Effects of X-ray and carbon ion beam irradiation on membrane permeability and integrity in *Saccharomyces cerevisiae* cells

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*Saccharomyces cerevisiae* has served as a eukaryotic model in radiation biology studies of cellular responses to ionizing radiation (IR). Research in this field has thus far mainly been focused on DNA strand breaks, DNA base damage, or inhibition of protein activity. However, the effects of IR on *S. cerevisiae* cell membranes have barely been studied. Here, we investigated the changes in the permeability and integrity of *S. cerevisiae* cell membranes induced by high–linear energy transfer carbon ion (CI) beam or low–linear energy transfer X-ray. After CI exposure, protein elution and nucleotide diffusion were more pronounced than after X-ray treatment at the same doses, although these features were most prevalent following irradiation doses of 25–175 Gy. Flow cytometry of forward scatter light versus side scatter light and double-staining with fluorescein diacetate and propidium iodide showed that CI and X-ray irradiation significantly affected *S. cerevisiae* cell membrane integrity and cellular enzyme activity compared with untreated control cells. The extent of lesions in CI-irradiated cells, which exhibited markedly altered morphology and size, was greater than that in X-ray-irradiated cells. The relationships between permeabilized cells, esterase activity, and non-viable cell numbers furthermore indicated that irradiation-induced increases in cell permeabilization and decreases in esterase activity are dependent on the type of radiation and that these parameters correspond well with cell viability. These results also indicate that the patterns of cell inactivity due to X-ray or CI irradiation may be similar in terms of cell membrane damage.

**Keywords:** cell membrane permeability; X-ray; carbon ion; flow cytometry; *Saccharomyces cerevisiae*

INTRODUCTION

Ionizing radiation (IR) has been extensively applied in research on microorganisms and eukaryotes over many decades. In investigations of the biological effects of various forms of radiation, carbon ion (CI) beams and X-rays have frequently been utilized as models of high–linear energy transfer (high-LET) and low–linear energy transfer (low-LET) radiation, respectively. CI beams can produce highly intense ionization along a given particle’s path. These dense distributions of energy generate high levels of relative biological effectiveness (RBE), induce a broader spectrum of mutations than low–LET X-rays [1], and bring about complex lesions that are deemed too difficult for cellular repair machinery to resolve. These cellular effects are primarily caused by DNA double-strand breaks (DSBs), clustered DNA damage, and large deletions [2–4]. Specific gene expression patterns of high-LET CI-irradiated *Saccharomyces cerevisiae* show that the DNA damage and oxidative lesions induced by CI can elicit broad cellular responses, including checkpoint pathway activation, cell cycle arrest, DNA repair, and oxidative stress response activation [5]. Simultaneously, the nature of the acute DNA damage induced by irradiation as well as the subsequent cellular responses and radioprotective processes depend on a variety of factors, including radiation quality, dose rate, cell type, cell cycle, and growth period [6, 7]. Research into the molecular mechanisms of the effects of IR on organisms has been focused on complex, irreparable, clustered DNA damage and the changes in single or multiple DNA bases
In comparison, the effects of IR on \textit{S. cerevisiae} cell membranes have not been paid much attention, despite the fact that, as the first protective screen, the cell membrane is damaged due to the interaction between ion beam irradiation and biological macromolecules of the membrane when \textit{S. cerevisiae} cells are subjected to CI or X-ray irradiation. These effects on cell membranes may also be one of the reasons for CI or X-ray irradiation leading to \textit{S. cerevisiae} apoptosis or cell death.

Damage to cell membranes mainly manifests as changes in membrane permeability and integrity, which can be assessed by measuring protein permeability, diffusion rates of nucleic acids [10, 11], and staining with specific fluorescent dyes. In microbiological studies, the plate count method is traditionally used to evaluate the effects of IR on microorganisms; however, when microorganisms are sublethally damaged, or when they are dormant or inactive because of changes in cell membrane integrity or metabolic activity, this technique has limitations [12]. Alternative techniques to adequately characterize this non-culturable population are thus needed for investigations into the effects of IR on microbial cell membranes. Flow cytometry (FCM) allows for physiological parameters of microorganisms to be studied: the physical form of microorganisms can be elucidated by light scattering patterns, in which forward scatter (FSC) and side scatter (SSC) of light by the cells provide insight into the size and granularity (respectively) of the analyzed cells. Furthermore, using specific fluorescent dyes, FCM can provide biological information regarding changes in cell function and structure based on changes in cellular targets, such as DNA, enzymes, cytoplasmic membranes, or membrane potential. Double-staining cells of interest with fluorescent dyes, for example, is frequently carried out to assess enzyme activity and membrane integrity [13].

To the best of our knowledge, the effects of CI or X-ray irradiation on \textit{S. cerevisiae} cell membranes have not yet been investigated by FCM. The objective of this study was to evaluate the changes in \textit{S. cerevisiae} cell membrane permeability and integrity after exposure to CI or X-ray radiation by assessing protein leakage and diffusion of nucleic acids as well as by applying FCM (dual-staining FDA–PI) in conjunction with the standard plate count technique. These methods were used to compare CI treatment with X-ray treatment in terms of the effects of irradiation on cell morphology, cell permeability, esterase activity, and cell viability.

**MATERIALS AND METHODS**

**Strain, medium and growth conditions**

The yeast strain used in this work was \textit{S. cerevisiae} strain CICC 1308 (\textit{MAT}a, budding, haploid) (obtained from the Center of Industrial Culture Collection of China). The \textit{S. cerevisiae} cells were inoculated into 20 ml of yeast peptone dextrose (YPD) medium (1% yeast extract, 2% peptone and 2% glucose) in 50-ml flasks incubated on a mechanical shaker (200 rpm) at 30°C. In order to obtain good strain vitality, cells were continuously cultured three times to the density of \textgreater{}\texttimes{}10^7 cells/ml (OD_{600} = 0.4).

**X-ray and CI beam irradiation**

\textit{Saccharomyces cerevisiae} cells were incubated at 30°C for 9 h (log phase) in YPD medium, centrifuged at 3500 rpm for 3 min to harvest yeast cells. The cells were resuspended in sterile water, and divided into eight groups at random. Each group was put into an individual sterilized dish of \textphi{}35 mm. These samples were irradiated with X-ray or CI beams at doses of 0, 25, 50, 75, 100, 125, 150 and 175 Gy, respectively. The irradiation experiments were conducted with the RX-650 X-ray biological irradiator (FAXITRON, USA) and the HIRFL (Heavy Ion Research Facility in Lanzhou) at IMPCAS (Institute of Modern Physics, Chinese Academy of Sciences). The energy and LET of CI beams are 100 MeV/u and 202 keV/\mu{}m, respectively. With regard to X-rays, the energy and dose rate are 100 kVp and 1.5 Gy/min, respectively. The solution was stable during irradiation and negative control was subjected to the same handling minus irradiation.

**Viable population determined by plate count method**

After irradiation, \textit{S. cerevisiae} cells were diluted by 10-fold aliquots to the desired concentrations using sterilized water and surface-plated to solid YPD medium for colony formation in order to measure their survival rate. Three replicates were used, but when irradiation treatment resulted in low counts, more plates were used as replicates. Plates were incubated for 48 h at 30°C. Counting was used for enumeration of colonies. Survival curves were generated from experimental data by plotting doses of irradiation versus \text{Log N}/\text{N}_0 [where \text{N} is the number of colony-forming units (CFUs) at a given dose and \text{N}_0 is the negative control number of CFUs]. The non-viable cells were shown by \text{Log N}_0–\text{Log N}.

**Evaluation of membrane integrity by protein leakage and diffusion of intracellular nucleotides**

After \textit{S. cerevisiae} cells were irradiated with X-ray or CI beams at different doses, the samples were harvested, washed and resuspended in PBS to a final OD_{600} = 0.2 (\textless{}1 \times{}10^8 cells/ml) and OD_{600} = 0.4 (\textless{}1 \times{}10^7 cells/ml), while the control was incubated with YPD medium alone. In order to prevent the activity of protease, all samples were kept on ice for 2 h and harvested by centrifugation at 10,000 rpm at 4°C for 10 min. Eluting protein from \textit{S. cerevisiae} cells was measured using a BCA protein assay kit (Beyotime, China) on the supernatants. The absorbances of irradiated cells, untreated control cells and BSA standards (range 0–200 \mu{}g/ml BSA) were measured at 560 nm after 2 h incubation at 37°C.
The BSA standard (µg/ml) was used to draw standard curves. In addition, for calculating diffusion of intracellular nucleotides, the absorbances of cell supernatants at 260 and 280 nm were measured. The calculating equation was as follows [14]: nucleotide (µg/ml) = (11.87 × A_{260} − 10.40 × A_{280}) × 100/9.

**Flow cytometric analysis of FSC–SSC and double-staining with FDA and PI**

Cells from the groups irradiated at 100 and 175 Gy and the control group (0 Gy) were injected into the FACSARia II flow cytometer (BD, USA) under the same voltage. Dot plots were drawn by FSC versus SSC. Moreover, double-staining with FDA (Sigma, USA) and PI (Sigma, USA) were used for FCM analysis. A blank control (non-stained and non-irradiated) was employed to determine the autofluorescence of the cells. The staining procedure was as follows: cells were incubated with 5 mg/ml (acetone) FDA at 37°C for 30 min, then cells were centrifugally washed twice by sterilized water (3000 rpm, 3 min) and suspended in 1 ml phosphate buffered saline solution (PBS buffer, pH 7.2). Afterwards, 1 mg/ml PI (dissolved in deionized water) was added to the system and incubated for 10 min at room temperature in the dark. Samples were immediately analyzed. Analysis was also performed on the FACSARia II flow cytometer (BD, USA). The flow rate was adjusted to keep the acquisition at 200 cells per second. A total of 10,000 events were registered per sample. Green fluorescence of cells stained with FDA was collected in the FL1 channel (525 ± 15 nm), and red fluorescence of cells labeled with PI was collected in the FL2 channel (620 ± 15 nm). Tests were replicated at least three times with three samples for each of the irradiation doses. The software FlowJo7.6 was used to analyze flow cytometric data. Dot plot analysis of FDA versus PI was applied to determine the fluorescence properties of the population.

**Data analysis**

Qualitative images of FDA–PI double-staining were acquired with BX53 epifluorescence microscopy (Olympus, Japan) for the 100- and 175-Gy irradiation groups and for the control group (0 Gy). Meanwhile, the corresponding bright-field images were also obtained. Residual esterase activity following irradiation was calculated using Eq. (1), in which the enzyme activity of the irradiated cells was set in relation to the activity of the untreated cells.

\[
\%EA_{\text{overall}} = \frac{1_{X/\text{CI}} + 2_{X/\text{CI}}}{1_{\text{Ctrl}} + 2_{\text{Ctrl}}} \times 100
\]  

(1)

Here, \(\%EA_{\text{overall}}\) is the percentage of residual enzyme activity in viable and non-viable cells; \(1_{X/\text{CI}}\) and \(2_{X/\text{CI}}\) are the percentages of the flow cytometric population in gates 1 and 2 following X-ray or CI treatment, and \(1_{\text{Ctrl}}\) and \(2_{\text{Ctrl}}\) are the percentages of the population in gates 1 and 2 in the control group, respectively. The permeabilized cells (PI-positive) were counted by the difference between Log N_{0PI} and Log N_X/CIPi (Log N_{0PI}/N_X/CIPi), where N_{0PI} and N_X/CIPi were the number of cells that did not take up PI (gate 1 + gate 4) at 0 Gy (untreated control) and at the different irradiation doses, respectively. Moreover, data are presented as the mean ± SD. Statistical analyses were performed using OriginPro 8.0 software.

**RESULTS**

**Cell inactivation following X-ray or CI exposure**

To evaluate the effects of X-ray and CI irradiation on *S. cerevisiae* cell viability, cells treated with different doses of either X-ray or CI irradiation were assessed in terms of viable cell number using the plate count method. The results of this assessment are shown in Fig. 1. With regard to CI exposure, the cell survival rate declined sharply with increasing irradiation dose and continued to drop until 150 Gy, at which point cell death had reached ~95%. Little to no further change in percentage cell survival was observed for 150–175 Gy doses of irradiation, suggesting that a small number of cells are more resistant to irradiation than the cells that are rapidly destroyed by ≤150 Gy irradiation. In contrast to CI-irradiated cells, cells exposed to X-ray irradiation exhibited almost 90% viability at doses of 25–75 Gy, and cell death was found to increase gradually with higher doses of irradiation. CI irradiation was therefore found to have a stronger lethal effect on *S. cerevisiae* cells than X-ray irradiation at identical doses. These results are in agreement with those reported by Matuo *et al.* [15].

![Fig. 1. Survival curves of *S. cerevisiae* cell with X-ray (circles) and CI (squares) irradiation (0, 25, 50, 75, 100, 125, 150 and 175 Gy). Each point is presented as the mean ± standard deviation of three determinations. The non-irradiated (0 Gy) stain is used as the negative control.](image-url)
Detection of protein leakage and intracellular nucleotide diffusion
Protein leakage and intracellular nucleotide diffusion were investigated in *S. cerevisiae* cells treated with X-ray or CI irradiation. As shown in Fig. 2, protein elution from cells increased with increasing CI dose (25–175 Gy), with significant differences being observed between the protein leakage measured at every dose ($P < 0.01$). Approximately $22.4 \pm 1.08 \mu g/ml$ protein leakage was measured for cells (OD$_{600} = 0.4$) treated with 175 Gy CI irradiation, a dose at which almost no cells survived. For cells exposed to X-ray radiation, on the other hand, protein elution did not change up until a dose of 75 Gy ($P < 0.05$), and differences only became pronounced ($P < 0.01$) with doses $\geq 100$ Gy. A dependence between cell density (OD$_{600} = 0.4$ vs OD$_{600} = 0.2$) and irradiation-induced protein leakage was evident: it appeared that protein leakage due to irradiation depends on cell density.

As shown in Fig. 3, nucleotide diffusion from *S. cerevisiae* cells was markedly enhanced by increasing doses of CI irradiation ($P < 0.01$). The cells exhibited dramatic diffusion of nucleotides: 57 and 175 $\mu g/ml$ nucleotide diffusion was measured for cells treated with 50 and 175 Gy CI, respectively, and as in the case of protein leakage, a multiple relationship was revealed between cell density (OD$_{600} = 0.4$ vs OD$_{600} = 0.2$) and irradiation-induced nucleotide diffusion. Nucleotide diffusion was distinctly lower in cells irradiated with X-ray radiation compared with those irradiated with CI radiation: in X-ray–irradiated cells, slight differences relative to untreated cells were only measured for doses of $\geq 75$ Gy.

Assessment of cell morphology and size
The effects of exposure to X-ray and CI irradiation on *S. cerevisiae* cell morphology and size were investigated using dot plots of FSC vs SSC generated from FCM analyses. Light scatter by *S. cerevisiae* cells before and after X-ray or CI radiation exposure at high doses (100 and 175 Gy) are shown in Fig. 4. Compared with untreated cells, the CI-treated *S. cerevisiae* population became more uniform, and on the dot plot the major cell population shifted to slightly lower FCS and SSC values, particularly with the highest CI dose (175 Gy). From the dot plots of FSC vs SSC, for cells treated with X-ray doses of 100 and 175 Gy it is clear that changes in cell morphology and size due to X-ray irradiation were less obvious compared with those induced by CI irradiation of equal doses. This difference in the effects of CI compared with X-ray radiation treatments is in line with the differences observed for the two radiation types in protein permeability and intracellular nucleotide leakage experiments (Figs 2 and 3).

Effects on membrane integrity and esterase activity
Double-staining of *S. cerevisiae* with FDA and PI allowed the effects of various doses of X-ray or CI irradiation on the cells’ capacity for fluorescein (F) accumulation and retention to be assessed. These measurements were used as indicators of membrane integrity and of enzyme activity in the cells, while PI uptake was monitored as a measure of cell membrane permeability. Dual parameter density plots of FDA and PI staining are shown in Fig. 5, in which green (y-axis) and red (x-axis) fluorescence intensities are plotted against one another. Untreated cells stained with FDA and PI (A, 0 Gy; B, 0 Gy) showed similar patterns in their fluorescence...
labeling properties, although not all cells appeared in quadrant 1 (F-PI-). With regard to treated cells, the data in Fig. 5 reveal that changes in fluorescence patterns due to irradiation depend on the radiation type: increasing doses of CI or X-ray irradiation from 0 to 175 Gy generally resulted in a gradual shift of the *S. cerevisiae* cell population from quadrant 1 to quadrant 3, suggesting that irradiation may indirectly decrease esterase activity and destroy cell membrane integrity in these cells, resulting in F accumulation declining and PI penetrating cells. In the case of CI exposure (Fig. 5B), the increasing shift of a cell population to quadrant 3 indicates that CI treatment strongly reduces the accumulation of F, presumably by inactivating intracellular esterase, allowing PI diffusion into the cytoplasm. For treatment doses of ≥25 Gy CI irradiation, the proportion of cells with permeabilized membranes increased. PI continued to accumulate inside cells, binding to DNA and thus yielding higher red fluorescence intensities. After 175 Gy of CI irradiation, as much as 90.3% of the whole cell population was stained with PI in conjunction with a notable decline in the green fluorescence subpopulation (quadrant 1). Data in Fig. 5A show that, as was the case for CI irradiation, increasing doses of X-ray irradiation resulted in an increasing number of *S. cerevisiae* cells gradually shifting from quadrant 1 to quadrant 3. Only cells treated with <75 Gy were mainly concentrated (>90%) in quadrants 1 and 2. For doses of ≥100 Gy, the proportion of permeabilized cells was shown to increase in a linear manner and more cells appeared to accumulate PI. In cells treated with X-ray irradiation, 33.2–61.9% of cells were stained with PI for doses of 100–175 Gy X-ray treatment, while corresponding doses of CI resulted in 61.9–90.3% of cells being stained with PI. Figure 5, furthermore, reveals a small fraction of cells that appeared in quadrant 2. In this region, red (PI+) and green (F+) fluorescence of cells is high, indicating that cell membrane integrity is affected, while cells retain their esterase activity. Since such cells have metabolic activity but are not detectable by plate counts, they are deemed viable but not culturable [12]. In this study, double-stained cells (PI-F+) represented ~4% of the cell population across all doses of X-ray exposure, while in the case of CI exposure, cells were not observed in quadrant 2 with any significant regularity.

Similar effects were observed in the qualitative epifluorescence microscopy images of FDA–PI double-stained cells.
Cells with esterase activity can produce F from FDA, thus emitting green fluorescence, and cells with compromised plasma membranes incorporate PI into their DNA (by intercalation between the DNA bases), thereby exhibiting red fluorescence. With increasing doses of irradiation, the number of cells emitting red fluorescence increased while the number of cells emitting green fluorescence declined.
Comparison of the effects of CI and X-ray irradiation on *S. cerevisiae*

To assess the differences between the cell membrane damage induced by CI irradiation compared with that induced by X-ray irradiation, the relationships between cell permeability, esterase activity, and non-viable cells were studied. The results of this assessment revealed that the number of PI\(^+\) *S. cerevisiae* cells (quadrants 2 and 3) expressed as logarithmic values increased as a function of irradiation dose (Fig. 7A). Residual esterase activity following X-ray or CI irradiation calculated using Eq. (1) is shown in Fig. 7B. After CI irradiation, the number of PI\(^+\) cells (permeabilized cells) increased, while the number of cells with residual esterase activity decreased with doses of \(\geq 25\) Gy. In contrast, the numbers of permeabilized cells and cells with residual esterase activity almost remained constant in cells treated with \(<75\) Gy X-ray irradiation. For doses of \(\geq 100\) Gy, the trends in the effects of X-ray treatment corresponded to those with CI treatment; however, at all doses, the observed changes were weaker for X-ray treatment than for CI treatment. In addition, to assess the relationships between viable and non-viable cells and the function of cell permeabilization and overall esterase activity in the inactivation of cells, the number of permeabilized cells (non-viable cells) and the number of cells with overall esterase activity (viable cells) were plotted against the increase in non-viable cells (Fig. 7C and D). These plots reveal that cells were determined almost as non-viable as cells with compromised membrane increased, namely the number of permeabilized cells was nearly consistent with the number of non-viable cells. Exposure to 175 Gy of X-ray irradiation, for example, resulted in 0.48 log reductions in the number of viable cells corresponding to 0.44 log increases in the number of permeabilized cells. Regarding CI treatment, a 1.47 log increase in the number of non-viable cells corresponds to a 1.53 log increase in the number of permeabilized cells (Fig. 7C).

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**Fig. 6.** Visualization of enzyme activity and membrane integrity with FDA–PI double-staining. Cells were irradiated with X-ray or CI radiation and stained using FDA–PI, as described in Materials and Methods. A (X-ray) and B (CI) represent X-ray and CI radiation exposure, respectively. Left panel: fluorescence micrographs of incorporated FDA and PI. Right panel: phase contrast micrographs of the same cells. Scale is 20 μm.
Changes in overall esterase activities also correlate well with increases in non-viable cell numbers: >90% enzyme activity was lost following 175 Gy CI irradiation treatment, while only 30% enzyme activity remained after treatment with X-ray irradiation (Fig. 7D).

**DISCUSSION**

In this study, *S. cerevisiae* cells were irradiated with either X-ray or CI beams, after which changes in cell membrane permeability and integrity were assessed. From the results described, the survival rate of *S. cerevisiae* cells subjected to irradiation was shown to depend on the type of irradiation applied. CI radiation had a markedly more damaging effect on cells compared with X-ray radiation (Fig. 1). Previous explanations for this difference are that high-LET irradiation leads to clustered damage to DNA, thus causing more serious DSBs than those caused by low-LET irradiation, and that this clustered damage is more difficult to repair [16]. Differences in the extent of cell membrane lesions caused by irradiation may also explain the distinct sensitivities that *S. cerevisiae* cells exhibit to the two radiation types. Membrane permeability is thought to be an important determinant of a cell’s ability to tolerate and resist irradiation, and it can be assessed by measuring protein leakage and the diffusion of intracellular nucleotides [10, 17]. In this study, the leakage of proteins and intracellular nucleotides increased with increasing doses of irradiation, and this effect was more pronounced with CI irradiation compared with X-ray irradiation (Figs 2 and 3). These findings demonstrate that CI exposure markedly alters the membrane permeability of *S. cerevisiae* cells (*P* < 0.01), in conjunction with inducing a commensurate decrease in cell viability (Fig. 1). It is likely that, in contrast to X-ray irradiation, CI beam irradiation, a high-LET form of irradiation, results in more DNA damage (especially irreparable DSBs, from which even more fragments of DNA are formed), which diffuse more readily into the cytoplasm [16, 18]. Furthermore, as a type of dense ionization irradiation, CI beam irradiation generates higher RBE, thus incurring more serious physical damage to cell membranes in comparison with X-ray irradiation.
In principle, unstained *S. cerevisiae* cells can be detected in FCM on the basis of their intrinsic light scattering properties (in terms of FSC and SSC). Light scatter levels often depend on cell morphology and size. Due to the rotational symmetry of *S. cerevisiae* cells, untreated cells (0 Gy) produced unimodal clustering with respect to SSC and FSC and were distributed from low to high. The majority of the cells were, however, concentrated in the range of smaller to intermediate values in terms of size and granularity. This could be attributable to the varying orientation of the coccoid yeasts as they traveled through the laser beam, which could vary in size range from the major to the minor in axis [13, 19]. After CI beam treatment, the *S. cerevisiae* population became more uniform and the major population shifted to lower FSC and SSC values, indicative of the coccoid *S. cerevisiae* cells becoming more symmetrical in shape, with smaller size and granularity (not obvious on X-ray exposure). This change could be (i) due to cell membrane damage caused by high CI beam doses (leading to protein leakage and intracellular nucleotide diffusion and consequent cell deformation and/or shrinking) or (ii) due to the refractive index of individual cells, which decreases as a consequence of cells decreasing in size [19].

FCM analysis was furthermore used to gain a better understanding of the mechanisms by which X-ray or CI irradiation inactivates cells. As a non-fluorescent precursor, FDA is often used to evaluate enzymatic activity and membrane integrity. FDA readily diffuses across intact cell membranes, and in active cells, FDA is hydrolyzed to F by non-specific esterase enzyme activity. F is a polar fluorescent compound and emits green fluorescence, and it is unable to diffuse out of intact cells. Since enzyme activity and membrane integrity...
are required for F labeling of cells, only viable cells are considered capable of accumulating F, whereas dead cells do not accumulate F. PI is a nucleotide-binding (DNA or RNA) probe which is commonly used to assess the membrane integrity of cells. With increasing cell membrane permeability, PI freely penetrates cells and combines with DNA, emitting red fluorescence [20, 21]. In FDA–PI double-stained, untreated cells (Fig. 5A, 0 Gy; Fig. 5B, 0 Gy), not all cells were found to be F+PI (active esterases, intact membranes). It is possible that the F in labeled cells is extruded out of the cells by an ATP-driven transport system, and in cases in which the proton-motive force is inhibited, the efflux of F is more serious. On the other hand, F efflux is a vitality marker of the metabolic performance of cells [22–24]. A subpopulation of cells identified using FDA–PI double-staining in this study provided further corroboration that CI or X-ray irradiation induces significant damage in the membrane integrity and enzyme activity of S. cerevisiae cells, and that the extent of CI lesions is greater than that of X-ray lesions (Fig. 5A and B). Our findings suggest that other targets (besides DNA) may be even more vital to the mechanisms by which CI or X-ray irradiation inactivates cells. It is interesting to note that changes in the uptake of PI by cells in this study (Figs 5 and 6) correlated well with the inactivation curve obtained from viability counts (Fig. 1), protein leakage experiments (Fig. 2), and intracellular nucleotide diffusion measurements (Fig. 3). The decline in esterase activity, however, may have been caused by IR-induced cell inactivation. In non-viable cells, protein activity appears rather low, and esterase activity could therefore also be a measure of cell activity. The possibility that mRNA or DNA damaged by IR results in decreased esterase expression, however, cannot be excluded.

The comparison of the effects of CI vs X-ray irradiation in this study demonstrated that changes in cell permeability and decreases in esterase activity are dependent on the type of irradiation and furthermore confirm that CI treatment leads to more severe lesions in cells than X-ray irradiation: minimal cell growth in media correlated well with smaller numbers of surviving cells, as observed in the inactivation curve (Fig. 1). Regardless of the type and dose of radiation exposure, the increasing non-viable cell numbers determined using the plate count method corresponded well with the increasing permeabilized cell numbers and decreasing overall esterase activity (Fig. 7C and D). These phenomena indicate that (i) loss of S. cerevisiae activity induced by CI or X-ray irradiation may be caused by damage to cell membranes; (ii) CI or X-ray irradiation doses that lead to S. cerevisiae cell death by DNA lesions (beyond the limit of DNA repair) are sufficient to lead to cell membrane permeabilization; in other words, membrane damage would occur before DNA lesions; and (iii) cell permeabilization and loss of metabolic activity as a result of X-ray or CI radiation exposure may be an irreversible phenomenon (irreparable lesions), which is not in agreement with former reports on non-IR [11, 25]. It has been shown that cell permeabilization caused by pulsed electric field or microwave irradiation is partially reversible and that cell membrane lesions result from structural changes in membranes rather than physiological or metabolic damage [26, 27]. Taken together, our findings and previous reports suggest that yeast cell membrane permeabilization induced by IR differs from that induced by non-IR and that the reversibility of cell permeabilization by irradiation is determined by the radiation type. Penetration of PI only reflects the state of cell membrane integrity and thus cannot be viewed as a comprehensive indicator of cell viability [28, 29]. Our findings show clearly that the trends for irradiation-induced cell inactivation are similar for X-ray and CI irradiation, but that CI treatment induces more severe cell inactivation compared with X-ray treatment.

In conclusion, the present study strongly suggests that the effects of irradiation on S. cerevisiae are dependent on the radiation type, and that CI irradiation results in more serious lesions than X-ray irradiation at the same doses. Besides DNA damage, cellular enzyme inactivation and changes in cell membrane integrity and permeability play pivotal roles in the cell death induced by irradiation. These fundamental insights may prove useful in further studies on the irradiation response mechanisms of microorganisms, especially in terms of cell membrane damage.

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