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Rad52 has a role in the repair of sodium selenite-induced DNA damage in *Saccharomyces cerevisiae*

Lucia Letavayová[a], Danuša Vlasáková[a], Viera Vlčková[b], Jela Brozmanová[a], Miroslav Chovanec[a,*]

[a] *Laboratory of Molecular Genetics, Cancer Research Institute, Vlárska 7, 833 91 Bratislava, Slovak Republic*
[b] *Department of Genetics, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic*

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ABSTRACT

Selenium (Se) is a chemo-preventive agent that has been shown to have a protective role against cancer. The inorganic form of Se, sodium selenite (Na_2SeO_3), has frequently been included in various chemo-prevention studies, and this commercially available form of Se is used as dietary supplement by the public. Because high doses of this Se compound can be toxic, the underlying molecular mechanisms of sodium selenite toxicity need to be elucidated. Recently, we have reported that sodium selenite is acting as an oxidizing agent in the budding yeast *Saccharomyces cerevisiae*, producing oxidative damage to DNA. This pro-oxidative activity of sodium selenite likely accounted for the observed DNA double-strand breaks (DSB) and yeast cell death. In this study we determine the genetic factors that are responsible for repair of sodium selenite-induced DSB. We report that the Rad52 protein is indispensable for repairing sodium selenite-induced DSB, suggesting a fundamental role of homologous recombination (HR) in this repair process. These results provide the first evidence that HR may have a fundamental role in the repair of sodium selenite-induced toxic DNA lesions.

1. Introduction

Selenium (Se) is an essential nutrient required for human health. Numerous epidemiological, clinical and experimental studies have indicated that Se may have potent chemo-preventive properties [1]. The most notable are the human studies from 1996, which showed a decrease in the incidence of prostate, lung and colorectal cancers in the Se-supplemented group [2,3]. Se acts as chemo-preventive agent either by directly altering cell metabolism through its different chemical and metabolic forms, such as methyl-selenol and methyl-selenic acid [4–6], or its incorporation into the family of selenoproteins, a unique class of proteins that contain the amino acid seleno-cysteine [7,8]. Indeed, polymorphisms in the genes for selenoproteins like glutathione peroxidase 1 (Gpx1) and selenoprotein 15 (Sep15) increase the risk for some cancers, and changes in the expression of Gpx1, Gpx2, selenoprotein P (SelP) and thioredoxin reductase 1 (Trx1) are associated with cancer development and tumorigenesis [9–11].

Cellular responses induced by Se are very diverse and encompass preventive action against cancer and other disease at low dietary dose, carcinostatic effects at supra-nutritional concentrations, and DNA damage and cell death induction at high doses (reviewed in [12–14]). These dose-dependent biological–toxicological responses are, however, strongly dependent upon the chemical form and metabolites of the particular Se compound [15,16].

Se in our environment exists in different chemical forms [17,18]. Seleno-methionine (SeM) is the major nutritional form and it is a component of most Se-rich diets. Cells do not distinguish between methionine and SeM during protein synthesis, so this natural seleno-amino acid gets incorporated into the general body proteins in place of methionine [3,19]. Some other organic Se compounds such as Se–methylseleno–cysteine (MSeC) are present primarily in Se accumulator plants. MSeC has recently been suggested as a Se form being potentially active in cancer prevention. Sodium selenite (Na_2SeO_3), an inorganic Se compound, was the first Se compound used in early chemoprevention studies. It naturally occurs infrequently and in very low concentrations. At present, sodium selenite is used as a component of some multivitamin preparations and animal feed. A few human epidemiological and clinical cancer-prevention trials have used inorganic Se. In comparison with organic forms of Se, sodium selenite is much more toxic. Its toxicity is caused by the generation of reactive oxygen species (ROS) [20–23]. Sodium selenite is reduced from the +4 valence state, which leads to the generation of hydrogen selenide (H_2Se) or elemental Se *via* selenodiglutathione (GSSeSG), its reduction being mediated by thiols and NADPH-dependent reductases [24,25]. Reduction *via* glutathione

* Corresponding author. Tel.: +421 2 59327 332; fax: +421 2 59327 350.
E-mail address: miroslav.chovanec@savba.sk (M. Chovanec).

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oxidation is tightly connected with the production of superoxide [26,27].

The Se compounds consumed by the public have entirely different pharmacokinetics based upon their dose-related cytotoxicity. It is a general tendency of people to prefer using the less toxic organic Se compounds [17,28]. However, sodium selenite was suggested to have a greater anti-carcinogenic potential than organic SeM and MSeC. This anti-carcinogenic potential is thought to be associated with its pro-oxidant activity, a likely consequence of which is the induction of apoptosis in tumor cells [29,30]. In accordance with this pro-oxidant activity, and similarly to other oxidative agents, it has been found that sodium selenite can induce oxidative damage to DNA, in the form of DNA strand-breaks and base lesions [31–34]. DNA damage induced by ROS includes structural alterations in DNA bases and deoxyribose, apurinic/apyrimidinic sites and DNA-protein cross-links, along with DNA single- and double-strand breaks (SSB and DSB, respectively). Oxidative DSB arise in DNA either directly or as a result of attempted and aborted repair of single or clustered oxidative DNA lesions. Moreover, an indirect way of oxidative DSB induction involves action of ROS in opposite DNA strands: a process that generates adjacent SSB that can be converted into DSB upon DNA replication [35–37]. Since un-repaired or mis-repaired DSB can initiate processes leading to mutagenesis, tumorigenesis and cell death, efficient DSB repair is crucial to maintain genome stability and cell viability. Principally, two key pathways have evolved to deal with DSB, i.e. homologous recombination (HR) and non-homologous end-joining (NHEJ) (for reviews, see [38,39]).

Recently, we have reported that DSB are the likely consequence of the toxic effects of sodium selenite in the budding yeast *Saccharomyces cerevisiae* [34]. In the present study we determined the genetic factors that are responsible for repair of the DSB generated by this Se compound. Here, we demonstrate that repair of sodium selenite-induced DSB requires the Rad52 protein function, indicating a possible role of HR in this process.

2. Materials and methods

2.1. Strains and media

The *S. cerevisiae* strains used in this study are listed in Table 1. All strains are isogenic derivatives of the W303 parental strain (this strain is referred to as wild type). The other strains differ only in their ability to carry out DSB repair due to disruptions in the key components of this process. While the W303αL strain is defective in DSB repair by NHEJ as a consequence of *YKU70* inactivation, the JDY1 strain is not able to perform DSB repair by HR due to *RAD52* inactivation. The JDY2 strain is deficient in both DSB repair pathways, as a result of concurrent inactivation of *YKU70* and *RAD52*. Media were the same as described previously [40].

2.2. Cell survival

Sodium selenite (Merck KGaA, Damstadt, Germany) treatment was carried out in the exponential phase of cell growth. Yeast cells were grown in YPD medium overnight. Overnight culture was used to inoculate fresh YPD. Incubation in YPD continued until the cell suspension reached a density of 2×10^7 cells/ml. The yeast culture was then collected by centrifugation, washed with and re-suspended in 0.1 M potassium phosphate (pH 7.4) at a density of 2×10^8 cells/ml and treated with increasing concentrations of sodium selenite at 30 °C for 3 h with shaking. After the treatment, the cells were collected by centrifugation, washed twice with and re-suspended in 0.1 M potassium phosphate (pH 7.4), diluted in physiological saline (0.9% NaCl) and plated onto YPD plates to determine cell viability.

Table 1
The *Saccharomyces cerevisiae* strains used in this study

Strain designation	Genotype	Source
W303	*MATα, ade2, leu2, his3, trp1, ura3, can1-100*	H. Feldmann
W303αL	W303 *yku70::LEU2*	H. Feldmann
JDY1	W303 *rad52::TRP1*	J.A. Downs
JDY2	W303 *yku70::LEU2 rad52::TRP1*	J.A. Downs

2.3. DSB induction and repair

DSB induction and repair was monitored using pulsed-field gel electrophoresis (PFGE). PFGE is a method that fractionates DNA molecules up to 10 Mbp, and therefore in yeast it separates chromosome-sized DNA molecules. In the DNA repair field, PFGE was mainly adopted to measure chromosomal fragmentation after exposure of cells to DNA-damaging agents as well as to follow DSB rejoining. PFGE experiments were performed as described previously [34,40]. For DSB induction experiments, untreated and sodium selenite-treated cells were washed twice with and re-suspended in 50 mM EDTA (pH 7.5) at a density of 6.25×10^8 cells/ml. Of the resulting suspension, 160 μl were then mixed with 40 μl of a buffer composed of 2 M sorbitol, 1 M citrate, 0.5 M EDTA pH 7.5 and 10% β-mercaptoethanol (β-ME). Thereafter, 5 μl of lyticase (10 mg/ml; Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 200 μl of 1% low melting-point agarose in 0.125 M EDTA (pH 7.5) were added. The cell suspension was equilibrated at 45 °C and subsequently transferred into the plug moulds and cooled until solidified. The plugs were first incubated in a buffer consisting of 0.5 M EDTA, 0.4% β-ME and 0.01 M Tris–HCl (pH 8.0) for 2 h and then lysed at 37 °C in 0.5 M EDTA, 0.01 M Tris–HCl (pH 8.0), 1% *N*-lauroylsarcosine and 0.5 mg/ml proteinase K (Amresco, Solon, OH, USA) overnight. The next day, they were incubated at 37 °C for 2 h in a buffer composed of 1 mM pefabloc (Serva Electrophoresis GmbH, Heidelberg, Germany), 1 mM EDTA and 10 mM Tris–HCl (pH 8.0) and then rinsed twice with 50 mM EDTA (pH 7.5). The plugs were stored in a buffer consisting of 1 mM pefabloc and 0.5 M EDTA (pH 8.0) at 4 °C until used. Just before the electrophoresis, the plugs were equilibrated twice in a buffer composed of 10 mM Tris–HCl and 1 mM EDTA (pH 7.5) and then loaded into 1% agarose gel. Electrophoresis was performed in TAE buffer (20 mM Tris acetate, 1 mM EDTA, pH 8.0) using either a transverse alternating field electrophoresis system (TAFE; Beckman Instruments) at 14 °C as follows: (i) constant current of 250 V/mA for 30 min with 4 s pulse time; (ii) constant current of 230 V/mA for 23 h with 90 s pulse time; (iii) constant current of 210 V/mA for 2 h with 150 s pulse time, or CHEF MAPPER® XA SYSTEM (Bio-Rad) with constant voltage 4.5 V/cm for 23 h at 14 °C with a switch time of 60–120 s. After electrophoresis, the gel was stained with 0.1 μg/ml ethidium bromide for 2 h, destained in TAE buffer containing RNase (2 μg/ml) overnight, visualized on a UV transilluminator and photographed with GDS 7500 Gel Documentation System (UVP). For repair experiments, cells after the treatment period were washed twice with 0.1 M potassium phosphate (pH 7.4) and re-suspended in YPD or phosphate-buffered saline (PBS). The cells were incubated at 30 °C with shaking and samples for PFGE analysis were removed after 0, 2, 4 and 24 h of post-incubation.

Computation of DSB was based upon the following formula:

$$N_{DSB} = -\ln \frac{IOD}{IOD_0}$$

where N_{DSB} is the number of DSB per chromosome represented by the chosen band shown in Fig. 2 (for further details, see Fig. 2), IOD is the integrated optical density of this band in treated samples and IOD_0 is the integrated optical density of the chosen band in untreated samples. Integrated optical densities were calculated using Gel-Pro analyzer 3.1 software.

3. Results

3.1. Cell survival after treatment with sodium selenite

To explore the role of HR and NHEJ in the cellular response to treatment with sodium selenite, the sensitivity of the *rad52* and *yku70* single and *rad52 yku70* double mutants to this Se compound relative to their isogenic parent was investigated. Only cells with inactivation of *RAD52* (i.e. *rad52* single and *rad52 yku70* double mutants) showed increased sensitivity to sodium selenite (Fig. 1). To verify that the effect of *RAD52* inactivation on sensitivity towards sodium selenite was indeed caused by this gene deletion, another wild type strain and *rad52* single mutant strain (FF 18734 and FF 18743, respectively) were checked for cell survival after exposure to sodium selenite. No significant differences between both wild types as well as *rad52* mutants were observed (data not shown), indicating that *RAD52* inactivation indeed renders the cells sensitive to sodium selenite. Notably, the *rad52 yku70* double mutant displays the same sensitivity as the *rad52* single mutant, suggesting epistatic interaction between *RAD52* and *YKU70* in the repair of sodium selenite-induced toxic DNA lesions.

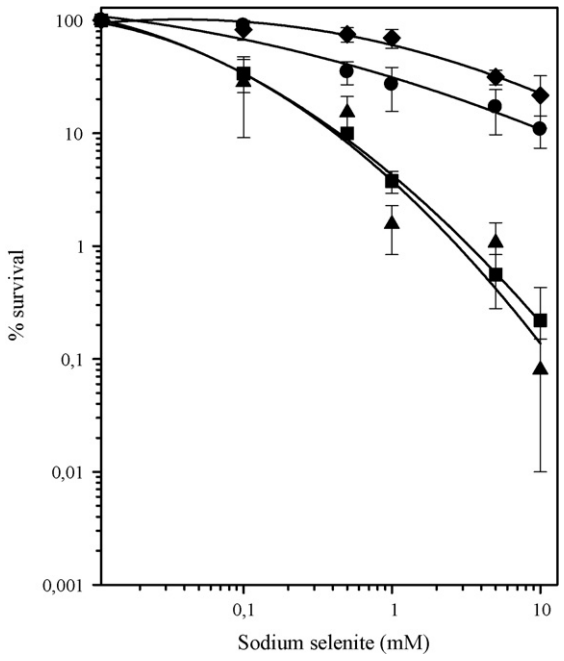


Fig. 1. Survival of the wild type (●) and the isogenic *yku70* (♦), *rad52* (■) and *yku70 rad52* (▲) mutant *S. cerevisiae* cells after exposure to sodium selenite. Data are the means of at least three independent experiments with standard deviations.

3.2. DNA double-strand break induction after treatment with sodium selenite

The survival data revealed that functional Rad52, and by inference HR, significantly contributes to the repair of the toxic DNA lesions induced by sodium selenite. This indicated that DSB may constitute the main toxic DNA lesion induced by this Se compound. Consequently, DSB induction following treatment with sodium selenite was determined. PFGE experiments revealed (Fig. 2) that sodium selenite effectively induces DSB, an observation that is in line with our previous findings [34]. The data imply that certain portions of the toxic DNA lesions induced by sodium selenite in yeast are represented by DSB and that the sensitivity towards sodium selenite of cells inactive in *RAD52* may be due to their inability to repair this particular type of DNA lesion.

3.3. DNA double-strand break repair in sodium selenite-treated cells

To gain more insight into the role of Rad52, and by inference HR, in the repair of DSB resulting from exposure to sodium selenite, the cells were treated with this Se compound and subsequently allowed to repair DSB in complete YPD medium for 2, 4 and 24 h (Fig. 3). Since only higher doses of sodium selenite effectively induced DSB (Fig. 2), treatments with 5 and 10 mM sodium selenite were used in DSB repair experiments. As is evident from Fig. 3, under growing conditions DSB are efficiently repaired in the wild type and *yku70* mutant cells where 20% of DSB remained after 24 h for the two doses tested. This is in sharp contrast to the situation in the *rad52* single or *yku70 rad52* double mutant cells, where no restoration of chromosomal-sized DNA was observed. Notably, PFGE results correlate well with the survival data (Fig. 1), both indicating that the inability to repair DSB determines cellular sensitivity to sodium selenite. Consequently, dividing cells proficient in the HR pathway (i.e. wild type and *yku70* mutant cells) are capable of repairing sodium selenite-induced DSB, which is the basis of their resistance to oxidative stress induced by this Se compound. However, when cells were cultivated in non-growing conditions after treatment with sodium selenite, i.e. in PBS, even HR-proficient cells failed to repair the sodium selenite-induced DSB (data not shown). This suggests that ongoing DNA replication and/or probably *de novo* protein synthesis may both be required for efficient DSB repair by HR in cells treated with sodium selenite.

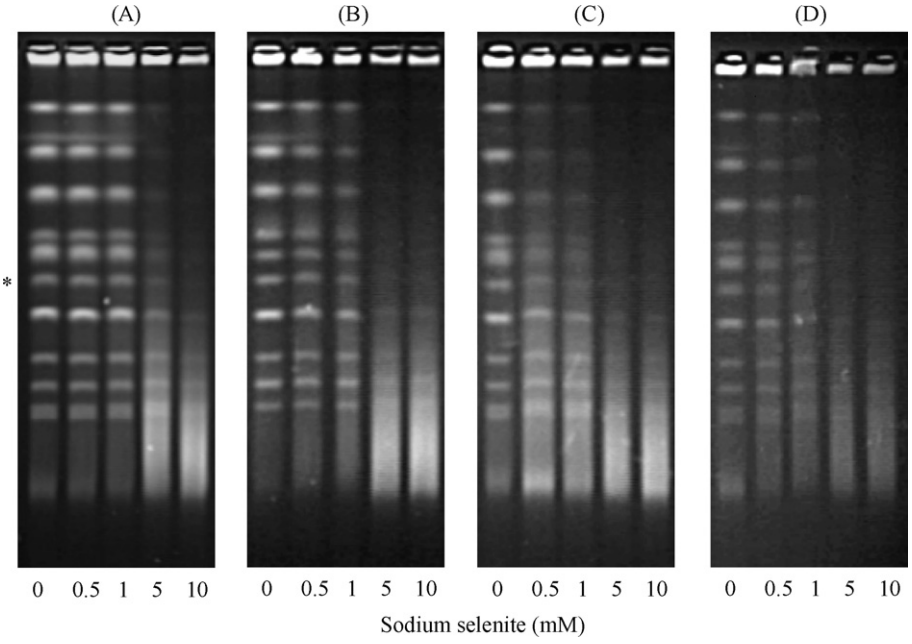
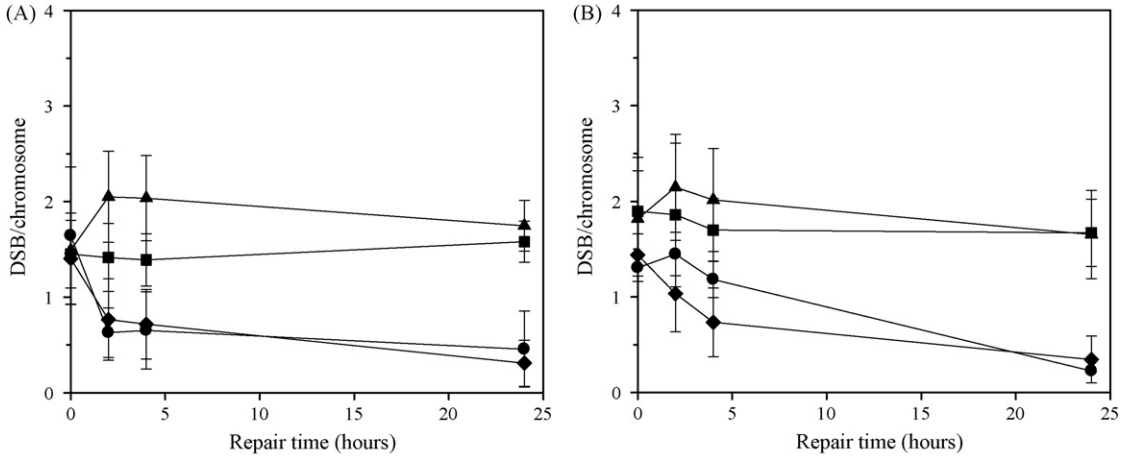


Fig. 2. DSB induction after treatment with sodium selenite in the wild type (A) and the isogenic *yku70* (B), *rad52* (C) and *yku70 rad52* (D) mutant *S. cerevisiae* cells. Representative gel for each strain is shown. *Indicates a band, whose IOD was used to estimate the yield of DSB. This band was chosen because the size of the chromosome it represents is about the average size of all *S. cerevisiae* chromosomes. Since the yield of DSB for different chromosomes displayed a mild variation dependent upon the size of the chromosome, we have chosen the chromosome of approximate average size to standardize the yield of DSB.



Strain	% remaining DSB			
	Repair time (hours)			
	0	2	4	24
wild type	100	38.3	39.7	27.7
yku70 mutant	100	54.5	51.1	22.0
rad52 mutant	100	97.5	95.8	>100
yku70 rad52 mutant	100	>100	>100	>100

Strain	% remaining DSB			
	Repair time (hours)			
	0	2	4	24
wild type	100	>100	90.6	17.3
yku70 mutant	100	71.9	51.0	23.9
rad52 mutant	100	98.0	89.6	88.1
yku70 rad52 mutant	100	>100	>100	91.0

Fig. 3. DSB repair in the wild type (●) and the isogenic *yku70* (♦), *rad52* (■) and *yku70 rad52* (▲) mutant *S. cerevisiae* cells treated with 5 mM (A) and 10 mM (B) sodium selenite. Samples for PFGE analysis were removed at 0, 2, 4 and 24 h of post-incubation in complete YPD medium, where 0 h means 3 h treatment with no repair time. DSB levels were calculated according to the formula provided in Section 2 using the IOD of the band indicated in Fig. 2. Error bars (standard deviations from at least three independent experiments) are indicated. Tables summarize the data plotted in the graphs.

4. Discussion

Selenium is an essential trace element for humans and, like some other trace elements, it can be toxic at higher concentrations than 800 μg/day, depending upon the chemical form. Although the toxic effects of sodium selenite, an inorganic Se compound capable of generating ROS, have already been well documented, the molecular mechanism of its toxicity has not thoroughly been investigated so far. Previously, we showed that sodium selenite manifests significant toxic effects in the budding yeast *S. cerevisiae*, likely through DSB induction, although contribution by other mechanism(s) and/or DNA lesion(s) could not entirely be excluded [34]. Our data were in line with the results reported by others, showing that treatment with sodium selenite causes DNA strand breaks and leads to chromosomal damage in carcinoma cell lines and human lymphocytes, respectively [41–43]. Notably, sodium selenite has also previously been shown to be toxic in *S. cerevisiae* [44,45].

In the present paper, we have examined the utilization of the DSB repair pathways after treatment of yeast with sodium selenite. In general, two main pathways are responsible for repairing DSB, HR and NHEJ (reviewed in [38]). Although both these pathways can in principle operate in yeast cells, specific factors may direct the DSB repair pathway choice, generally favoring HR in dividing and diploid cells [46,47]. As previously reported [34], DSB induction after exposure to sodium selenite is significantly higher in exponentially growing yeast cells than in the same cells in the stationary phase of growth. Higher DSB induction in dividing cells may be, at least in part, caused by the fact that another DNA damage type(s) (SSB and/or DNA base damage processed *via* SSB intermediates) can undergo conversion to DSB upon DNA replication. Such DSB have an indirect origin and are expected to be repaired primarily by HR [47]. In support of this, we show that HR-compromised, exponentially growing cells (i.e. *rad52* single and *rad52 yku70* double mutants) are hypersensitive to sodium selenite (Fig. 1) and unable to repair sodium selenite-induced DSB (Fig. 3).

Notably, the yield of DSB after exposure to sodium selenite was very similar in all strains examined (Fig. 2), with a slightly higher variability in the case where cells were exposed to 10 mM sodium selenite and allowed to repair DSB in complete YPD medium (Fig. 3B). This indicated that none of the DSB repair defects significantly increased the cells' ability to undergo DNA double-strand breakage. Therefore, the observed impairment in DSB repair of the HR-debilitated cells cannot be attributed to the higher yield of DSB in these cells. However, we noticed a difference in the yield of DSB in the exponentially growing wild type cells, with the yield of DSB being higher in our previous study [34]. As all experimental procedures were followed exactly in both studies, the basis for this difference likely lies in the genetic background of the strains used. In support of this explanation, the SJR 751 strain used in our previous study [34] was more sensitive to the toxic effects of sodium selenite compared with the W303 strain used in this study. In addition, the sensitivity of the FF 18734 strain towards sodium selenite was comparable to that of the W303 strain. Accordingly, these two wild type strains displayed the same DSB induction kinetics (data not shown).

DSB belong to the main oxidative DNA lesions induced by the radiomimetic drug bleomycin (BLM). Previously, we reported that both HR and NHEJ are involved in the repair of BLM-induced DSB, although their contribution to the process was not equal, with HR being the predominant pathway [40]. In contrast to DSB induced by sodium selenite, DSB generated after BLM exposure were shown to be eliminated under non-growing conditions in the wild type strain [40,48]. This suggests that repair of sodium selenite-induced DSB may also require DNA replication and/or *de novo* protein synthesis. Further experiments will be required to verify this assumption.

The toxic effects of Se compounds may become strongly beneficial if they can be selectively targeted against cancer cells.

Spallholz et al. have undertaken an approach to develop a therapy against cancer or bacterial and viral infections using redox-cycling selenides covalently attached to site-directing molecules such as polyclonal and monoclonal antibodies as well as peptides, steroids and solid polymer surfaces [49]. Another target for this Se drug action is the modulation of the internal cellular redox tone. As previously shown [50], altering the redox environment of prostate cancer cells with sodium selenite increases their apoptotic potential and sensitizes them to radiation-induced killing. In addition, exposure to sodium selenite has been reported to influence the balance between thioredoxin and thioredoxin reductase, two key Se enzymes in the control of the cell's redox metabolism, which increases the sensitivity of transformed mesothelial cells to therapy by doxorubicin [51].

In conclusion, the present results show that exposure to sodium selenite causes DSB induction in *S. cerevisiae* and that sodium selenite-induced DSB require Rad52, and by inference HR, but not NHEJ, to be efficiently repaired. Since the toxic effects of sodium selenite are mediated through generation of ROS, it is of considerable interest to extend the present work to mutant strains defective in base excision repair in order to evaluate the contribution of oxidative DNA damage and its repair to the toxic and mutagenic effects of sodium selenite. Such experiments are the focus of our research and are currently being carried out in our laboratories.

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