lines) conditions. The curves are taken from Figs. 3 and 4. $\bar{N} \cdot m_r^{-1}$: average number ($\bar{N}$) of DSBs per relative molar mass of DNA, m_r. $DSB_{ind.}$: induced DSBs; $DSB_{unrej.}$: unrejoined DSBs.

joined DSBs at low doses, the OER at low doses can only be estimated and is presented by a dashed line.

DISCUSSION

Double-Strand Breaks Induced by Radiation under Oxic and Anoxic Conditions Are Rejoined in Yeast

Rejoining of DSBs induced in yeast by irradiation under oxic conditions has been demonstrated to occur during post-irradiation incubation of cells in growth medium (*11–13*) and nongrowth medium, i.e., under PLD repair conditions (*3, 4, 6, 13, 14*). However, only a few studies were concerned with the rejoining of DSBs induced in yeast under anoxic conditions. Geigl[2] showed that DSBs induced in diploid yeast irradiated under anoxic conditions were rejoined during incubation of cells under PLD repair conditions. In the present paper we have extended such studies (Fig. 3) and compared the results with those obtained for DSBs induced under oxic conditions (Fig. 4) which were published recently (*4*). As already reported for DSBs induced under oxic conditions (*3, 6*), the linear relationship between DSBs induced by radiation under anoxic conditions was also converted into a nonlinear relationship in the course of rejoining processes. However, significant differences could be observed in the rejoining kinetics of DSBs induced under anoxic and oxic conditions. The kinetics of rejoining of DSBs induced under anoxic conditions revealed the formation of secondary DSBs (Figs. 3 and 5) which presumably arise by enzymatic repair processes acting on radiation-induced DNA lesions other than DSBs which are formed predominantly in anoxic cells. In contrast to high doses no secondary DSBs were detectable within the first hour of incubation after low-dose irradiation (250 and 500 Gy) (Fig. 5), probably because of a simultaneously occurring rapid rejoining of radiation-induced DSBs, proceeding with a $t_{1/2}$ not higher than about 60 min. Taking into consideration the simultaneous formation of secondary DSBs, the initial component of rejoining of radiation-induced DSBs should have a $t_{1/2}$ even shorter than 60 min. After the formation of secondary DSBs, both radiation-induced and enzymatically formed DSBs were rejoined in a dose-dependent manner ($t_{1/2} \approx 9$ h at 2000 Gy and ≈ 3 h at 1200 Gy) (Fig. 5).

There are at least two reasons for this dose-dependent complex kinetic pattern. First, there may operate a dose-dependent processing of lesions which gives rise to enzymatically formed secondary DSBs. Second, the rapid component of rejoining of DSBs induced under anoxic conditions may decrease with dose (as already described for DSBs induced under oxic conditions (4)).

In contrast, the rejoining of DSBs induced under oxic conditions was less complex and proceeded much slower as already described (4). The biphasic kinetics showed for the initial rapid component of rejoining a $t_{1/2}$ of 3.8 h and for the slow component a $t_{1/2}$ of about 10 h (*4*).

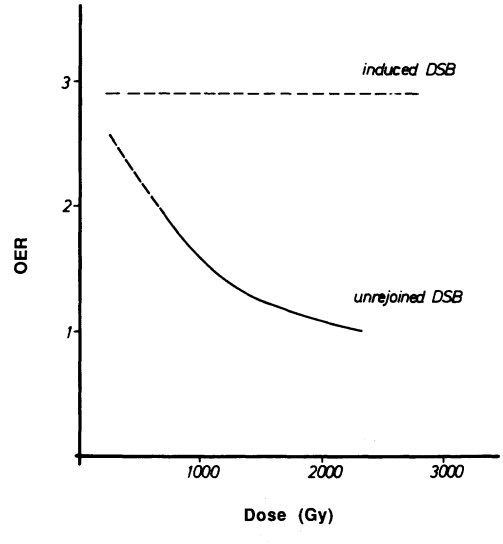

FIG. 7. The dose relationship of the OER for induced and unrejoined DSBs, as evaluated from Fig. 6. The OER values for unrejoined DSBs at low doses were estimated and thus are represented by a dashed line.

[2] E. M. Geigl, *Untersuchung von S1-Nuclease-sensitiven Stellen im Genom der Hefe Saccharomyces cerevisiae nach in vivo-Bestrahlung mit Gamma-Strahlen.* Thesis, GSF-Bericht, 18/87, 1987.

Based on the different rates of rejoining determined for DSBs induced under anoxic and oxic conditions, it is concluded that there is a qualitative difference between DSBs induced in cells irradiated under anoxic or oxic conditions.

DNA Lesions Preferably Induced by Radiation in Anoxic Cells

The nature of DNA lesions which are preferably induced in anoxic cells and which may give rise to secondary DSBs remains to be elucidated. DNA–protein crosslinks are known to be induced by radiation at a higher frequency in anoxic cells compared to oxic cells (*15–17*). However, it is not known if and under which conditions DSBs may be formed in the course of repair of these crosslinks. Bulky lesions, which are structural disturbances of the DNA double-helix involving clustered base damage (*18*) with at least three unpaired bases (*19*), may be another candidate. These lesions gave rise to DSBs when incised *in vitro* by nuclease S1 of *Aspergillus oryzae* as shown for yeast cells irradiated under anoxic conditions[2] (*20*). It is still unknown whether bulky lesions are formed predominantly in yeast cells irradiated under anoxic conditions and whether these are incised *in vivo*.

Controversial Reports on Qualitative Differences between DNA Strand Breaks Induced by Radiation under Oxic and Anoxic Conditions

The different rates of rejoining of DSBs induced under oxic and anoxic conditions described in this paper suggest that a different type(s) of DSB is induced in yeast under oxic compared to anoxic conditions. Van der Schans *et al.* (*21*) also studied the induction and rejoining of DSBs (measured by the neutral elution technique) in primary human skin fibroblasts after irradiation under oxic (50 Gy) or anoxic conditions. However, these authors did not detect any differences in the rate of rejoining of DSBs induced under oxic or anoxic conditions during a 2-h postirradiation incubation of cells in growth medium. Likewise, Vos *et al.* (*22*) reported that logarithmically growing CHO cells rejoin DSBs induced under oxic (100 Gy) or anoxic (200 Gy) conditions during postirradiation incubation for up to 2 h in growth medium with the same kinetics. There may be several explanations of the controversial results on the rejoining kinetics of DSBs induced under oxic or anoxic conditions reported in this paper and in those of Van der Schans *et al.* (*21*) and Vos *et al.* (*22*). Apart from the different eukaryotic cell types used which may differ in their prevalence for the mechanism of rejoining of DSBs (i.e., recombination repair versus ligation of DSBs), different postirradiation incubation conditions were applied in these studies, and the method to measure DSBs may also be important. It is still unclear whether the neutral filter elution technique measures solely DSBs.

The qualitative difference between DSBs induced under oxic and anoxic conditions indicated for yeast in the present paper is in agreement with other findings reported for total DNA breaks, i.e., single-strand breaks (SSBs) and DSBs. About 80% of SSBs induced under oxic conditions were rejoined after 30 min of postirradiation incubation of Chinese hamster V79 cells in growth medium, whereas only about 50% of breaks induced under hypoxic conditions were rejoined under the same conditions (irradiation with X rays in the dose range of 10 to 13 Gy) (*23*). Likewise, Koch and Painter (*24*) found a larger fraction of unrejoined SSBs in Chinese hamster V79 cells irradiated under hypoxic compared to oxic conditions (dose range 50 to 180 Gy) after a 60-min postirradiation incubation in growth medium. A similar result was reported by Roots and Smith (*25*) for exponentially growing Chinese hamster ovary cells irradiated under oxic (47 Gy) or anoxic (188 Gy) conditions and incubated in growth or nongrowth medium for up to 10 h.

By contrast, other authors found no qualitative difference between total breaks induced under oxic and anoxic conditions. For example, Lennartz *et al.* (*26*) found the same proportion of different strand-break end groups (deoxyribose-3′-OH, -5′-phosphate, and -5′-OH) in thymocytes irradiated under oxic or anoxic conditions with 10-MeV electrons at doses between 10,000 and 60,000 Gy. However, differences in the proportion of end groups pertaining to DSBs may have been masked in their study because of the about 25-fold greater frequency of SSBs compared to DSBs, and also because of the fact that with the enzymic analyses applied only about 50% of the total end groups can be identified (*27*). Rejoining of SSB was investigated during postirradiation incubation of thymocytes in growth medium. A decrease of 3′-OH and 5′-phosphate end groups was observed which leveled off after 15 min, irrespective of the presence or absence of oxygen during irradiation (*27*). Likewise, Ormerod and Stevens (*28*) did not find a difference in the rejoining of SSBs induced under oxic or anoxic conditions in murine lymphoma cells incubated in growth medium for up to 125 min after exposure to doses of 50 to 500 Gy of X rays.

Thus, for DSBs as well as for total breaks, i.e., mainly SSBs, there are conflicting results with respect to differences of the rejoining kinetics of breaks induced under oxic or anoxic conditions. It should be mentioned, however, that all mammalian cell studies were performed at supralethal doses, in contrast to the yeast work presented in this paper (see the legend of Fig. 5). Although higher doses are used in yeast survival studies than in mammalian cells (because of the smaller DNA target size in yeast), doses up to 2400 Gy did not impair the activity of enzymes involved in DSB rejoining, as demonstrated previously (*3*).

The OER of Double-Strand Breaks Decreases in a Dose-Dependent Manner Due to Repair Processes

The OER determined from survival data is usually lower than that for the induction of DSBs, as has been shown, for example, for yeast (*1*). These findings may be interpreted as a result of a less efficient rejoining of DSBs induced under anoxic conditions which, as a consequence, lead to a lower OER for unrejoined DSBs (*1*). Alternatively, it may be proposed that other radiation-induced lesions, for example, base damage, which exhibit a lower OER (e.g., (*16, 29*)) than induced DSBs, may contribute to cell lethality.

The data in Fig. 7 show that the OER for unrejoined DSBs is smaller than that for induced DSBs, suggesting that the reduced extent of DSB rejoining (or increased *fraction* of unrejoined DSBs) detectable in cells irradiated under anoxia with doses greater than about 1000 Gy (Fig. 6) may be responsible for the decreased OER observed for unrejoined DSBs. Thus the consideration solely of radiation-induced and unrejoined DSBs supports the first interpretation. However, in view of the kinetics of DSB rejoining shown in Fig. 5, the second interpretation is favored. The observed *increase* in DSBs during the first hours seems to be due to other DNA lesions induced by radiation in anoxic cells which give rise to secondary DSBs during enzymatic repair processes. The increased number of DSBs (radiation- as well as enzymatically induced DSBs) observed in cells irradiated under anoxia may increase the probability of interaction between DSBs, resulting in a greater fraction of un- or misjoined DSBs (see (*9, 14*)). Consequently, as illustrated in Fig. 7, the OER for un- or misrejoined DSBs decreases with increasing dose, i.e., with the number of radiation-induced DSBs and other DNA lesions which are converted into DSBs by repair processes. It is interesting to note that for the yield of dicentrics a dose-dependent decrease of the OER has also been observed by Prosser *et al.* (*30*) in X-irradiated human lymphocytes.

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REFERENCES

1. M. FRANKENBERG-SCHWAGER, D. FRANKENBERG, D. BLÖCHER, and C. ADAMCZYK, The influence of oxygen on the survival and yield of DNA double-strand breaks in irradiated yeast cells. *Int. J. Radiat. Biol.* **36,** 261–270 (1979).
2. M. FRANKENBERG-SCHWAGER, DNA double-strand breaks: Their repair and relationship to cell killing in yeast. *Int. J. Radiat. Biol.* **57,** 1252 (1990). [Abstract]
3. M. FRANKENBERG-SCHWAGER, D. FRANKENBERG, D. BLÖCHER, and C. ADAMCZYK, Repair of DNA double-strand breaks in irradiated yeast cells under nongrowth conditions. *Radiat. Res.* **82,** 498–510 (1980).
4. M. FRANKENBERG-SCHWAGER, D. FRANKENBERG, R. HARBICH, and C. ADAMCZYK, A comparative study of the rejoining of DNA double-strand breaks in yeast irradiated with 3.5 MeV α-particles or with 30 MeV electrons. *Int. J. Radiat. Biol.* **57,** 1151–1168 (1990).
5. G. W. HÖLTZ and M. BRENDEL, Incorporation of (methyl ^{3}H) 5′ dTMP into DNA of diploid yeast cells as tracer of liquid holding recovery after x-ray irradiation. In *Radiation and Cellular Control Processes* (J. Kiefer, Ed.), pp. 111–116. Springer-Verlag, Berlin/Heidelberg/New York, 1976.
6. M. FRANKENBERG-SCHWAGER, D. FRANKENBERG, D. BLÖCHER, and C. ADAMCZYK, The linear relationship between DNA double-strand breaks and radiation dose (30 MeV electrons) is converted into a quadratic function by cellular repair. *Int. J. Radiat. Biol.* **37,** 207–212 (1980).
7. W. POHLIT and I. R. HEYDER, Growth of cells on solid culture medium. II. Cell physiological data of stationary yeast cells and the initiation of cell cycle in nutrient free buffer solution. *Radiat. Environ. Biophys.* **14,** 213–230 (1977).
8. D. FRANKENBERG, M. FRANKENBERG-SCHWAGER, D. BLÖCHER, and R. HARBICH, Evidence for DNA double-strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic or anoxic conditions. *Radiat. Res.* **88,** 524–532 (1981).
9. M. FRANKENBERG-SCHWAGER and D. FRANKENBERG, DNA double-strand breaks: Their repair and relationship to cell killing in yeast. *Int. J. Radiat. Biol.* **58,** 569–575 (1990).
10. D. FRANKENBERG, B. D. MICHAEL, M. FRANKENBERG-SCHWAGER, and R. HARBICH, Fast kinetics of the oxygen effect for DNA double-strand breakage and cell killing in irradiated yeast. *Int. J. Radiat. Biol.* **57,** 485–501 (1990).
11. K. HO, Induction of DNA double-strand breaks by x-rays in a radiosensitive strain of the yeast *Saccharomyces cerevisiae. Mutat. Res.* **30,** 327–443 (1975).
12. M. A. RESNICK and P. MARTIN, Repair of DNA double-strand breaks in nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. *Mol. Gen. Genet.* **143,** 119–129 (1976).
13. A. N. LUCHNIK, V. M. GLASER, and S. V. SHESTAKOV, Repair of DNA double-strand breaks requires two homologous DNA duplexes. *Mol. Biol. Rep.* **3,** 437–442 (1977).
14. M. FRANKENBERG-SCHWAGER, D. FRANKENBERG, D. BLÖCHER, and C. ADAMCZYK, Effect of dose rate on the induction of DNA double-strand breaks in eukaryotic cells. *Radiat. Res.* **87,** 710–717 (1981).
15. A. J. FORNACE and J. B. LITTLE, DNA crosslinking induced by x-rays and chemical agents. *Biochim. Biophys. Acta* **477,** 343–355 (1977).
16. I. R. RADFORD, Effect of radiomodifying agents on the ratios of x-ray-induced lesions in cellular DNA: Use in lethal lesions determination. *Int. J. Radiat. Biol.* **49,** 621–637 (1986).
17. L. Y. XUE, L. R. FRIEDMAN, and N. L. OLEINICK, Repair of chromatin damage in glutathione-depleted V79 cells: Comparison of oxic and hypoxic conditions. *Radiat. Res.* **116,** 89–99 (1988).
18. E. KOHFELDT, H. BERTRAM, and U. HAGEN, Action of gamma endonuclease in clustered lesions in irradiated DNA. *Radiat. Environ. Biophys.* **27,** 123–132 (1988).
19. J. R. SILBER and L. L. LOEB, S1 nuclease does not cleave DNA at single-base mis-matches. *Biochim. Biophys. Acta* **656,** 256–264 (1982).
20. J. ANDREWS, H. MARTIN-BERTRAM, and U. HAGEN, S1 nuclease-

sensitive sites in yeast DNA: An assay for radiation-induced base damage. *Int. J. Radiat. Biol.* **45,** 497–505 (1984).

21. G. P. VAN DER SCHANS, H. B. CENTEN, and P. H. M. LOHMAN, DNA lesions induced by ionizing radiation. In *Progress in Mutation Research* (A. T. Natarajan, G. Obe, and H. Altmann, Eds.), Vol. 4, pp. 285–299. Elsevier, Amsterdam, 1982.
22. O. VOS, G. P. VAN DER SCHANS, and W. S. D. ROS-VERHEJ, Reduction of intracellular glutathione content and radiosensitivity. *Int. J. Radiat. Biol.* **50,** 155–165 (1986).
23. H. G. MODIG, M. EDGREN, and L. REVESZ, Dual effect of oxygen on the induction and repair of single-strand breaks in the DNA of x-irradiated mammalian cells. *Int. J. Radiat. Biol.* **26,** 341–353 (1974).
24. C. J. KOCH and R. B. PAINTER, The effect of extreme hypoxia on the repair of DNA single-strand breaks in mammalian cells. *Radiat. Res.* **64,** 256–269 (1975).
25. R. ROOTS and K. C. SMITH, Rejoining of DNA single-strand breaks in mammalian cells incubated in buffer or in medium after aerobic or anaerobic x-irradiation. *Int. J. Radiat. Biol.* **27,** 595–602 (1975).
26. M. LENNARTZ, T. COQUERELLE, A. BOPP, and U. HAGEN, Oxygen effect on strand breaks and specific endgroups in DNA of irradiated thymocytes. *Int. J. Radiat. Biol.* **27,** 577–585 (1975).
27. M. LENNARTZ, T. COQUERELLE, and U. HAGEN, Modification of endgroups in DNA strand breaks of irradiated thymocytes during early repair. *Int. J. Radiat. Biol.* **28,** 181–185 (1975).
28. M. G. ORMEROD and U. STEVENS, The rejoining of x-ray-induced strand breaks in the DNA of a murine lymphoma cell (L5178Y). *Biochim. Biophys. Acta* **232,** 72–82 (1971).
29. M. C. PATERSON, B. P. SMITH, P. H. M. LOHMAN, A. K. ANDERSON, and L. FISHMAN, Defective excision repair of gamma-ray damaged DNA in human (ataxia telangiectasia) fibroblasts. *Nature* **260,** 444–446 (1976).
30. J. S. PROSSER, C. M. WHITE, and A. A. EDWARDS, The effect of oxygen concentration on the x-ray induction of chromosome aberrations in human lymphocytes. *Mutat. Res.* **61,** 287–295 (1979).
31. G. D. LAUER, T. ROBERTS, and L. C. KLOTZ, Determination of the nuclear DNA content of *Saccharomyces cerevisiae* and implications for the organisation of DNA in yeast chromosomes. *J. Mol. Biol.* **114,** 507–526 (1977).