Research Article

Analysis of *Saccharomyces cerevisiae* null allele strains identifies a larger role for DNA damage *versus* oxidative stress pathways in growth inhibition by selenium

Eden Seitomer, Bharvi Balar, Dongming He, Paul R. Copeland and Terri Goss Kinzy

Department of Molecular Genetics, Microbiology and Immunology, UMDNJ Robert Wood Johnson Medical School, NJ, USA

Selenium toxicity is a growing environmental concern due to widespread availability of high-dose selenium supplements and the development of high-selenium agricultural drainage basins. To begin to analyze the effects of selenium toxicity at the genetic level, we have systematically determined which genes are involved in responding to high environmental selenium using a collection of viable haploid null allele strains of *Saccharomyces cerevisiae* representing three major stress pathways: the *RAD9*-dependent DNA repair pathway, the *RAD6/RAD18* DNA damage tolerance pathway, and the oxidative stress pathway. A total of 53 null allele strains were tested for growth defects in the presence of a range of sodium selenite and selenomethionine (SeMet) concentrations. Our results show that ~64–72% of the strains lacking *RAD9*-dependent DNA repair or *RAD6/RAD18* DNA damage tolerance pathway genes show reduced growth in sodium selenite *versus* ~28–36% in SeMet. Interestingly both compounds reduced growth in ~21–25% of the strains lacking oxidative stress genes. These data suggest that both selenite and SeMet are likely inducing DNA damage by generating reactive species. The anticipated effects of loss of components of the oxidative stress pathway were not observed, likely due to apparent redundancies in these gene products that may keep the damaging effects in check.

Keywords: DNA damage / Oxidative stress / *Saccharomyces cerevisiae* / Selenium / Selenite

Received: August 31, 2007; revised: October 24, 2007; accepted: November 14, 207

1 Introduction

Selenium (Se) was first identified by the Swedish chemist Jakob Berzelius in 1817 [1]. Initially, it was deemed a highly toxic agent. In 1957, a breakthrough study reported that Se in the form of inorganic salts such as sodium selenite (hereafter referred to as selenite) is effective in preventing liver necrosis degeneration in rats at low dietary levels [2]. The only known function for dietary Se is its utilization as selenocysteine in a select group of proteins, many of which are oxidoreductases. Among these selenoproteins, the glutathione peroxidase family of enzymes are responsible for maintaining cellular redox homeostasis by reducing a multitude of peroxides that form as a result of both small molecule and macromolecular oxidation [3].

Se toxicity is a re-emerging public health issue because of two relatively recent developments: (i) high-Se agricultural drainage basins have developed in several locations in the US [4] and (ii) Se has become a common dietary supplement. The latter development is a consequence of the Nutritional Prevention of Cancer trial in which 1312 former skin cancer patients supplemented their diets with 200 μg/day Se in the form of Se-enriched yeast in the hopes of reducing the recurrence of nonmelanoma skin cancer. Although no benefit was derived in terms of skin cancer recurrence, total cancer mortality decreased by 52%; total cancer incidence decreased by 39%; and incidences of lung, colorectal, and prostate cancers decreased by 44, 61, and 65%, respectively [5]. More recent follow-up has demonstrated that only those subjects whose baseline Se levels were in the lower tertile

Correspondence: Dr. Terri Goss Kinzy, Department of Molecular Genetics, Microbiology and Immunology, UMDNJ Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854-5635, USA
E-mail: kinzytg@umdnj.edu
Fax: +1-732-235-5223

Abbreviations: ROS, reactive oxygen species; **SeMet,** selenomethionine

received maximum benefit from Se supplementation. In fact, those subjects with baseline Se levels in the upper tertile suffered nonsignificant but strikingly higher total cancer incidence rates [6]. This trend was even more striking when the primary endopoint was re-analyzed and it was found that there was a statistically significant increase in squamous cell carcinoma and total nonmelanoma skin cancer incidence [7]. While these data seem to suggest that Se supplementation may only be required for individuals who may have a slight Se deficiency, the predominant model for Se chemoprevention is one in which high levels of small selenocompounds are able to selectively induce apoptosis in transformed cells [8]. This paradox will likely be resolved upon the completion of an ongoing Se intervention clinical trial focused on chemoprevention as the primary endpoint [7]. Overall, it is clear that unregulated access to Se supplements has the potential to be a serious public health concern directly related to Se toxicity.

Se is proposed to be toxic because it is a potent oxidant. Most of the dietary Se consumed is in the form of selenomethionine (SeMet) which is nonspecifically substituted for methionine in plants grown in Se-rich soils. SeMet itself is considered nontoxic, but its metabolites have strong oxidant potential [8]. For example, the production and subsequent oxidation of methylselenol to form superoxide has been proposed to be the basis for SeMet toxicity in fish [9]. In support of this hypothesis, yeast lacking *S*-adenosyl methionine metabolism genes are resistant to SeMet treatment at high doses [10]. Selenite, which is also utilized as a dietary supplement, is much more reactive and has been shown to be highly cytotoxic. A recent study indicates that selenite induces superoxide-dependent mitochondrial damage as a trigger for autophagy in human glioma cells [11]. A third form of Se that is sometimes used as a dietary supplement is sodium selenate. While considerably less toxic to mammals than selenite, presumably because it is not enzymatically reduced to selenite, selenate has also been reported to be an environmental toxin, particularly in species that have been shown to be more sensitive to selenate than selenite [12]. This along with the fact that Se cytotoxicity can be circumvented in some species of plants that have evolved to utilize Se hyperaccumulation as a defense mechanism [13, 14] provide strong evidence for a highly complex relationship between Se status and toxicity depending on environmental conditions and the species being analyzed. Interestingly, all three Se compounds are potent inducers of selenoprotein production at concentrations substantially below the toxic levels [15], suggesting that production of the selenide precursor for selenocysteine synthesis may be distinct from the production of selenide as an inducer of reactive oxygen species (ROS).

Not surprisingly, Se toxicity has been closely associated with the induction of an oxidative stress response in several organisms including the budding yeast *Saccharomyces cerevisiae* (reviewed in ref. [16]). Although there is some evidence that the pathways leading to oxidative stress may be compound-specific [17], it is generally believed that the generation of ROS as a result of the reaction of selenocompounds with thiols is responsible for cellular injury, especially DNA damage [18, 19]. Consistent with these findings, it has been found that selenite induces *RAD9*-dependent cell cycle arrest in yeast [20], providing evidence that genomic approaches in yeast may provide significant insight into the mechanisms of and ultimately the prevention of Se toxicity.

In this report, we used the *S. cerevisiae* collection of viable haploid null allele strains to determine which gene families were associated with selenite or SeMet toxicity. This study revealed that the deletion of a subset of members of three pathways resulted in reduced growth in the presence of Se: the *RAD6/RAD18*-dependent DNA damage tolerance pathway, the *RAD9*-dependent DNA repair pathway and the oxidative stress pathway. These results shed new light on the specific pathways that contribute to Se toxicity and likely tolerance.

2 Materials and methods

2.1 Strains and media

The parental wild-type strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and haploid deletions for nonessential genes in the *MATa* BY4741 strain background were obtained form Open Biosystems (Huntsville, AL) [21]. Standard yeast genetics methods were employed (Sherman, 1986). Yeast cells were grown in either YEPD (1% Bacto yeast extract, 2% peptone, 2% dextrose) or defined synthetic complete media (C-) supplemented with 2% dextrose as a carbon source.

2.2 SeMet growth assays

Yeast strains were grown overnight in 5 mL of YEPD or C-media to mid log phase. The OD_{600} was taken by UV – Vis spectroscopy (Thermo) and diluted to 0.1 OD_{600}. Concentrations from 0 to 0.075 mM of SeMet and 0 to 0.5 mM of selenite were made in C-media and filter sterilized. Equal volumes of the Se medium and the diluted yeast were added to a 96-well microtiter plate for a final volume of 200 µL in triplicate. The final OD_{600} was monitored and was approximately 0.05 and the final concentration varied from 0 to 0.0375 mM and 0 to 0.25 mM for SeMet and selenite, respectively. Plates were incubated at 30°C with constant shaking and the OD_{600} was taken using the ELx 800 microplate reader (Bio-Tek Instruments) at 0 and 20 h. All experiments were performed in a minimum of duplicate and errors reported as SDs.

3 Results

Since yeast is a well-understood and genetically tractable organism, this model system provides a unique opportunity to easily screen for molecular and cellular functions involved in Se metabolism. The availability of a library of viable haploid *S. cerevisiae* null allele strains [21] has allowed for examining the effects of loss of function on growth in the presence of SeMet and selenite. Three biological pathways were analyzed based on the proposed mechanisms of Se toxicity. These include the *RAD9*-dependent DNA repair pathway, the *RAD6/RAD18*-dependent DNA damage tolerance pathway, and the oxidative stress pathway. These pathways were chosen due to the interactions observed with SeMet and selenite. Using the available library of null allele strains (Open Biosystems), individual strains with a nonessential gene deleted from the chromosome were assayed for growth in complete defined synthetic media containing SeMet or selenite. Each strain was grown in varying concentrations of the Se compound in triplicate in a 96-well plate-based assay. An incubation time of 20 h was chosen to optimally show relative growth, calculated by comparison to the strain grown in the absence of the Se compound to account for any slow growth phenotypes associated with the loss of the gene product of interest. These results were compared to the isogenic wild-type strain, and reduced or enhanced growth is relative to the wild-type strain at the same concentration.

The growth of the null allele strains was classified as reduced, wild-type, or enhanced for either SeMet or selenite (Tables 1 – 3). Listed with each yeast gene is the Ensembl (http://www.ensembl.org/index.html) annotation for the best match to the *Homo sapiens* genome identified by P-POD: Princeton Protein Orthology Database (http://ortholog.princeton.edu/findorthofamily.html). The classification of reduced indicates the null allele strains had a consistently lower percent growth at all of the Se concentrations where cells were viable, compared to the percent growth of the wild-type strain. Likewise, the classification of enhanced indicates the null allele strains had a consistently higher percent growth at all of the Se concentrations where cells were viable, compared to the percent growth of the wild-type strain. The classification of wild-type specifies that there was no appreciable difference in percent growth between the null allele strains and the wild-type strain. Interestingly, the growth of the wild-type yeast strain used was reduced more by SeMet than selenite, with an average IC_{50} of approximately 0.028 mM for SeMet *versus* 0.15 mM for Selenite.

3.1 *RAD9*-dependent DNA repair pathway

A recent study has shown that SeMet induces a p53-dependent DNA repair response that protects cells from subsequent UV-induced DNA damage [22]. In *S. cerevisiae*, we expected that deleting a gene from the DNA repair pathway would still demonstrate wild-type growth in the presence of SeMet. A reduced growth phenotype would possibly be observed if damage had indeed occurred upon SeMet treatment, leading to cell toxicity and inhibition of growth. Consistent with the second model, 5 out of the 18 null allele strains tested showed reduced growth with SeMet (*asf1*Δ, *bmh1*Δ, *chk1*Δ, *csm3*Δ, and *swi6*Δ) while two null allele strains had a resistant phenotype (*bmh2*Δ and *mrc1*Δ) compared to wild-type (Table 1). The most reduced growth was seen with an *asf1*Δ strain and the most enhanced was *mrc1*Δ (Fig. 1A). Asf1p is a histone H3-H4 chaperone protein present in a complex that repairs dsDNA damage [23]. Lack of the protein leads to a lag in the metaphase cell cycle due to DNA damage checkpoint activation [24]. The Mrc1p is an activator of a DNA replication stress response. Presence of the protein creates a lag in the metaphase cell cycle and leads to DNA repair [25]. As is seen in Fig. 2, the genes affected by SeMet treatment do not cluster within a specific section of the DNA repair pathway.

Selenite treatment induces DNA damage in the form of chromosome abnormalities at low concentrations [26] and likely induces DNA double strain breaks in yeast [27]. Se treatment is also proposed to induce abnormal DNA repair in human cells [28]. As predicted, a reduced growth phenotype was observed with many null allele strains in the *RAD9*-dependent DNA repair pathway. Thirteen out of the eighteen strains displayed a reduced growth phenotype compared to wild-type upon selenite treatment: *asf1*Δ, *bmh2*Δ, *chk1*Δ, *csm3*Δ, *ddc1*Δ, *dun1*Δ, *ptc2*Δ, *rad9*Δ, *rad17*Δ, *rad24*Δ, *rfx1*Δ, *tel1*Δ, and *swi6*Δ (Table 1). These thirteen strains encompassed four out of the five null allele genes found to have a reduced growth phenotype to SeMet, excluding *bmh1*Δ. The greatest reduction in growth was observed with a *tel1*Δ strain (Fig. 1B). The Tel1p is involved in DNA damage repair and telomere length regulation [29]. It is also a homolog of the human ataxia telangiectasia gene, which when present as an autosomal recessive mutation leads to cerebellar ataxia, immune defects, and an increased risk of cancer [30]. As is seen in Fig. 2, a consistent trend where selenite treatment leads to growth inhibition, upon loss of the *RAD9*-dependent DNA repair pathway is observed.

3.2 *RAD6/RAD18*-dependent DNA damage tolerance pathway

To further investigate the effect that SeMet and selenite have upon DNA damage, we looked at an offshoot of the DNA repair pathway, the *RAD6/RAD18*-dependent DNA damage tolerance pathway. Tolerance to SeMet treatment was largely unaffected by the loss of most of the genes in this pathway, as only 4 out of the 11 null allele strains assayed had a reduced growth phenotype, compared to wild-type: *rad5*Δ, *rad6*Δ, *ubc13*Δ, and *rad52*Δ (Table 2).

Table 1. The *RAD9*-dependent DNA repair pathway *S. cerevisiae* null allele strains, proposed human homologs, and their phenotype upon SeMet or selenite treatment

Growth	Ensembl human homolog	Sensitive		Wild-type		Resistant	
Compound		SeMet	SeO_3	SeMet	SeO_3	SeMet	SeO_3
*asf1*Δ	ENSP00000229595 (ASF1A) ENSP00000263382 (ASF1B)	X	X	–	–	–	–
*bmh1*Δ	ENSP00000217069 (YWHAB) ENSP00000264335 (YWHAE) ENSP00000297569 (YWHAZ) ENSP00000300161 (YWHAB) ENSP00000309503 (YWHAZ)	X	–	–	X	–	–
*bmh2*Δ	ENSP00000217069 (YWHAB) ENSP00000264335 (YWHAE) ENSP00000297569 (YWHAZ) ENSP00000300161 (YWHAB) ENSP00000309503 (YWHAZ)	–	X	–	–	X	–
*chk1*Δ	ENSP00000278916 (CHEK1)	X	X	–	–	–	–
*csm3*Δ	ENSF00000261881	X	X	–	–	–	–
*ddc1*Δ	No	–	X	X	–	–	–
*dun1*Δ	No	–	X	X	–	–	–
*mec3*Δ	No	–	–	X	X	–	–
*mrc1*Δ	No	–	–	–	X	X	–
*pin4*Δ	No	–	–	–	X	–	–
*ptc2*Δ	ENSP00000342778 (PPM1G) ENSP00000264714 (PPM1G)	–	X	X	–	–	–
*rad9*Δ	No	–	X	X	–	–	–
*rad17*Δ	No	–	X	X	–	–	–
*rad24*Δ	ENSP00000303134 ENSP00000346938 ENSP00000346271 ENSP00000282891 ENSP00000350725 (RAD17) ENSP00000324164 ENSP00000355226 ENSP00000311227	–	X	X	–	–	–
*rfx1*Δ	ENSP00000350552 (RFX4) ENSP00000266774 ENSP00000229387	–	X	X	–	–	–
*tel1*Δ	ENSP00000278616 (ATM)	–	X	X	–	–	–
*swi6*Δ	No	X	X	–	–	–	–
*yuh1*Δ	ENSP00000284440 (UCHL1) ENSP00000237800 (UCHL3)	–	–	X	X	–	–

The most reduced growth was observed for a *rad52*Δ strain (Fig. 3A). Rad52p is engaged in DNA repair and recombination, and is necessary for efficient annealing of complimentary ssDNA after a double strand break [31]. The protein is present only during meiosis or in response to DNA damage [32]. Two null allele strains, *rad30*Δ and *rev1*Δ, showed enhanced growth in SeMet.

Selenite treatment on the other hand induced a reduced growth phenotype upon loss of twice as many genes, similar to the differential effect compared to SeMet seen upon loss of components of the *RAD9*-dependent DNA repair pathway. Seven out of the eleven null allele strains demonstrated reduced growth phenotype upon selenite treatment compared to wild-type: *mms2*Δ, *pol32*Δ, *rad5*Δ, *rad6*Δ, *rad18*Δ, *rad52*Δ, and *rev7*Δ (Table 2). Three out of the four null alleles strains that show reduce growth in SeMet are also slower growing in selenite. The *rad5*Δ strain was showed the most reduced growth to selenite (Fig. 3B). Rad5p eliminates the formation of DNA minor groove adducts through excision repair [33]. Similar to the results found for the *RAD9*-dependent DNA repair pathway, loss of components within the *RAD6/RAD18*-dependent DNA damage tolerance pathway caused greater growth inhibition in selenite (Fig. 4).

3.3 Oxidative stress pathway

SeMet, *via* its interaction with a methionase, produces superoxide radicals that can lead to oxidative stress [34]. However, only 5 out of the 24 null allele strains studied

Table 2. The *RAD6/RAD18*-dependent DNA damage tolerance pathway *S. cerevisiae* null allele strains, proposed human homologs, and their phenotype upon SeMet or selenite treatment

Growth	Ensembl homolog	Sensitive		Wild-type		Resistant	
Compound		SeMet	SeO_3	SeMet	SeO_3	SeMet	SeO_3
*mms2*Δ	ENSP00000354653 (UBE2V1) ENSP00000340305 ENSP00000352224 (UBE2V2) ENSP00000326473 ENSP00000344166 (UBE2V1)	–	X	X	–	–	–
*pol32*Δ	No	–	X	X	–	–	–
*rad5*Δ	ENSP00000308944 (SMARCA3) ENSP00000330929 (SMARCA3)	X	X	–	–	–	–
*rad6*Δ	ENSP00000161284 (UBE2A) ENSP00000265339 (UBE2B)	X	X	–	–	–	–
*rad18*Δ	ENSP00000264926 (RAD18)	–	X	X	–	–	–
*rad30*Δ	ENSP00000306591 (POLH)	–	–	–	X	X	–
*rad52*Δ	ENSP00000305577 (RAD52) ENSP00000351284 (RAD52)	X	X	–	–	–	–
*rev1*Δ	ENSP00000258428 (REV1L)	–	–	–	X	X	–
*rev3*Δ	ENSP00000351697 (REV3L) ENSP00000352783	–	–	X	X	–	–
*rev7*Δ	No		X	X	–	–	–
*ubc13*Δ	ENSP00000316176 (UBE2N)	X	–	–	X	–	–

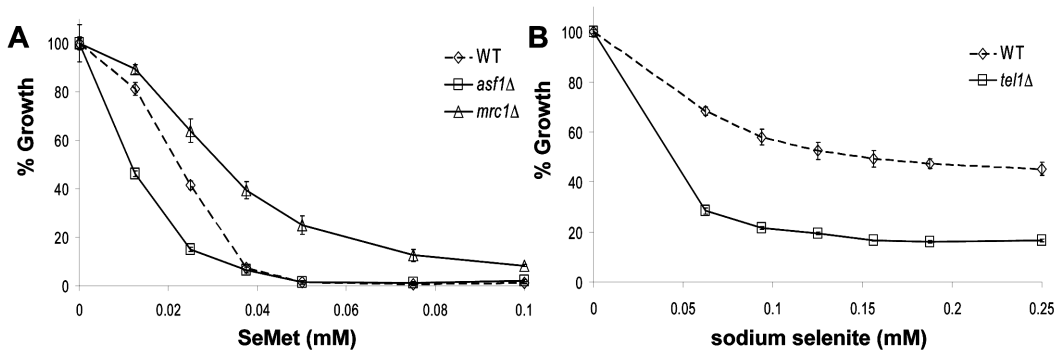

Figure 1. The *RAD9*-dependent DNA repair pathway null allele genes that show the greatest reduction or enhancement of growth with SeMet and reduced growth with selenite. Haploid wild-type strain BY4741 and a series of strains lacking a nonessential component gene were grown overnight at 30°C in YEPD and diluted to 0.1 OD_{600} in C-medium. Cells were grown for 20 h at 30°C in triplicate in varying concentrations of (A) SeMet or (B) selenite in a mitrotiter assay plate. Growth was monitored by change in OD_{600} between 0 and 20 h and plotted as% growth ± SEM normalized to growth in 0 mM Se.

showed a reduced growth phenotype upon SeMet treatment: *grx3*Δ, *grx5*Δ, *sod1*Δ, *tsa1*Δ, and *zwf1*Δ (Table 3). The greatest reduction in growth was observed for a *grx3*Δ strain (Fig. 5A). Grx3p belongs to a family of glutaredoxins and is highly sensitive to oxidative agents such as menadione and hydrogen peroxide [35].

Selenite has also been shown to produce ROS within the cell [11]. In parallel with SeMet results, only 6 out of the 24 null allele strains exhibited a reduced growth phenotype upon selenite treatment, compared to wild-type: *glr1*Δ, *grx3*Δ, *grx5*Δ, *sod2*Δ, *tsa1*Δ, and *yap1*Δ (Table 3). The greatest reduction in growth was observed for a *grx5*Δ strain (Fig. 5B). Grx5p belongs to the same family of glutaredoxins as Grx3p and absence of this protein leads to a high basal protein carbonyl content, indicative of oxidative protein damage [35]. Interestingly, unlike the *RAD9*-dependent DNA repair and *RAD6/RAD18*-dependent DNA damage tolerance pathways, none of the null allele strains resulted in enhanced growth in SeMet or selenite. As seen in Fig. 6, the genes affected by either SeMet or selenite treatment are differential for loss of the two superoxide dismutases tested, and tend to occur upon loss of genes involved in the reduction of reactive oxygen, nitrogen, and sulfur species.

Table 3. The oxidative stress pathway *S. cerevisiae* null allele strains, proposed human homologs, and their phenotype upon SeMet or Selenite treatment

Growth	Human homolog	Sensitive		Wild-type		Resistant	
Compound		SeMet	SeO_3	SeMet	SeO_3	SeMet	SeO_3
*ahp1*Δ	ENSP00000265462 (PRDX5) ENSP00000335334 ENSP00000335363	–	–	X	X	–	–
*ccp1*Δ	No	–	–	X	X	–	–
*cta1*Δ	ENSP00000241052 (CAT)	–	–	X	X	–	–
*ctt1*Δ	No	–	–	X	X	–	–
*glr1*Δ	ENSP00000221130 (GSR) ENSP00000334518 ENSP00000347020 (TXNRD1) ENSP00000353329 (TXNRD3) ENSP00000354511 (TXNRD2)	–	X	X	–	–	–
*grx1*Δ	ENSP00000237858 (GLRX) ENSP00000025176 ENSP00000280254 (GLRX2)	–	–	X	X	–	–
*grx2*Δ	ENSP00000237858 (GLRX) ENSP00000025176 ENSP00000280254 (GLRX2)	–	–	X	X	–	–
*grx3*Δ	ENSP00000225000 (TXNL2)	X	X	–	–	–	–
*grx4*Δ	ENSP00000225000 (TXNL2)	–	–	X	X	–	–
*grx5*Δ	ENSP00000328570	X	X	–	–	–	–
*gpx1*Δ	ENSP00000346103 (GPX4) ENSP00000296734	–	–	X	X	–	–
*gpx2*Δ	ENSP00000346103 (GPX4) ENSP00000296734	–	–	X	X	–	–
*gtt1*Δ	No	–	–	X	X	–	–
*gtt2*Δ	No	–	–	X	X	–	–
*hyr1*Δ	ENSP00000346103 (GPX4) ENSP00000296734	–	–	X	X	–	–
*sod1*Δ	ENSP00000270142 (SOD1)	X	–	–	X	–	–
*sod2*Δ	ENSP00000351054 (SOD2) ENSP00000337127	–	X	X	–	–	–
*trx1*Δ	ENSP00000259332 (TXN)	–	–	X	X	–	–
*trx2*Δ	ENSP00000259332 (TXN)	–	–	X	X	–	–
*trx3*Δ	ENSP00000259332 (TXN)	–	–	X	X	–	–
*tsa1*Δ	ENSP00000262746 (PRDX1) ENSP00000301522 (PRDX2) ENSP00000298510 (PRDX3) ENSP00000319964 (PRDX4)	X	X	–	–	–	–
*tsa2*Δ	ENSP00000262746 (PRDX1) ENSP00000301522 (PRDX2) ENSP00000298510 (PRDX3) ENSP00000319964 (PRDX4)	–	–	X	X	–	–
*yap1*Δ	No	–	X	X	–	–	–
*zwf1*Δ	ENSP00000342362 (G6PD) ENSP00000291567	X	–	–	X	–	–

4 Discussion

SeMet is the main organic Se form found in dietary supplements, however inorganic selenite is still prevalent in other dietary sources. To gauge the differential effect that both Se compounds have upon the cell, we examined the outcome of treatment of *S. cerevisiae* strains bearing null alleles of nonessential genes in DNA repair and oxidative stress pathways. This system also has the advantage of not incorporating Se into proteins *via* the selenocysteine pathway, and thus can separate out the effects of Se metabolism from Se function within the active sites of proteins. Previous studies indicated that SeMet interacts with the p53 protein to induce a DNA repair response in mammalian cells [22] and selenite treatment can lead to DNA damage, even at relatively low doses [26]. Both SeMet and selenite have been shown to cause oxidative stress within the cell (reviewed in ref. [16]). We thus determined the requirement for specific

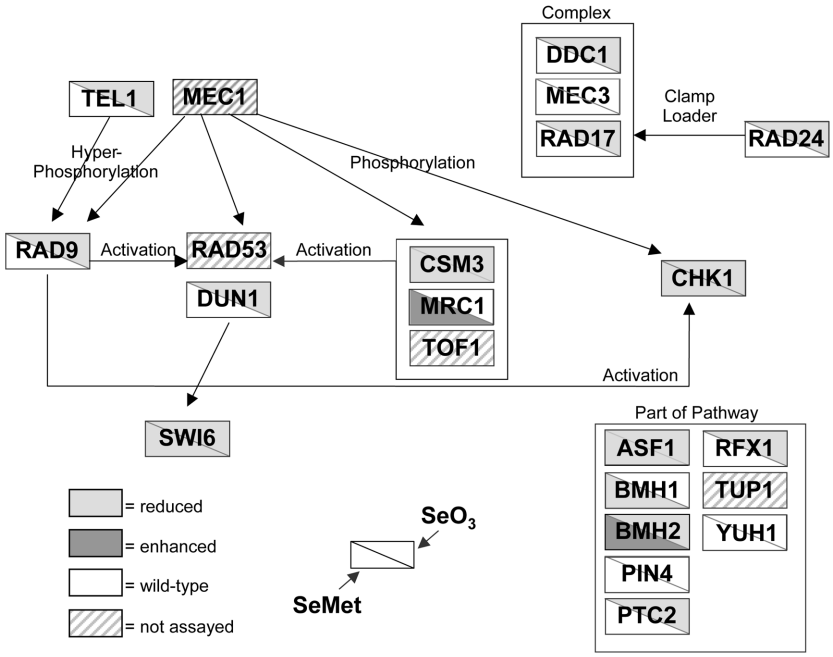

Figure 2. Diagram of the relationship of the *RAD9*-dependent DNA repair pathway null allele strains tested (bisected boxes) and select genes not tested due to growth defects or inviability (hatched boxes). The phenotype of the null allele strain relative to the isogenic wild-type strain in the presence of SeMet (lower section) or selenite (upper section) are indicated as wild-type (white), reduced (light gray), and enhanced (dark gray) growth.

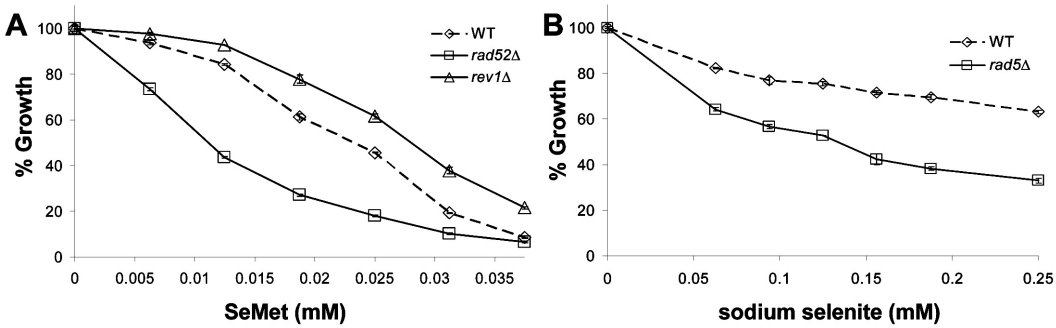

Figure 3. The *RAD6/RAD18*-dependent DNA damage tolerance pathway null allele genes that show the greatest reduction or enhancement of growth with SeMet and reduced growth with selenite. Haploid wild-type strain BY4741 and a series of strains lacking a nonessential gene were grown overnight at 30°C in YEPD and diluted to 0.1 OD_{600} in C-medium. Cells were grown for 20 h at 30°C in triplicate in varying concentrations of (A) SeMet or (B) selenite in a mitrotiter assay plate. Growth was monitored by change in OD_{600} between 0 and 20 h and plotted as% growth ± SEM normalized to growth in 0 mM SeMet.

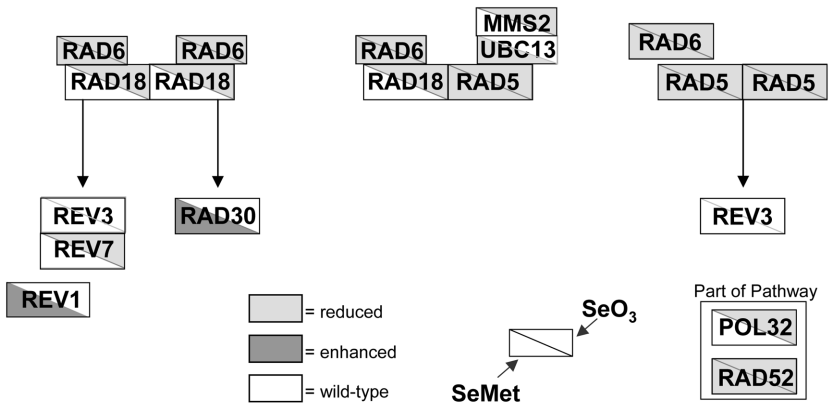

Figure 4. Diagram of the relationship of the *RAD6/RAD18*-dependent DNA damage tolerance pathway null allele strains tested (bisected boxes) and select genes not tested due to growth defects or inviability (hatched boxes) adapted from ref. [44, 45]. The phenotype of the null allele strain relative to the isogenic wild-type strain in the presence of SeMet (lower section) or selenite (upper section) are indicated as wild-type (white), reduced (light gray), and enhanced (dark gray) growth.

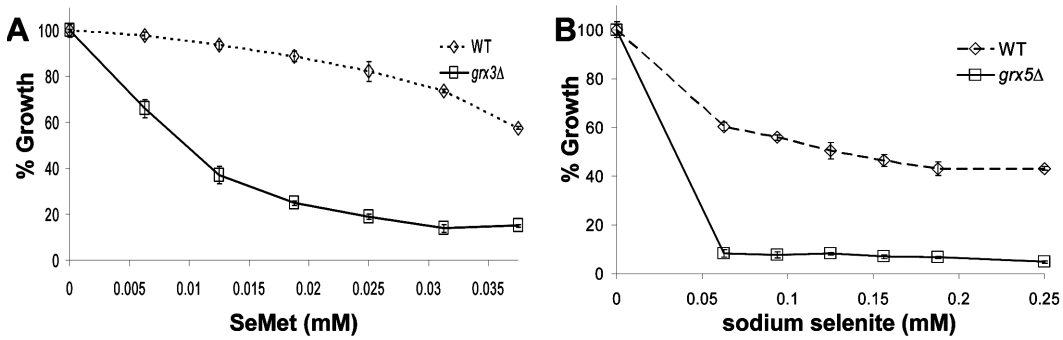

Figure 5. The oxidative stress pathway null allele genes that show the greatest reduction of growth with Se compounds. Haploid wild-type strain BY4741 and a series of strains lacking a nonessential gene were grown overnight at 30°C in YEPD and diluted to 0.1 OD_{600} in C-medium. Cells were grown for 20 h at 30°C in triplicate in varying concentrations of (A) SeMet or (B) selenite in a mitrotiter assay plate. Growth was monitored by change in OD_{600} between 0 and 20 h and plotted as% growth ± SEM normalized to growth in 0 mM SeMet.

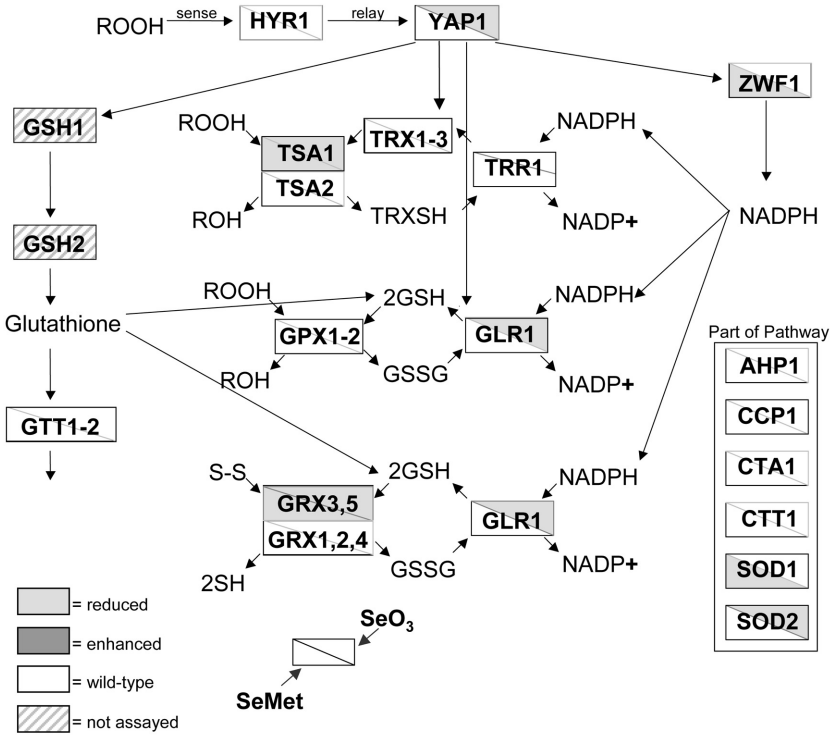

Figure 6. Diagram of the relationship of the oxidative stress pathway null allele strains tested (bisected boxes) and select genes not tested due to growth defects or inviability (hatched boxes). The phenotype of the null allele strain relative to the isogenic wild-type strain in the presence of SeMet (lower section) or selenite (upper section) are indicated as wild-type (white), reduced (light gray), and enhanced (dark gray) growth.

proteins in two sets of nonessential genes involved in DNA repair as well as those in the oxidative stress pathway in the eukaryotic model system *S. cerevisiae*.

Analysis of strains lacking components of the *RAD9*-dependent DNA repair pathway shows that while SeMet treatment leads to a reduced growth phenotype upon loss of a few genes (28%), with selenite treatment of a majority (72%) of these strains results in a growth defect. Thus, the effects of selenite are likely countered by this repair pathway *in vivo*. The null allele strains with the most reduced growth in SeMet lacked *ASF1* and for selenite lacked *TEL1*. Asf1p is linked to chromatin metabolism during transcription, and thus may have many effects on gene expression and the DNA damage checkpoint [36]. *TEL1* mutants affect telomere length and also the DNA damage checkpoint [29]. *MEC1* is functionally redundant with *TEL1*, however, as an essential gene it was not assayed in this screen. A cell cycle block at the S/G2-M phase in mammalian cells is associated with increased levels of selenite concentration, which may be a response to selenite-induced DNA damage [37]. selen-

ite also induces chromosome aberrations such as single-strand breaks, chromosome breaks, and spindle disturbances in the bone marrow cells of albino mice [38].

Reduced growth was associated with the loss of a significant number of genes associated with the *RAD6/RAD18*-dependent DNA damage tolerance pathway. The greatest growth reduction was observed in a null allele strain lacking *RAD52* for SeMet and *RAD5* for selenite, however, reduced growth was observed for both Se compounds upon loss of either gene. Interestingly *RAD5*, as well as *RAD6* and *RAD18*, function in the postreplicative repair pathway, which allows cells to survive without removing damage [40]. Loss of any of these three genes results in reduced growth in selenite. Similar to the results for the loss of *RAD9*-dependent DNA repair genes, reduced growth was more pronounced for selenite (64%) than SeMet (36%). These percentages are nearly identical in both sets of strains, indicating the strong bias toward reduced growth with selenite treatment. Compared to prior work, reduced growth in selenite was observed for *rad9*Δ, *rad18*Δ, and *rad52*Δ strains [20]. While reduced growth in selenite was observed for a *rev1*Δ strain in that study *versus* wild-type growth in our work, the former used a nearly ten-fold higher concentration of selenite.

SeMet and selenite treatment induced a small number of reduced growth phenotypes upon loss of components of the oxidative stress pathway, with loss of 21 and 25% of the analyzed oxidative stress genes, respectively, resulting in sensitivity. The loss of 50% of these genes (three out of six) conferred sensitivity to both compounds. One interesting finding was that a null allele strain lacking the *ZWF1* gene showed reduced growth in SeMet but not selenite. Null mutants of *ZWF1* require an organic sulfur source and display methionine auxotrophy, which may indicate a link to sulfur amino acid metabolism [40]. We expected a more pronounced phenotype upon loss of the oxidative stress response genes, due to the reports that SeMet and selenite produce superoxide radicals both *in vivo* and *in vitro*. It is possible that the oxidative stress agents are removed readily from the cell by a secondary mechanism that acts primary to the oxidative stress pathway. In addition, only nonessential and single gene deletions were analyzed. Further analysis of multiple gene disruptions for redundant gene products or analysis of mutant alleles in essential genes may demonstrate additional factors in these pathways that contribute to Se tolerance or detoxification.

Surprisingly, a few genes in the *RAD9*-dependent DNA repair and *RAD6/RAD18*-dependent DNA damage tolerance pathways assayed resulted in enhanced growth in SeMet, but none resulted in enhanced growth in selenite. Loss of the *BMH2*, *MRC1*, *RAD30*, or *REV1* genes shows a modest increase in growth in SeMet. These last two genes of the *RAD6/RAD18*-dependent DNA damage tolerance pathway are particularly interesting, in that loss of two of these gene products has previously been demonstrated to reduce error frequencies. The finding that *rev1*Δ induced enhanced growth is particularly interesting since studies have shown that Rev1p and Rev3p co-operate in recombination-independent repair of interstrand crosslinks and mammalian cell lines lacking *REV1* or *REV3* show reduced DNA interstrand crosslink repair-induced mutations [41]. The loss of *REV3* did not affect growth with either Se compound, consistent with the fact that loss of this gene product resulted in a reduced mutagenesis rate [42]. *RAD30* encodes DNA polymerase η, which synthesizes DNA *in vitro* with low fidelity. Somewhat like the effect of the *rev1*Δ, it has previously been shown that deletion of *RAD30* does not affect the spontaneous mutagenesis rate and may in fact result in a slightly lower rate under some conditions [43].

Overall these findings support the model that SeMet may induce DNA damage within the cell but to a lesser extent than selenite. This is perhaps unexpected as wild-type yeast show greater growth inhibition with SeMet than selenite. However, the finding of differential pathway components necessary to provide growth tolerance to these compounds highlights the fact that the cell metabolizes them differently, in particular with SeMet entering the amino acid pool and thus nonspecifically incorporated into proteins. Specifically, the fact that the loss of *RAD52* results in a reduced growth phenotype provides good evidence that both selenite and SeMet are inducing a DNA damage response. The effects of loss of the *RAD9*-dependent DNA repair and *RAD6/RAD18*-dependent DNA damage tolerance pathways on growth inhibition with Se compounds is clearly more pronounced than loss of the components of the oxidative stress pathway, indicating oxidative stress damage by either of these two compounds is perhaps secondary to the DNA damage that might be occurring. Together these results indicate that the *S. cerevisiae* model system provides a genetically tractable model system to allow for expanded analysis of the role of DNA damage in Se toxicity in eukaryotic cells.

We thank members of the Kinzy and Copeland labs and Kiran Madura for helpful comments and Anthony Esposito for developing the oxidative stress null allele set. T. G. K. and P. R. C. are supported by NIH GM074180.

The authors have declared no conflict of interest.

5 References

[1] El-Bayoumy, K., The protective role of selenium on genetic damage and on cancer. *Mutat. Res.* 2001, *475*, 123 – 139.

[2] Schwarz, K., Foltz, C. M., Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Am. Chem. Soc.* 1957, *79*, 3292 – 3293.

[3] Rayman, M. P., The importance of selenium to human health. *Lancet* 2000, *356*, 233 – 241.

[4] Wu, L., Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. *Ecotoxicol. Environ. Saf.* 2004, *57*, 257 – 269.

[5] Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., *et al.*, Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional prevention of cancer study group. *JAMA* 1996, *276*, 1957 – 1963.

[6] Reid, M. E., Duffield-Lillico, A. J., Garland, L., Turnbull, B. W., *et al.*, Selenium supplementation and lung cancer incidence: An update of the nutritional prevention of cancer trial. *Cancer Epidemiol. Biomarkers Prev.* 2002, *11*, 1285 – 1291.

[7] Klein, E. A., Lippman, S. M., Thompson, I. M., Goodman, P. J. *et al.*, The selenium and vitamin E cancer prevention trial. *World J. Urol.* 2003, *21*, 21 – 27.

[8] Schrauzer, G. N., Selenomethionine: A review of its nutritional significance, metabolism and toxicity. *J. Nutr.* 2000, *130*, 1653 – 1656.

[9] Palace, V. P., Spallholz, J. E., Holm, J., Wautier, K., *et al.*, Metabolism of selenomethionine by rainbow trout (Oncorhynchus mykiss) embryos can generate oxidative stress. *Ecotoxicol. Environ. Saf.* 2004, *58*, 17 – 21.

[10] Malkowski, M. G., Quartley, E., Friedman, A. E., Babulski, J., *et al.*, Blocking S-adenosylmethionine synthesis in yeast allows selenomethionine incorporation and multiwavelength anomalous dispersion phasing. *Proc. Natl. Acad. Sci. USA* 2007, *104*, 6678 – 6683.

[11] Kim, E. H., Sohn, S., Kwon, H. J., Kim, S. U., *et al.*, Sodium selenite induces superoxide-mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells. *Cancer Res.* 2007, *67*, 6314 – 6324.

[12] Somogyi, Z., Kiss, I., Kadar, I., Bakonyi, G., Toxicity of selenate and selenite to the potworm Enchytraeus albidus (annelida: enchytraeidae): A laboratory test. *Ecotoxicology* 2007, *16*, 379 – 384.

[13] Freeman, J. L., Lindblom, S. D., Quinn, C. F., Fakra, S., *et al.*, Selenium accumulation protects plants from herbivory by orthoptera via toxicity and deterrence. *New Phytol.* 2007, *175*, 490 – 500.

[14] Morris, V. H., Neiswander, C. R., Sayre, J. D., Toxicity of selenium-containing plants as a means of control for red spiders. *Plant Physiol.* 1941, *16*, 197 – 202.

[15] Rebsch, C. M., Penna, F. J., IIIrd, Copeland, P. R., Selenoprotein expression is regulated at multiple levels in prostate cells. *Cell Res.* 2006, *16*, 940 – 948.

[16] Letavayova, L., Vlckova, V., Brozmanova, J., Selenium: From cancer prevention to DNA damage. *Toxicology* 2006, *227*, 1 – 14.

[17] Terada, A., Yoshida, M., Seko, Y., Kobayashi, T., *et al.*, Active oxygen species generation and cellular damage by additives of parenteral preparations: Selenium and sulfhydryl compounds. *Nutrition* 1999, *15*, 651 – 655.

[18] Lu, J., Jiang, C., Kaeck, M., Ganther, H., *et al.*, Dissociation of the genotoxic and growth inhibitory effects of selenium. *Biochem. Pharmacol.* 1995, *50*, 213 – 219.

[19] Lu, J., Kaeck, M., Jiang, C., Wilson, A. C., *et al.*, Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells. *Biochem. Pharmacol.* 1994, *47*, 1531 – 1535.

[20] Pinson, B., Sagot, I., Daignan-Fornier, B., Identification of genes affecting selenite toxicity and resistance in Saccharomyces cerevisiae. *Mol. Microbiol.* 2000, *36*, 679 – 687.

[21] Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., *et al.*, Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 1999, *285*, 901 – 906.

[22] Fischer, J. L., Mihelc, E. M., Pollok, K. E., Smith, M. L., Chemotherapeutic selectivity conferred by selenium: A role for p53-dependent DNA repair. *Mol. Cancer Ther.* 2007, *6*, 355 – 361.

[23] Tyler, J. K., Adams, C. R., Chen, S. R., Kobayashi, R., *et al.*, The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* 1999, *402*, 555 – 560.

[24] Ramey, C. J., Howar, S., Adkins, M., Linger, J., *et al.*, Activation of the DNA damage checkpoint in yeast lacking the histone chaperone anti-silencing function 1. *Mol. Cell Biol.* 2004, *24*, 10313 – 10327.

[25] Alcasabas, A. A., Osborn, A. J., Bachant, J., Hu, F., *et al.*, Mrc1 transduces signals of DNA replication stress to activate Rad53. *Nat. Cell Biol.* 2001, *3*, 958 – 965.

[26] Biswas, S., Talukder, G., Sharma, A., Chromosome damage induced by selenium salts in human peripheral lymphocytes. *Toxicol. In Vitro* 2000, *14*, 405 – 408.

[27] Letavayova, L., Vlasakova, D., Spallholz, J. E., Brozmanova, J. *et al.*, Toxicity and mutagenicity of selenium compounds in Saccharomyces cerevisiae. *Mutat. Res.* 2008, *638*, 1 – 10.

[28] Abul-Hassan, K. S., Lehnert, B. E., Guant, L., Walmsley, R., Abnormal DNA repair in selenium-treated human cells. *Mutat. Res.* 2004, *565*, 45 – 51.

[29] Craven, R. J., Greenwell, P. W., Dominska, M., Petes, T. D., Regulation of genome stability by TEL1 and MEC1, yeast homologs of the mammalian ATM and ATR genes. *Genetics* 2002, *161*, 493 – 507.

[30] Greenwell, P. W., Kronmal, S. L., Porter, S. E., Gassenhuber, J. *et al.*, TEL1, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. *Cell* 1995, *82*, 823 – 829.

[31] Paques, F., Haber, J. E., Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. *Microbiol. Mol. Biol. Rev.* 1999, *63*, 349 – 404.

[32] Cole, G. M., Schild, D., Lovett, S. T., Mortimer, R. K., Regulation of RAD54- and RAD52-lacZ gene fusions in Saccharomyces cerevisiae in response to DNA damage. *Mol. Cell. Biol.* 1987, *7*, 1078 – 1084.

[33] Kiakos, K., Howard, T. T., Lee, M., Hartley, J. A., *et al.*, Saccharomyces cerevisiae RAD5 influences the excision repair of DNA minor groove adducts. *J. Biol. Chem* 2002, *277*, 44576 – 44581.

[34] Spallholz, J. E., Palace, V. P., Reid, T. W., Methioninase and selenomethionine but not Se-methylselenocysteine generate methylselenol and superoxide in an in vitro chemiluminescent assay: Implications for the nutritional carcinostatic activity of selenoamino acids. *Biochem. Pharmacol.* 2004, *67*, 547 – 554.

[35] Rodriguez-Manzaneque, M. T., Ros, J., Cabiscol, E., Sorribas, A., *et al.*, Grx5 glutaredoxin plays a central role in protection against protein oxidative damage in Saccharomyces cerevisiae. *Mol. Cell. Biol.* 1999, *19*, 8180 – 8190.

[36] Mousson, F., Ochsenbein, F., Mann, C., The histone chaperone Asf1 at the crossroads of chromatin and DNA checkpoint pathways. *Chromosoma* 2007, *116*, 79 – 93.

[37] Sinha, R., Said, T. K., Medina, D., Organic and inorganic selenium compounds inhibit mouse mammary cell growth in vitro by different cellular pathways. *Cancer Lett.* 1996, *107*, 277 – 284.

[38] Biswas, S., Talukder, G., Sharma, A., Selenium salts and chromosome damage. *Mutat. Res.* 1997, *390*, 201 – 205.

[39] Lehoczky, P., McHugh, P. J., Chovanec, M., DNA interstrand cross-link repair in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 2007, *31*, 109 – 133.

[40] Thomas, D., Cherest, H., Surdin-Kerjan, Y., Identification of the structural gene for glucose-6-phosphate dehydrogenase in yeast. Inactivation leads to a nutritional requirement for organic sulfur. *EMBO J.* 1991, *10*, 547 – 553.

[41] Shen, X., Jun, S., O'Neal, L. E., Sonoda, E., *et al.*, REV3 and REV1 play major roles in recombination-independent repair of DNA interstrand cross-links mediated by monoubiquitinated proliferating cell nuclear antigen (PCNA). *J. Biol. Chem.* 2006, *281*, 13869 – 13872.

[42] Endo, K., Tago, Y., Daigaku, Y., Yamamoto, K., Error-free RAD52 pathway and error-prone REV3 pathway determines spontaneous mutagenesis in Saccharomyces cerevisiae. *Genes Genet. Syst.* 2007, *82*, 35 – 42.

[43] Pavlov, Y. I., Nguyen, D., Kunkel, T. A., Mutator effects of overproducing DNA polymerase eta (Rad30) and its catalytically inactive variant in yeast. *Mutat. Res.* 2001, *478*, 129 – 139.

[44] Cejka, P., Vondrejs, V., Storchova, Z., Dissection of the functions of the Saccharomyces cerevisiae RAD6 postreplicative repair group in mutagenesis and UV sensitivity. *Genetics* 2001, *159*, 953 – 963.

[45] Xiao, W., Chow, B. L., Broomfield, S., Hanna, M., The Saccharomyces cerevisiae RAD6 group is composed of an error-prone and two error-free postreplication repair pathways. *Genetics* 2000, *155*, 1633 – 1641.

