BioMetals (2006) 19:705–714
DOI 10.1007/s10534-006-9007-1
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Sensitivity to Sn^{2+} of the yeast *Saccharomyces cerevisiae* depends on general energy metabolism, metal transport, anti-oxidative defences, and DNA repair

C. Viau[1], C. Pungartnik[2], M.C. Schmitt[1], T.S. Basso[2], J.A.P. Henriques[1] & M. Brendel[2,*]
[1]*Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, RS, Brasil;* [2]*Departamento de Biology, Universidade Estadual de Santa Cruz (UESC), Rodovia Ilhéus-Itabuna Km 16, CEP-45662-000, Ilhéus, Bahia, Brasil; *Author for correspondence (E-mail: martinbrendel@yahoo.com.br)*

Received 10 October 2005; accepted 18 March 2006

Key words: cytochrome oxidase, DNA repair, genotoxicity, glucose repression, membrane transport, oxidative stress, stannous chloride, yeast

Abstract

Resistance to stannous chloride ($SnCl_2$) of the yeast *Saccharomyces cerevisiae* is a product of several metabolic pathways of this unicellular eukaryote. Sensitivity testing of different null mutants of yeast to $SnCl_2$ revealed that DNA repair contributes to resistance, mainly via recombinational (Rad52p) and error-prone (Rev3p) steps. Independently, the membrane transporter Atr1p/Snq1p (facilitated transport) contributed significantly to Sn^{2+}-resistance whereas absence of ABC export permease Snq2p did not enhance sensitivity. Sensitivity of the superoxide dismutase mutants *sod1* and *sod2* revealed the importance of these anti-oxidative defence enzymes against Sn^{2+}-imposed DNA damage while a catalase-deficient mutant (*ctt1*) showed wild type (WT) resistance. Lack of transcription factor Yap1, responsible for the oxidative stress response in yeast, led to 3-fold increase in Sn^{2+}-sensitivity. While loss of mitochondrial DNA did not change the Sn^{2+}-resistance phenotype in any yeast strain, cells with defect cytochrome *c* oxidase (CcO mutants) showed gradually enhanced sensitivities to Sn^{2+} and different spontaneous mutation rates. Highest sensitivity to Sn^{2+} was observed when yeast was in exponential growth phase under glucose repression. During diauxic shift (release from glucose repression) Sn^{2+}-resistance increased several hundred-fold and fully respiring and resting cells were sensitive only at more than 1000-fold exposure dose, i.e. they survived better at 25 mM than exponentially growing cells at 25 μM Sn^{2+}. This phenomenon was observed not only in WT but also in already Sn^{2+}-sensitive *rad52* as well as in *sod1*, *sod2* and CcO mutant strains. The impact of metabolic steps in contribution to Sn^{2+}-resistance had the following ranking: Resting WT cells > membrane transporter Snq1p > superoxide dismutases > transcription factor Yap1p ≥ DNA repair ≫ exponentially growing WT cells.

Introduction

Trace amounts of different metals play a crucial role in cellular metabolism as they constitute ligands of diverse enzymes (Eide & Guerinot 1997). At higher concentrations metal ions, especially some of heavy metals, interfere negatively with cellular metabolism as they may inactivate proteins and damage DNA (McMurray & Tainer 2003). Evolution thus favoured survival of organisms which had developed mechanisms that guaranteed optimal intracellular metal concentration by balancing metal uptake from the environment and metal excretion/neutralization (metal homeostasis (Tomsett & Thurmann 1988)). Modern food preservation relies on sterilization and

packaging of food, and tin plays an important role in this process as it is used for the inner lining of metal containers and for conservation of soft drinks (McLean *et al*. 1983a). Tin thus comes into contact with the packaged food and may form stannous salts. Increased consumption of canned foods, therefore, is held responsible for tin accumulation in humans of wealthy countries (Schroeder *et al*. 1964).

Stannous chloride ($SnCl_2$) is a weak mutagen as defined by its genotoxicity in unicellular prokaryotes (Bernardo-Filho *et al*. 1994; Dantas *et al*. 1996) and eukaryotes (Pungartnik *et al*. 2005) as well as its DNA interactions in mammalian cells (McLean & Kaplan 1979; McLean *et al*. 1983b). Mutagenicity and mitotic gene conversion induced by Sn^{2+} in *Saccharomyces cerevisiae* points to the involvement of error-prone repair mechanisms in the removal of DNA lesions and the involvement in repair of the recombinational Rad52-controlled pathway was also shown (Pungartnik *et al*. 2005).

Metal uptake and metal homeostasis in yeast are controlled by a complex system of metabolic steps, most prominently by membrane transporters (Eide & Guerinot 1997; Van Ho *et al*. 2002) and by intracellular neutralization with thiol-oligopeptides and metallothioneins (Heuchel *et al*. 1994). Thus it is likely that changes in Sn^{2+} uptake or excretion via membrane transporters might also influence sensitivity of yeast cells to this metal. Once in the cell, the genotoxic potential of Sn^{2+} might also be significantly modulated by other, non-DNA repair or membrane transport-related physiological parameters, i.e., the quality and quantity of enzymatic and non-enzymatic scavengers of metal-induced reactive oxygen species (ROS). $SnCl_2$ is known to produce ROS (Dantas *et al*. 1999) most probably via Fenton-like reactions (McLean *et al*. 1983b) and thus the genetic endowment of yeast with anti-oxidative defence systems, e.g., superoxide dismutases, catalase, glutathione, and their oxidative stress-induced expression might contribute to Sn^{2+}-resistance.

Anaerobically growing microorganisms, especially obligate anaerobes, are known to have a higher metal sensitivity than aerobically living microbial species. The facultative anaerobe yeast *S. cerevisiae* can grow both in presence or absence of respiratory metabolism, and thus might be a good model organism to test the influence of general energy metabolism on sensitivity to Sn^{2+}. Mitochondrial activity plays a crucial role in aerobic energy metabolism of eukaryotes and it is thus likely that defects in the respiratory chain located within the inner mitochondrial membrane might directly or indirectly contribute to the generation of ROS (Barros *et al*. 2003) which might be altered by the presence of Sn^{2+}.

All above-mentioned metabolic steps are controlled by proteins encoded in the yeast cell's genome and thus can be influenced by genetic manipulation. Therefore, this unicellular fungus offers itself as an ideal eukaryotic model for the observation of Sn^{2+}-induced effects on its DNA, allowing to determine the relative contribution to Sn^{2+}-resistance of individual protective metabolic pathways.

Materials and methods

Yeast strains and growth conditions

The relevant genotypes of the yeast strains used in this work are given in Table 1. Media, solutions and buffers were prepared according to Burke *et al*. (2000). Complete medium (YPD) was used for routine growth of yeast cells and minimal medium (MM) was supplemented with the appropriate amino acids (synthetic complete medium, SC). To ascertain yeast respiratory competence and for elimination of spontaneously accumulated *petites* all strains were pre-grown on YPglycerol media (glucose replaced by 2% glycerol) before being grown in YPD.

Yeast exposure to $SnCl_2$ and survival

Stationary (STAT) cells were grown in YPD at 30 °C for 72 h. Different times of growth of STAT cells in fresh medium yielded cells in exponentially phase of growth (LOG). LOG cells were microscopically checked for bud appearance and the bud index (% budded cells) was established. Sensitivity of twice saline-washed yeast suspensions to $SnCl_2$ was routinely determined in liquid saline (0.9% NaCl, pH 5.0). Exposure concentration was 25 mM for STAT cells and 25 μM for LOG cells. Exposure time was for 60 min at 30 °C. Thereafter, $SnCl_2$-mediated cell aggregates were de-clumped in phosphate buffer (PB, pH 7.4, 0.067 M) followed by vigorous vortexing before

Table 1. Strains used in this study and their relevant genotypes.

Yeast strains	Genotype	Reference
XS2316 (WT)	*MAT***a** + *leu1 − 1trp5 − 48 + + + his1 − 208* *MAT*α *ade6 leu1-12 + cyh2 met13 lys5-1 his1-208*	Machida & Nakai (1980)
XV185-14c (WT)	*MAT*α *ade2-2 his1-798 lys1-1 trp5-48 hom3-10 arg4-17*	von Borstel *et al.* (1971)
BY10000 (WT)	*MAT*α *his3*Δ1 *lys2*Δ0 *leu2*Δ0 *ura3*Δ0	EUROSCARF
4 BY *rad* mutants	Same genotype as BY10000 but *rad52*Δ, *rad2*Δ, *rad4*Δ, *rad6*Δ	See above
YPH98 (WT)	*MAT***a** *ura3-52 lys2-801 ade2-101 leu2-*Δ*1 trp1-*Δ*1*	Wehner *et al.* (1983)
4NQO sensitive	Same genotype as YPH98 but *snq1*Δ, *snq2*Δ, *snq3*Δ/*yap1*Δ	See above
q1	Same genotype as YPH98 but rho^0	M. Grey, Frankfurt/Main
q2	Same genotype as q1 but *gsh1*Δ	See above
q3	Same genotype as q1 but *gsh1*Δ*lwg1*Δ	See above
q4	Same genotype as q1 but *lwg1*Δ	See above
EG103 (WT)	*MAT*α it leu2-3, 112 his3Δ*1 trp1-289 ura3-52 GAL*$^+$	E.B. Gralla, Los Angeles
EG118 (*sod1*Δ)	*sod1::URA3* all others markers as EG103	See above
EG110 (*sod2*Δ)	*sod2::TRP1* all others markers as EG103	See above
EG133 (*sod1*Δ*sod2*Δ)	*sod1::URA3 sod2*::*TRP1* double mutant, all others markers as EG103	See above
EG223 (*ctt1*Δ)	*ctt1::TRP1* all others markers as EG103	See above
BER/NER (WT)	*MAT*α *ade2-101 his3*Δ*200 ura3*ΔNco lys2ΔBgl	Swanson *et al.* (1999)
Base excision repair mutants	Same genotype as BER/NER WT but *ntg1*Δ, *ntg2*Δ, *ntg1*Δ*ntg2*Δ, *ntg1*Δ*ntg2*Δ*apn1*Δ, *ntg1*Δ*ntg2*Δ*apn1*Δ*rad52*Δ, *ntg1*Δ*ntg2*Δ*apn1*Δ*rad1*Δ, *ntg1*Δ*ntg2*Δ*apn1*Δ*rev3*Δ	See above
W303 (WT)	*MAT***a** *ade2-1 leu2-3, 112 his3-11,15 trp1-1 ura3-1 can1-100*	A. Tzagoloff, New York
Mitochondrial mutants	Same genotype as W303 but *cox14*Δ, *cox15*Δ, *cox16*Δ, *cox17*Δ, *cox18*Δ, *cox20*Δ, *shy1*Δ, *sco1*Δ, *pet100*Δ, *pet117*Δ	See above

dilution in PB and plating (Pungartnik *et al.* 2005). Cells were plated on YPD and survival was determined after 3 d at 30 °C. Presented results are the mean of at least three independent experiments, the standard deviation and statistical analyses were calculated by GraphPad Prism® program.

Spontaneous mutation of mitochondrial mutants

STAT cells were grown in YPD at 30 °C for 72 h, washed twice with saline (0.9% NaCl, pH 5.0) and yeast suspensions plated on media SC (survival) and SC-Trp (spontaneous mutation). Following incubation for 7 d at 30 °C, colonies appearing on SC medium yielded data on cell survival, while those grown on SC-Trp represented the spontaneous mutations. Frequencies of spontaneous genomic mutation in different mitochondrial mutants (deficient of functional CcO) were scored per 10^7 cells. Results are means of three independent experiments, the standard deviation and statistical analyses was performed using the GraphPad Prism® software.

Results and discussion

Repair of Sn^{2+}-induced DNA lesions

The sensitivity of 35 different yeast strains (STAT cells of WT and isogenic mutants) to 60 min exposure at 25 mM of $SnCl_2$ is given in Tables 2 and 3. The range of killing of mutant cells as compared with the WT varied not more than 2 decades (survival between 1 and 90%, Table 2) or not at all (Table 3). Amongst the seven different DNA repair WT strains, survival varied from 25 to 90%, depending on the genetic background of each strain. Six of the WT strains can be roughly allocated to two sensitivity groups (survival either around 25 or 85%), with strain W303 in between (Table 2). These WT sensitivity variations were neutralized when comparing to sensitivities of

Table 2. Sensitivity to $SnCl_2$ (25 mM, 60 min) of STAT cells of different yeast strains.

Strains	$SnCl_2$	*p* value[a]	Relative sensitivity increase[b]
WT (XS2316)	**90.3 ± 3.3**		–
WT (XV185-14c)	**85.0 ± 8.0**		–
WT (W303)	**57.0 ± 5.4**		–
WT (Y10000)	**24.1 ± 1.1**		–
*rad2*Δ	13.5 ± 2.8	<0.05	1.4×
*rad4*Δ	10.3 ± 4.8	<0.01	1.5×
*rad6*Δ	8.1 ± 0.0	<0.0001	1.7×
*rad52*Δ	2.8 ± 0.9	<0.0001	2.5×
WT (YPH98)	**28.2 ± 6.2**		–
*snq1*Δ	14.5 ± 0.5	<0.05	1.5×
*snq2*Δ	23.0 ± 0.4	n.s.	1.1×
*snq3*Δ/*yap1*Δ	1.9 ± 0.2	<0.05	3.0×
WT (EG103)	**26.2 ± 6.2**		–
*sod1*Δ	5.3 ± 0.5	<0.01	2.5×
*sod2*Δ	17.5 ± 0.5	n.s.	1.4×
*sod1sod2*Δ	2.5 ± 0.2	<0.01	3.0×
*ctt1*Δ	49.0 ± 6.1	n.s.	0.6×
*ctt1*Δ*sod1*Δ	26.0 ± 2.3	n.s.	1.0×
WT	**87.2 ± 0.7**		–
*ntg1*Δ	77.4 ± 2.8	<0.01	1.7×
*ntg2*Δ	69.2 ± 2.2	<0.001	2.5×
*ntg1*Δ*ntg2*Δ	70.3 ± 1.6	=0.001	2.5×
*ntg1*Δ*ntg2*Δ*apn1*Δ	70.0 ± 3.4	=0.001	2.5×
*ntg1*Δ*ntg2*Δ*apn1*Δ*rad52*Δ	55.0 ± 1.3	<0.0001	4.0×
*ntg1*Δ*ntg2*Δ*apn1*Δ*rad1*Δ	31.3 ± 0.8	<0.0001	7.0×
*ntg1*Δ*ntg2*Δ*apn1*Δ*rev3*Δ	19.5 ± 0.6	<0.0001	11.0×

[a]Unpaired *t* test (95% interval confidence); statistical analyses comparing each mutant to its isogenic WT.[b]Sensitivity is defined by the inclination of an idealized linear inactivation curve in a semi-log plot. If WT survives 10% and mutant 1%, sensitivity increase of the mutant is by factor 2.n.s. not significantly different from the isogenic WT.

mutant strains, as always a set of WT and WT-derived isogenic mutant strains were compared. Sensitivities (or relative resistance) of mutant strains were calculated by comparison with the WT of semi-log graphs of respective survival curves.

It is known that $SnCl_2$ sensitivity in yeast increases from WT (RAD) < *rad2*Δ < *rad4*Δ < *rad6*Δ < *rad52*Δ in the Y10000 (EUROSCARF) background (Pungartnik *et al*. 2005). The recombination repair-deficient mutant *rad52*Δ had a 2.5-fold higher sensitivity as compared to the WT (Table 2). The relative resistance of mutant strains *rad2* and *rad4*, deficient in nucleotide excision repair (NER) was rather high, indicating a minor but significant contribution to repair of Sn^{2+} -induced DNA lesions by this repair pathway.

A series of mutants defective in different base excision repair (BER) pathways, combined with nucleotide excision repair (BER/NER, constructed elsewhere) were used to indirectly determine the type of $SnCl_2$-produced DNA lesion. Three DNA *N*-glycosylases, encoded by yeast genes *NTG1, NTG2*, and *OGG1* are known to be involved in repair of oxidative DNA damage that results in abasic sites in DNA (You *et al*. 1999, Alseth *et al*. 1999, Boiteux and Guillet 2004). Mutant allele *ntg2*Δ conferred the highest sensitivity (not to be enhanced in the *ntg1*Δ*ntg2*Δ*apn1*Δ triple mutant) demonstrating the necessity of the nucleus-located Ntg2p (Alseth *et al*. 1999) for repair of Sn^{2+} -induced DNA lesions (Table 2), whereas the apurinic site endonuclease Ntg1p, mainly localized in the mitochondria (You *et al*. 1999) and the

Table 3. Mutant alleles not affecting Sn^{2+}-resistance (25 mM, 60 min) of STAT cells.

Strains
q1 WT
q2* *gsh1ΔLWG1*
q3 *gsh1Δlwg1*
q4 *GSH1 lwg1*
*erg3*Δ [EUROSCARF]
*ogg1*Δ [EUROSCARF]
*mag1*Δ [EUROSCARF]
*gsh1*Δ* [EUROSCARF]
*ctt1*Δ [EUROSCARF]

*Grown in SynCo media supplemented with 100 μg GSH/ml.

OGG1-encoded *N*-glycosylase seem dispensable for repair of Sn^{2+}-induced DNA lesions (Tables 2 and 3).

Although all mutants containing *ntg2*Δ in conjunction with mutant alleles of other repair pathways (NER, error-prone or recombinational repair) had statistically significant higher sensitivity than the WT, the *rev3*Δ mutant allele-containing quadruple mutant strain was the most sensitive, thus indicating that the error-prone repair pathway (translesion synthesis (Lawrence 2002)) may make the highest contribution to repair of $SnCl_2$-induced DNA lesions (Table 2); this could explain the observed mutagenicity of $SnCl_2$ (Pungartnik *et al*. 2005). The response to $SnCl_2$-induced oxidative DNA damage thus differs from that introduced by hydrogen peroxide where the contribution of translesion synthesis is smaller than that of recombinational repair (Salmon *et al*. 2004). Introduction of a *rad52*Δ mutant allele, conferring lack of recombinational repair, into the BER triple knockout mutant *ntg1*Δ*ntg2*Δ*apn1*Δ led to a significant increase in sensitivity, demonstrating that the two repair modes contribute (at least in part) independently to removal of $SnCl_2$-induced DNA lesions (Table 2). The same can be said after the introduction of a *rad1*Δ mutant allele that yields an even more sensitive quadruple mutant strain. This indicates that NER, independently from BER or recombinational repair, can remove part of Sn^{2+}-induced DNA damage, most probably abasic sites (Torres-Ramos *et al*. 2000). These overlapping specificities of BER, NER, recombination and error-prone translesion synthesis in repair of damaged bases has already been shown by Swanson *et al*. (1999). Since DNA repair mechanisms were largely conserved during evolution (Eisen & Hanawalt 1999), the repair of Sn^{2+}-induced DNA damage via several different repair pathways in yeast might suggest a similar repair scenario in humans.

Membrane transport proteins influence Sn^{2+} toxicity

Two types of yeast membrane transporters were tested for their putative contribution to Sn^{2+}-uptake/homeostasis. Cells deficient in the facilitated transporter Atr1p/Snq1p (Kanazawa *et al*. 1988, Gömpel-Klein & Brendel 1990) showed significantly increased Sn^{2+}-sensitivity (factor 1.5) while a deletion mutant of the ABC transport protein Snq2p that so far has been shown to mediate resistance to structurally unrelated chemicals like 4-Nitro-quinoline oxide, sulphometuron methyl, triaziquone, and phenanthroline (Servos *et al*. 1993) was practically as resistant as the WT (resistance ranking was WT ≥ *snq2*Δ > *snq1*Δ). Complexity of metal ion homeostasis (Van Ho *et al*. 2002) however, makes it highly likely that other, hitherto unknown transport protein are also involved in Sn^{2+} transport (import/export). Indeed, it has been shown that $SnCl_2$ facilitates the Ca^{2+} entry through the L-type calcium channel under the condition of the membrane depolarization. There is the possibility that Ca^{2+} release from intracellular Ca^{2+} pools is involved in the action of $SnCl_2$ (Hattori *et al*. 2001) and that tin induces considerable changes in the metabolism of endogenous metals such as zinc and copper (Chmielnicka *et al*. 1981).

Lack of adaptive response to oxidative stress leads to Sn^{2+}-sensitivity

Mutants lacking yeast transcription factor Yap1p displayed a 3-fold higher Sn^{2+}-sensitivity than the isogenic WT (Table 2). Under oxidative stress Yap1 is oxidized and rapidly accumulated in the nucleus where it regulates the expression of up to 70 genes encoding proteins involved in oxidative stress response (Wood *et al*. 2004). Thus the yap1 mutant's sensitivity response indicates that anti-ROS defence systems of WT yeast are transcriptionally activated after Sn^{2+}-exposure. A similar response to oxidative stress exists in bacteria where the transcription activator OxyR induces the genes coding for

anti-stress proteins. Bacterial strains lacking a functional OxyR gene are used in the Mutoxitest to detect, via their specific sensitivity phenotype, ROS producing chemicals (Martínez *et al*. 2000). Such mutants also display a significantly higher sensitivity to Sn^{2+} (Pungartnik *et al*. 2005).

Yeast mutants lacking one or two genes encoding anti-oxidative defences (superoxide dismutase mutants *sod1*Δ, *sod2*Δ, and the double mutant *sod1*Δ*sod2*Δ) revealed 2–3-fold higher sensitivity to $SnCl_2$. The yeast strain containing both *sod* mutant alleles exhibited about additive sensitivities of the respective single mutants (Table 2). Judged by the higher sensitivity of *sod1*Δ mutant (Pungartnik *et al*. 2005), cytosolic Sod1p seems more important than mitochondrial Sod2p in protecting against the toxic effects of Sn^{2+} in STAT cells. On the other hand cytoplasmic catalase Ctt1p (Hartig & Ruis 1986) is apparently not involved in detoxification of any Sn^{2+}-induced ROS. In bacteria, H_2O_2 induces a cross-adaptive response to ROS-producing agents, amongst them $SnCl_2$ (Assis *et al*. 2002) suggesting that the OxyR transcription activator, which induces expression of catalase, alkyl hydroperoxide reductase and superoxide dismutase protects against Sn^{2+}-generated oxidative stress. The Yap1 transcription activator in yeast may have the same function (Wu & Mowe-Rowley 1994). However, this protective response to oxidative stress does not render a yeast cell about 1,000-fold more resistant (as calculated in exposure dose necessary for a like-wise killing) to Sn^{2+} as is seen when changing from glucose-repressed LOG to the glucose de-repressed STAT phase (cf. below and Figures 3 and 4). It has been suggested that two independently acting anti-ROS protective systems (one mediated by glucose repression/de-repression, the other via ROS-inducible transcription activators) are working in yeast (Maris *et al*. 2001), and our data imply that both are contributing to Sn^{2+}-resistance.

Defects in respiratory chain lead to Sn^{2+}-sensitivity

Yeast strains containing mutant alleles of genes encoding proteins of mitochondrially located cytochrome c oxidase (CcO-deficient mutants), also showed enhanced and variable sensitivity to

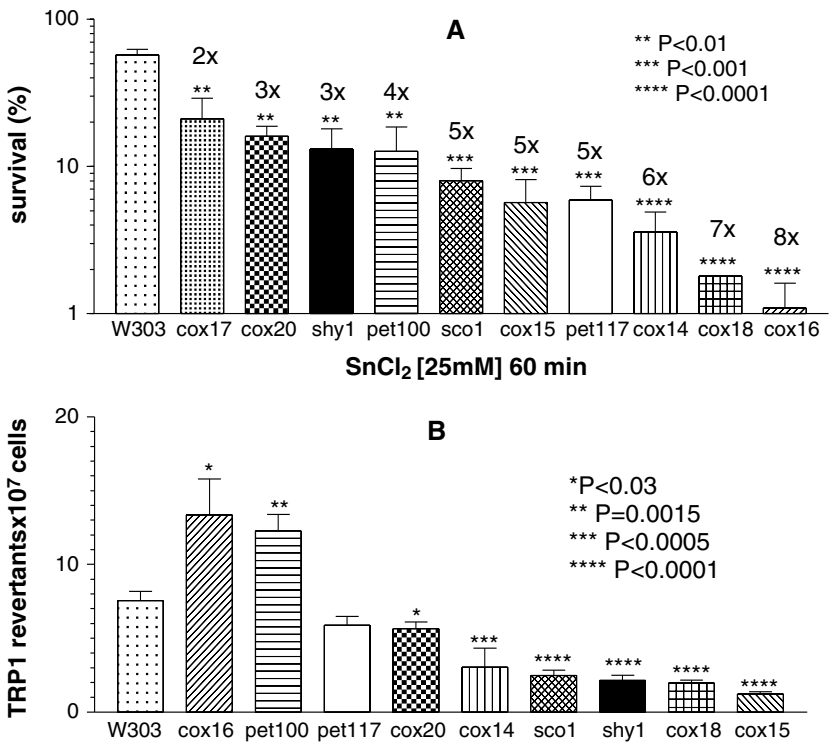

Figure 1. (A) Survival of STAT cells of WT and 9 mitochondrial mutants after 60 min $SnCl_2$ exposure (25 mM). (B) Reversion of tryptophan mutant allele *trp1-1* in mitochondrial mutant strains *(trp1-1)*, per 10^7 survivors. Numbers above the error bar of each column (A) gives the sensitivity increase of the respective mutant. Cells were diluted in PB.

$SnCl_2$ (Figure 1A). Interestingly, this sensitivity could be strongly enhanced in a *cox11/pso7-1* mutant (Pungartnik *et al.* 1999) when introducing the *erg3/pso6* mutant allele (Schmidt *et al.* 1999), as a second mutation (Brendel *et al.* 2003). Alone, the *erg3/pso6* mutant allele confers ergosterol deficiency and renders the mutant not sensitive to Sn^{2+} (Table 3), suggesting a non-protective role of this membrane constituent against the oxidative stress induced by this metal. In combination with *cox11/pso7-1* mutant allele, however, *erg3/pso6* showed a dramatic sensitivity effect. Generally CcO-deficient mutants are thought to produce more H_2O_2 by letting electrons escape from the respiratory chain (Barros *et al.* 2003). This elevated H_2O_2 might act as a mutagen on genomic DNA. We assayed, therefore, for spontaneously induced mutations in the *trp1-1* locus (reversion to trp^+) in mutant alleles of 9 different CcO-encoding genes (Figure 1B). While 2 CcO mutants, *cox16* and *pet100* had indeed higher-than-WT mutability in *trp1-1*, six others, i.e., the majority showed lower-than-WT mutability (Figure 1B), so that a general assumption of higher spontaneous mutation in CcO mutants could not be verified. It is known that Sn^{2+} generates ROS via Fenton-like reactions (McLean *et al.* 1983) and that there is variable content of ROS being produced in different CcO mutants (Barros *et al.* 2003). This might be the reason for, or at least contribute to, the observed variation of Sn^{2+}-sensitivity in the CcO mutants (Figure 1A). It must be emphasized, however, that total lack of respiratory chain activity in rho^0 mutants does not lead to enhanced Sn^{2+}-sensitivity, as isogenic rho^+ and rho^0 strains (YPH98 and q1, respectively) have identical WT-like survival (Tables 2 and 3).

Diauxic shift-induced Sn^{2+}-resistance

The highest $SnCl_2$ -sensitivity, however, was observed in glucose-repressed pre-diauxic shift exponentially growing cells (LOG cells (Figure 2)). On the basis of comparison to the $SnCl_2$ exposure dose required for likewise inactivation of STAT cells we found a more than 1000-fold increase in sensitivity (LOG slightly more sensitive to 25μM Sn^{2+} than STAT at 25 mM (Figure 3)). The significant increase of sensitivity of sod mutants as compared to the WT was the same in LOG cells at 25 μM Sn^{2+} exposure as seen in STAT cells at the

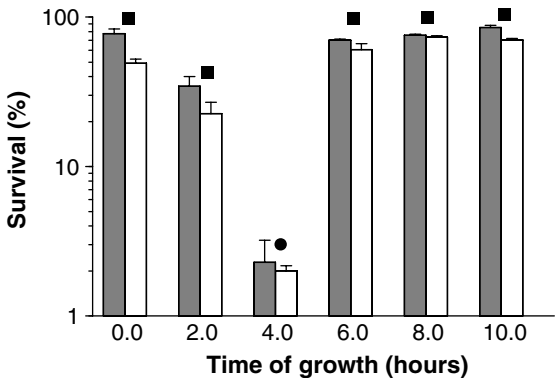

Figure 2. Sensitivity to 20 minutes Sn^{2+}-exposure of haploid WT strain Y10000 (grey column) and *rad52*Δ (white column); (■) STAT cells exposed to 25 mM; (●) LOG cells exposed to 2.5 mM. Cells were diluted in PB.

1000-fold Sn^{2+} exposure dose (Table 2). The high resistance to Sn^{2+} is acquired during and after the diauxic shift, i.e., when the yeast LOG cells are released from glucose repression and many cellular functions are adapted to respiratory metabolism. This resembles the response of yeast cells during diauxic shift-induced resistance against hydroperoxides (Maris *et al.* 2001). This process is independent of one tested repair function (Rad52p) (Figure 2), of the presence of superoxide dismutases (Figure 3), functional cytochrome c oxidase (Figure 4), and the presence of any mitochondrial respiratory metabolism (rho^0 mutants).

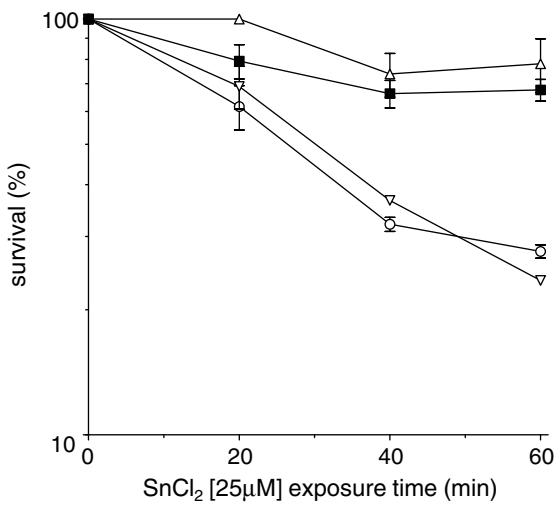

Figure 3. Sensitivity to 25 μM Sn^{2+}-exposure of LOG cells of haploid WT strain EG103 (■); and its isogenic mutants *sod2*Δ (△); *sod1*Δ (▽); and the double mutant *sod1*Δ*sod2*Δ (○).

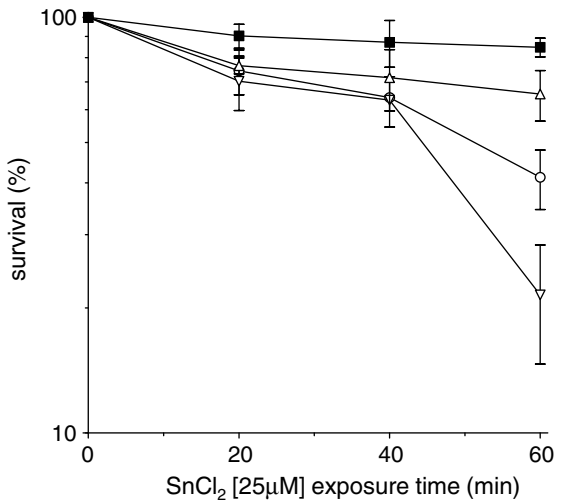

Figure 4. Sensitivity to 25 μM Sn^{2+}-exposure of LOG cells with different defects in cytochrome *c* oxidase WT W303 (■); *cox15*Δ (□); *sco1*Δ (○) and *cox17*Δ (▽).

Glutathione not needed to protect against short-term Sn^{2+}-exposure

Interestingly, mutants with low and extremely low glutathione (GSH) pools showed no enhanced sensitivity to Sn^{2+} (Table 3). Mutant strain q2 (*gsh1*Δ) that lacks the first step of the two-step GSH biosynthesis and relies totally on externally offered (low) GSH showed the same Sn^{2+}-resistance as the q1 WT. The same was true for mutant q3 which is isogenic with mutant strain q2 but, due to a mutational change in the second enzyme of the proline biosynthetic pathway produces a little amount of the dipeptide γ-glutamylcysteine (the product lacking in the *gsh1* mutant (Spector *et al.* 2001)), and hence GSH, and is thus independent of external GSH supplementation. Finally mutant q4 that is WT for GSH biosynthesis and contains only the altered enzyme of the proline pathway, was also WT-like in its Sn^{2+}-resistance phenotype. Thus, GSH is not needed for the protection of yeast against acute, short-term (i.e. 1 h) Sn^{2+}-exposure (Table 3), most probably because catalase provides an overlapping defence system against metal-induced ROS (Grant *et al.* 1998).

The combined action of all above-mentioned protective mechanisms, whose functions were shown in STAT cells, i.e. DNA repair, membrane transport, and defences against ROS, can hardly explain the difference of three orders of magnitude in $SnCl_2$-sensitivity between isogenic LOG and STAT cells. Actually, some protective mechanisms, e.g., Sod1 and Sod2 as well as functionality of cytochrome c oxidase may be totally discounted in this comparison, as the *sod1* and *sod2* mutant alleles (Figure 3) as well as CcO mutants (Figure 4) conferred enhanced $SnCl_2$-sensitivity in STAT and LOG cells alike, regardless of their 1000-fold difference in sensitivity. Thus, this extreme sensitivity of LOG cells (alternatively, the extreme resistance of STAT cells (De Winde *et al.* 1997)) either suggests that cells growing under glucose repression lack at least one, most probably several, unknown mechanism(s) protecting against ROS or other stress (Fuge & Werner-Washburne 1997) or that the protection factors already known to us (c.f. above) have an extremely synergistic interaction (i.e. overlapping functionality) in STAT cells; this would not show us the real protective potential of a single metabolic contribution (as is suggested for the contribution of different repair mechanisms to removal of Sn^{2+}-induced DNA lesions (cf. above and Swanson *et al.* 1999)), but a joint suppression of several of these protective mechanisms (e.g. under glucose repression) would render a LOG cell extremely sensitive. Alternatively or additionally, we might speculate that the high sensitivity of LOG cells could also be, at least partially, due to a more efficient uptake of Sn^{2+} ions as rapidly growing cells might have a more active membrane transport. One step towards clarifying this last question might be the quantitative determination of Sn^{2+}-uptake by molecular dosimetry methods, e.g. via PIXE (particle induced X-ray emission) in isogenic LOG and STAT cells (Viau *et al.* in press). This would also allow us to better assess the genotoxic potential of intracellular Sn^{2+} at different physiological states of the yeast cell. Clearly, there is need for clarifying the types of ROS being directly or indirectly formed by Sn^{2+}, and more information on this may be gained by studying the response of all yeast strains known to have a defect in anti-ROS defence (single or multiple allele mutants) and by complementing this info by *in vitro* biochemical studies.

Despite of the LOG/STAT cells Sn^{2+}-sensitivity/resistance riddle we may summarize our results to partially answer two questions: (1) What type of DNA damage is induced by Sn^{2+}? We know that strand breaks are formed *in vitro* (Dantas *et al.* 1999), and this would best explain the contribution

of recombinational repair; oxidized base damage would explain the necessity of BER repair; some bulky adducts could explain involvement of NER in repair; finally, and most important, translesion synthesis would allow resumption of DNA synthesis at stalled replication forks, at the cost of error-prone repair (mutation). (2) Which species of ROS are generated by intracellular Sn^{2+}? Clearly superoxide anion, as Sod1p, and to a lesser extent, Sod2p are protecting the cells; hydrogen peroxide is most probably generated only in little quantity (or not at all) as cytosolic catalase and GSH are not necessary for protection. Direct base oxidation may occur as indicated by the role of BER in repair, but 8-hydroxyguanine, the specific substrate of Ogg1p, is apparently not formed.

Acknowledgements

We thank Dr. A. Tzagoloff for kindly providing the mitochondrial mutant strains and Dr. M. Grey for constructing the q mutants. Research supported by Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and GENOTOX. C. P. held a fellowship by FAPESB/CNPq (PRODOC Program). M.B. is Visiting Scientist supported by FAPESB. Part of the data is from the Master thesis of C.V. (PPGBCM-UFRGS).

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