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In yeast redistribution of Sod1 to the mitochondrial intermembrane space provides protection against respiration derived oxidative stress

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ABSTRACT

The antioxidative enzyme copper–zinc superoxide dismutase (Sod1) is an important cellular defence system against reactive oxygen species (ROS). While the majority of this enzyme is localized to the cytosol, about 1% of the cellular Sod1 is present in the intermembrane space (IMS) of mitochondria. These amounts of mitochondrial Sod1 are increased for certain Sod1 mutants that are linked to the neurodegenerative disease amyotrophic lateral sclerosis (ALS). To date, only little is known about the physiological function of mitochondrial Sod1. Here, we use the model system *Saccharomyces cerevisiae* to generate cells in which Sod1 is exclusively localized to the IMS. We find that IMS-localized Sod1 can functionally substitute wild type Sod1 and that it even exceeds the protective capacity of wild type Sod1 under conditions of mitochondrial ROS stress. Moreover, we demonstrate that upon expression in yeast cells the common ALS-linked mutant $Sod1^{G93A}$ becomes enriched in the mitochondrial fraction and provides an increased protection of cells from mitochondrial oxidative stress. Such an effect cannot be observed for the catalytically inactive mutant $Sod1^{G85R}$. Our observations suggest that the targeting of Sod1 to the mitochondrial IMS provides an increased protection against respiration-derived ROS.

1. Introduction

Reactive oxygen species (ROS) are byproducts of many oxygen-dependent redox reactions. Increased levels of ROS can result in deleterious consequences for the cell including lipid peroxidation, protein carbonylation and DNA damage [1]. Cells contain multiple systems to counteract and degrade ROS. The superoxide anion is a highly reactive ROS that is a product of the transfer of a single electron onto molecular oxygen. It is detoxified by two superoxide dismutases, Sod1 and Sod2. Both proteins use metal cofactors to catalyze the disproportionation of superoxide anions to oxygen and the less toxic hydrogen peroxide (H_2O_2). The manganese-containing Sod2 is located in the mitochondrial matrix, while the copper–zinc enzyme Sod1 is an abundant cytosolic protein. In addition, Sod1 has also been found in the nucleus [2], in lysosomes [3], in the endoplasmic reticulum [4] and more recently in the intermembrane space (IMS) of mitochondria [5,6]. The latter compartment contains approximately 1% of the cellular Sod1 [6]. The import of Sod1 into the IMS has recently been demonstrated to be linked to the mitochondrial disulfide relay machinery [7,8]. The disulfide relay component Mia40 thereby participates in the import of the copper chaperone for Sod1 (Ccs1) which in turn mediates the import, folding and activation of Sod1.

Given the tiny volume of the IMS the concentration of Sod1 in the IMS presumably exceeds that of the cytosol. It has been hypothesized that the mitochondrial fraction of Sod1 protects cellular structures against superoxide radicals that are released into the IMS by the respiratory chain [6]. This is supported by the finding that the knockout of *SOD1* results in increased free radical damage in mitochondria [6,9]. Moreover, in yeast increased cellular amounts of Sod1 lead to prolonged stationary phase survival, which is limited by mitochondrial oxidative stress [6]. However, contrary to these results it also has been suggested that the presence of Sod1 in the IMS might promote the production of damaging free radicals [10]. Thus, the significance of the presence or absence of Sod1 from the IMS remains unclear.

The localization of Sod1 to the IMS has attracted significant attention because certain Sod1 mutants that are linked to the neurodegenerative disease familial amyotrophic lateral sclerosis [11] show an increased association with mitochondria [12]. These Sod1 mutant proteins were reported to accumulate in the IMS, in the matrix and on the outer membrane of mitochondria [13–15]. The increased accumulation of Sod1 mutants with mitochondria leads to a decreased mitochondrial protein import [13] as well as to shifts of the mitochondrial glutathione redox potential and impairments of respiratory chain complexes [16]. These harmful effects of Sod1 mutants are attributed to a still unidentified toxic gain-of-function since many mutants exhibit full enzymatic

Abbreviations: ALS, amyotrophic lateral sclerosis; DNPH, 2,4-dinitrophenylhydrazine; IMS, intermembrane space; NBT, nitro blue tetrazolium; ROS, reactive oxygen species; Sod, superoxide dismutase.

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activity *in vivo* [17] and *in vitro* [18], and have structures like the wild type protein [12]. Due to this wild type-like activity it is conceivable that the accumulation of Sod1 mutants in mitochondrial fractions results in beneficial effects under specific conditions. However, this has not yet been assessed experimentally.

In this work, we aimed to understand the specific physiological roles of Sod1 and Sod1 mutants in mitochondria. To this end, we constructed an exclusively IMS-targeted variant of wild type Sod1 that we expressed in the model system *Saccharomyces cerevisiae*. This Sod1 variant fully complements a *Δsod1* mutant and provides increased protection under conditions of mitochondrial oxidative stress. Moreover, we characterized two ALS-linked Sod1 mutants: $Sod1^{G93A}$ (a mutant that still exhibits activity and shows an increased accumulation in mitochondria) and $Sod1^{G85R}$ (a mutant without activity that does not accumulate in mitochondria) [19,20]. Similar to the situation in patient cells, the $Sod1^{G93A}$ mutant accumulated in mitochondria when expressed in yeast. Surprisingly, this mutant also efficiently protected cells from ROS that are derived from the respiratory chain. Thus, we demonstrate that both a mitochondria-associated ALS-linked Sod1 mutant and IMS-targeted wild type Sod1 are beneficial for cells during mitochondrial oxidative stress.

2. Materials and methods

2.1. Yeast strains and media

For primers and plasmids see Table S1. The *Δsod1* strain was derived from the wild type strain W303 (MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*) by replacement of the *SOD1* open reading frame (ORF) with a *HIS3* cassette. Homologous recombination was verified by PCR and controlled by immunoblotting against Sod1. The *Δsod1* strains expressing Sod1 variants were generated by transformation with the respective plasmids. Strains were grown in S(Lac) medium (0.85% yeast nitrogen base, 2.5% $(NH_4)_2SO_4$, 9.9% lactate, 20 mg/l adenine sulfate, 20 mg/l uracil, 100 mg/l L-leucine, 30 mg/l L-lysine, pH 5.5) with 0.2% galactose at 30 °C. For drop dilution assays 5 μl of tenfold dilutions of 1 OD/ml cells were plated on YPD. To induce oxidative stress plates were supplemented with 4 mM H_2O_2 or 0.5 mM paraquat.

2.2. Antibodies

The following antibodies were used: anti-Cox2 [21], anti-DNP (Sigma–Aldrich), anti-Mrp20 [21], and anti-Pgk1 (Invitrogen). To generate the Sod1 antibody yeast Sod1 was purified and used for immunization of rabbits.

2.3. Preparation of yeast mitochondria; cell fractionation

Wild type and mutant yeast strains were grown to exponential phase in S(Lac) medium, and mitochondria were prepared as described [22].

2.4. Protein carbonylation assay

Sodiumdodecyl sulfate (SDS) was added to isolated mitochondria or whole cell lysate to a concentration of 6% SDS. Samples were reacted with 2,4-dinitrophenylhydrazine (DNPH) (3.96 mg/ml 2,4-dinitrophenylhydrazine, 0.154 g/ml trifluoroacetic acid) for 10 min at 25 °C. After precipitation with trichloroacetic acid the pellets were resuspended in SDS-loading buffer (60 mM Tris/HCl pH 6.8, 10% glycerine, 2% SDS, 0.01% bromophenol blue). Proteins were separated on SDS–polyacrylamide gels, transferred to nitrocellulose membranes, and carbonyl groups were detected by Western blotting with a DNP antibody.

2.5. Amplex red assays

For the measurement of H_2O_2 production 100 μg mitochondria were dissolved in 100 μl Amplex red buffer (0.6 M sorbitol, 50 μM Amplex red, 1 mM EDTA, 2 μl horseradish peroxidase (50 U/ml), 20 mM KP_i pH 8.0; optionally containing 10 mM TEMED or 10 mM paraquat). The reaction was started by addition of 10 mM succinate pH 7.0 and was followed in a Fluoroscan Ascent CF Fluorometer (ThermoLabsystems) at an emission wavelength of 587 nm (excitation wavelength of 563 nm).

3. Results

3.1. IMS-localized Sod1 improves viability during respiratory chain-derived ROS stress

To understand the specific physiological role of Sod1 in mitochondria, we generated yeast cells in which Sod1 is exclusively localized to the IMS. To this end, a *SOD1* deletion strain was transformed with either an empty vector (Δ*sod1*), a plasmid encoding for wild type yeast Sod1 (Sod1), or an IMS-targeted yeast Sod1 variant, respectively (Fig. 1A). The latter plasmid encoded an extended bipartite mitochondrial targeting signal of cytochrome b_2 fused to wild type Sod1 (b_2-Sod1). This bipartite targeting signal consists of a matrix-targeting signal followed by a hydrophobic sorting sequence ensuring transfer into the inner membrane, and an additional IMS-localized domain that prevents accidental matrix import. After import into mitochondria a part of the bipartite targeting signal is removed and the mature b_2-Sod1 protein localizes to the IMS.

In a first set of experiments we verified the expression, localization and activity of the different Sod1 variants. As expected no Sod1 was detected in Western blots of lysates from the *Δsod1* strain, while the strains expressing the Sod1 and the b_2-Sod1 variants contained proteins that migrated at sizes of approximately 20 and 30 kDa, respectively (Fig. S1). Next, we analyzed these strains by a cell fractionation approach. We separated cells into cytosolic and mitochondrial fractions and demonstrated by Western blots the dual localization of wild type Sod1 (Fig. 1B). We thereby confirmed previous findings that around 1% of total endogenous Sod1 localize to the IMS (see Fig. 3C and [6]). Notably, b_2-Sod1 was exclusively localized to the mitochondrial fraction. b_2-Sod1 as well as Sod1 also exhibited Sod activity in cell extracts and mitochondrial fractions (Fig. S2). The b_2-Sod1 activity was considerably enriched in the latter fraction due to its exclusive presence in mitochondria. Taken together, the expressed b_2-Sod1 protein is functional and exclusively localized to mitochondria.

We next asked whether this exclusively IMS-localized Sod1 can functionally replace wild type Sod1 (Fig. 1C). To this end, we spotted cells grown to log phase on full medium and assessed their growth. In the absence of oxygen all strains exhibited a similar growth. Conversely, under aerobic conditions the Δ*sod1* strain containing an empty plasmid exhibited a strongly reduced growth, while the expression of Sod1 or b_2-Sod1 suppressed the Δ*sod1* defect. However, the strain expressing Sod1 grew better than the strain expressing b_2-Sod1. This suggests that the cytosol-localized fraction of Sod1 provides an improved ROS protection under these conditions. We next tested whether b_2-Sod1 can replace wild type Sod1 also under conditions of oxidative stress. To this end, we exposed cells to either of two ROS-producing reagents, H_2O_2 or paraquat (Fig. 1C). While H_2O_2 leads to amplified ROS levels in the whole cell, paraquat induces ROS production through mitochondrial NADPH dehydrogenases and at the level of complex III of the respiratory chain [23–25] (see also Figs. S3 and S6). As expected all strains exhibited a diminished growth upon exposure

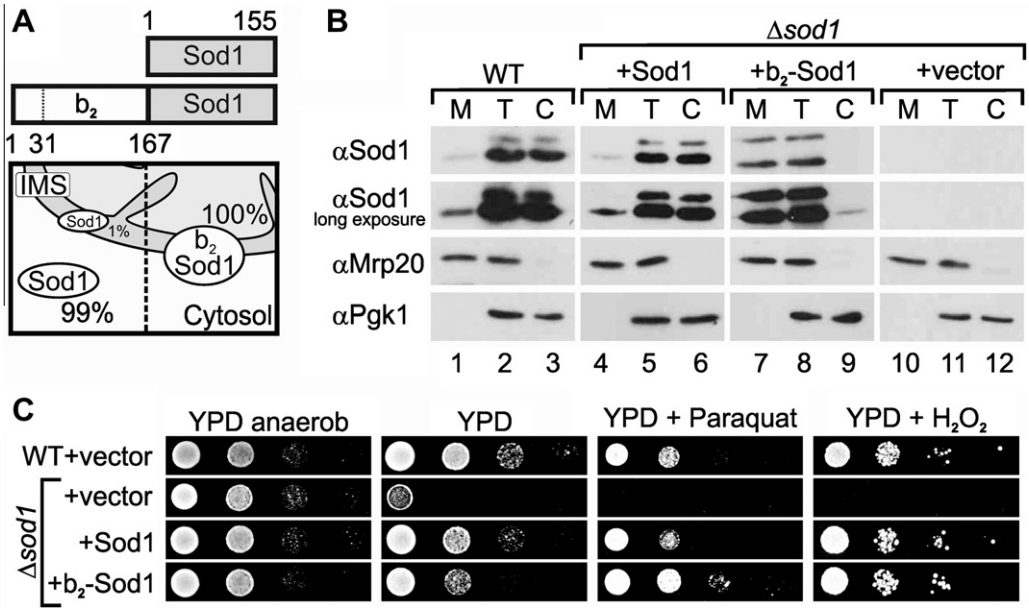

Fig. 1. Mitochondrial Sod1 improves viability under conditions of mitochondrial ROS stress. (A) Scheme of the Sod1 constructs used in this study. For efficient targeting to the IMS the N-terminal 167 amino acids of cytochrome b_2 were fused to Sod1. The resulting b_2-Sod1 (mature protein starting from residue 31, dashed line) is exclusively present in the IMS. (B) Subcellular localization of Sod1. Wild type (W303) as well as Δ*sod1* yeast cells expressing wild type or b_2-Sod1 were grown to mid-log phase in S(Lac) medium. Cells were lysed and fractionated into post-mitochondrial supernatant (C) and mitochondria (M). Fractions were analyzed by SDS–PAGE and immunoblotting using antibodies directed against Sod1, Mrp20 (mitochondrial marker) and Pgk1 (cytosolic marker). M, mitochondria; C, post-mitochondrial supernatant; T total. (C) The indicated strains were grown to log phase. Tenfold serial dilutions were spotted on plates containing glucose as carbon source and incubated at 30 °C for 2–4 days. Plates were incubated either in the presence or absence of oxygen, or in the presence or absence of either 4 mM H_2O_2 or 0.5 mM paraquat. The results in (C) are representative for three independent experiments.

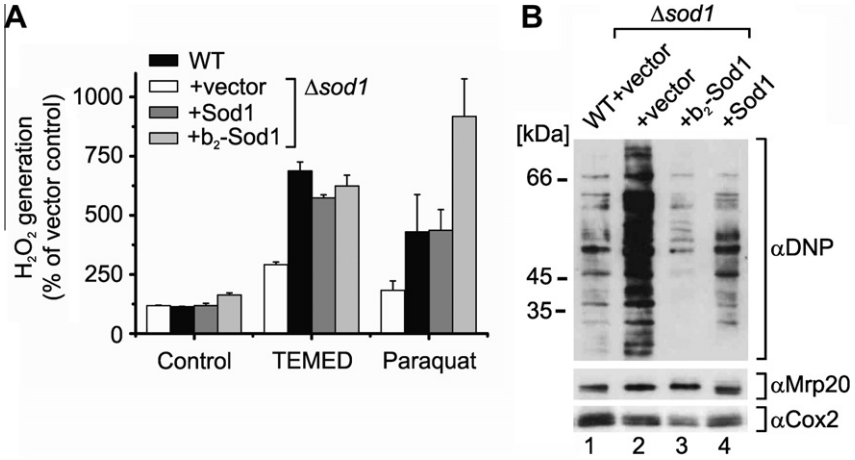

Fig. 2. Mitochondria-targeted Sod1 is able to counteract oxidative damage to mitochondrial proteins. (A) Mitochondria isolated from the indicated strains were subjected to an amplex red assay to detect the generation of H_2O_2. Mitochondria were either left untreated or preincubated with 10 mM paraquat or 10 mM TEMED. The data are represented as percentage of the vector control. (B) Mitochondria isolated from the indicated cells were incubated with DNPH. DNPH-reactive carbonyl groups were detected by immunoblotting with a DNP antibody. Equal loading was confirmed by immunoblotting against Mrp20 and Cox2. The results in (A) and (B) are representative for three independent experiments.

to oxidative stress when compared with unstressed conditions. Again the expression of wild type Sod1 as well as b_2-Sod1 conferred viability to the respective strains. Cells expressing the dually localized wild type Sod1 exhibited a better growth upon exposure to general oxidative stress. Surprisingly however, b_2-Sod1 protected cells better from mitochondrial ROS than Sod1 (Fig. 1C). Taken together, we find that b_2-Sod1 can replace wild type Sod1. Noteworthy, b_2-Sod1 even provides increased protection of cells against mitochondrial oxidative stress. Mitochondria-localized Sod1, however, is apparently less protective against oxidative stress that affects the whole cell.

3.2. IMS-localized Sod1 provides increased protection of proteins from carbonylation by respiratory chain-derived ROS

Next, we aimed to analyze the impact of the different Sod1 variants on the production of H_2O_2 and the amount of carbonylated proteins. H_2O_2 production is an indicator for the detoxifying activity of Sod1, and protein carbonylation represents a marker for the cellular damage caused by ROS. We applied a fluorescence-based assay to determine the H_2O_2 production in mitochondria isolated from the different strains (Fig. 2A). Under unstressed conditions the H_2O_2 production was generally very low, and only slightly

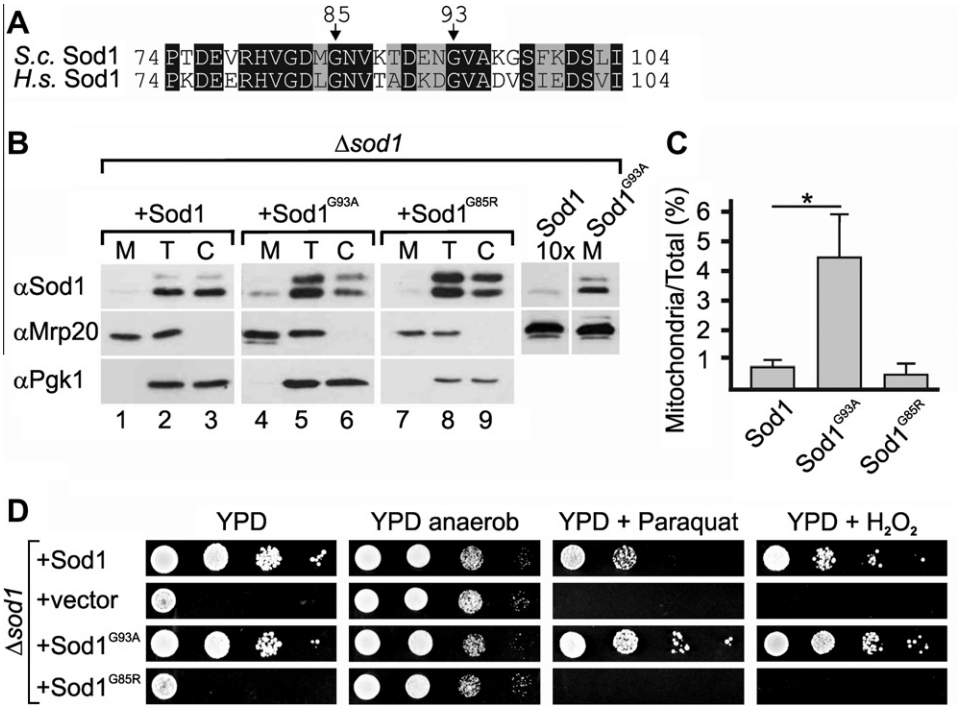

Fig. 3. Sod1^{G93A} is enriched in the mitochondrial fraction compared to wild type Sod1 and improves viability under conditions of mitochondrial ROS stress. (A) Alignment of human and yeast Sod1. The positions of the glycin residues mutated in the G93A and the G85R mutations are indicated. (B) Subcellular localization of Sod1. Δ*sod1* yeast cells expressing wild type, Sod1^{G93A} or Sod1^{G85R} were grown to mid-log phase in S(Lac) medium. Cells were lysed and fractionated into total (T), post-mitochondrial supernatant (C) and mitochondria (M), and fractions were analyzed by SDS–PAGE and immunoblotting using antibodies directed against Sod1, Mrp20 (mitochondrial marker) and Pgk1 (cytosolic marker). (C) Quantification of three independent experiments as described in (B). (D) The indicated strains were grown to log phase. Tenfold serial dilutions were spotted on plates containing glucose as carbon source and incubated at 30 °C for 2–4 days. Plates were incubated either in the presence or absence of oxygen, or in the presence or absence of either 4 mM H_2O_2 or 0.5 mM paraquat. The results in (D) are representative for three independent experiments.

increased in mitochondria from the b_2-Sod1 strain. However, upon application of the ROS-producing agents TEMED or paraquat we observed an increased release of H_2O_2. In this case, the generation of H_2O_2 was mainly dependent on Sod1 since mitochondria isolated from the Δ*sod1* strain hardly increased their H_2O_2 production (Fig. 2A, compare WT with Δ*sod1*). The residual H_2O_2 production is presumably due to the activity of Sod2 in the mitochondrial matrix. During incubation with TEMED mitochondria containing Sod1 and b_2-Sod1 released similar amounts of H_2O_2. Interestingly, upon application of paraquat mitochondria containing b_2-Sod1 exhibited a strongly increased release of H_2O_2 compared to mitochondria isolated from strains expressing wild type Sod1. This is consistent with an improved accessibility of b_2-Sod1 to mitochondria-generated ROS.

Sustained production of superoxide anions leads to oxidative damage of proteins that can be monitored by the detection of protein carbonylation. We therefore determined the amount of protein carbonylation in mitochondrial fractions using a dinitrophenylhydrazine (DNPH) modification assay (Fig. 2B). DNPH adds to sites of carbonylation in proteins and can subsequently be detected using an anti-DNP antibody. Hereby, we found that carbonylated proteins accumulated in the absence of Sod1. This suggests that proteins in mitochondrial fractions are especially sensitive to the lack of Sod1 presumably due to their proximity to the respiratory chain. The expression of either of the Sod1 variants diminished the level of protein carbonylation to wild type levels. Apparently, the mitochondrially localized Sod1 protected mitochondrial proteins from carbonylation even better than wild type Sod1 presumably by providing a higher local Sod activity close to the respiratory chain. This observation is consistent with an improved antioxidative capacity of b_2-Sod1 towards mitochondrial oxidative stress.

3.3. The ALS-associated mutant Sod1^{G93A} is enriched in mitochondria and provides increased protection from mitochondrial ROS

Some ALS-linked Sod1 mutants were shown to accumulate in mitochondria (*e.g.* by immunogold electron microscopy). We therefore asked whether these variants would exert a similar protective effect as b_2-Sod1. To test this we generated two strains expressing mutants of yeast Sod1 that correspond to the G93A and G85R mutations in human Sod1 (Fig. 3A). First, we compared the expression levels of both Sod1 mutants with that of wild type Sod1, and found for all three Sod variants similar protein levels (Fig. S4). The distribution of Sod1^{G93A} significantly shifted towards the mitochondrial fraction compared to wild type Sod1 (4.4% of total Sod1^{G93A} in the mitochondrial fraction compared to 0.7% in the case of Sod1), while the distribution of Sod1^{G85R} remained unchanged (Fig. 3B and C). Moreover, we confirmed that Sod1^{G85R} is inactive whereas Sod1^{G93A} is active (Fig. S5). However, despite similar protein levels in whole cells the activity of the Sod1^{G93A} mutant is diminished compared to the wild type protein indicating a decreased specific activity of Sod1^{G93A}. Notably, in line with the mitochondrial accumulation of the Sod1^{G93A} protein the activity of wild type Sod1 and Sod1^{G93A} in mitochondria is similar (Fig. S5). Taken together, the data support that like b_2-Sod1 Sod1^{G93A} is enriched in mitochondrial fractions.

Next, we tested whether these Sod1 mutants can functionally replace Sod1 (Fig. 3D). As expected the strain expressing the non-functional Sod1^{G85R} variant grew like the *SOD1* deletion strain under all conditions tested. Conversely, the Sod1^{G93A} variant complemented wild type Sod1 (Fig. 3D). Interestingly, under conditions of mitochondrial oxidative stress Sod1^{G93A} grew better than wild type resembling the phenotype of the strains expressing IMS-localized wild type Sod1 (Figs. 3D and S6).

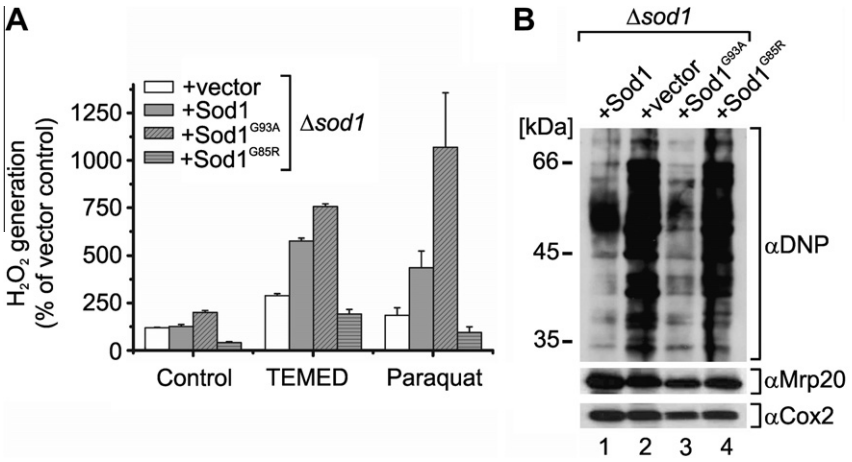

Fig. 4. Mitochondria isolated from the Sod1^{G93A} strain exhibit an increased Sod activity. (A) Mitochondria isolated from the indicated strains were subjected to an amplex red assay to detect the generation of H_2O_2. Mitochondria were either left untreated or preincubated with either 10 mM paraquat or 10 mM TEMED. (B) Mitochondria isolated from the indicated cells were reacted with DNPH. DNPH-reactive carbonyl groups were detected by immunoblotting with a DNP antibody. Equal loading was confirmed by immunoblotting against Mrp20 and Cox2. The results depicted in (A) and (B) are representative for three independent experiments.

3.4. Sod1^{G93A} provides increased protection of proteins from carbonylation

The H_2O_2 release from mitochondria of Sod1^{G85R}-expressing strains resembled the release from mitochondria isolated from the *SOD1* deletion strain (Fig. 4A). Conversely, mitochondria isolated from the Sod1^{G93A} strain released similar amounts of H_2O_2 as b_2-Sod1-containing mitochondria (compare Fig. 4A and Fig. 2A). Moreover, these correlations were confirmed by our analysis of the level of protein carbonylation in mitochondria (Fig. 4B). Taken together, we find that Sod1^{G93A} endows cells with a similarly increased antioxidative capacity against mitochondria-derived ROS as b_2-Sod1, a finding that can be explained by the enrichment of the Sod1 mutant in the mitochondrial fraction (Fig. 3B and C).

4. Discussion

The antioxidative enzyme Sod1 is distributed between two cellular compartments, the cytosol and the mitochondrial IMS. The relevance of the mitochondrial fraction for cellular functionality remained unclear, in particular since only a rather minute fraction of the enzyme is located in mitochondria and since the mitochondrial matrix harbours a second superoxide dismutase enzyme (Sod2) which overlaps with Sod1 in its role in oxidative stress defence [26,27]. In this study we focused on the capacity of IMS-localized Sod1 to combat oxidative damage. Our results demonstrate that, even if the entire cytoplasmic fraction was shifted into the IMS, Sod1 can still efficiently counteract the deleterious effects of oxidative agents. Surprisingly, this re-direction of Sod1 even provided increased cellular resistance against mitochondria-derived ROS. Such an improved stress resistance was also observed for the ALS-linked Sod1^{G93A} mutant that accumulated in mitochondria.

In fungi as in many animal cells, the respiratory chain of mitochondria is the predominant source of ROS [1]. Oxygen radicals are thereby released to both sides of the inner membrane. In the matrix, Sod2, peroxiredoxins and other enzymes degrade these radicals [1]. Our results suggest that Sod1 in the IMS plays a major role in the oxidative stress defence of eukaryotic cells. Expression of the b_2-Sod1 protein almost completely complemented the *Δsod1* phenotype on glucose-containing medium. Under these conditions, yeast cells produce their energy by fermentation. It therefore appears unlikely that differences in the damaging of mitochondrial components account for the observed growth defects. It seems more likely that the IMS-located Sod1 can efficiently reduce the ROS burden not only within the mitochondria but also in extra-mitochondrial compartments of the cell. However, the exclusive presence of Sod1 in mitochondria is not sufficient to fully complement wild type Sod1 under all conditions. The decreased viability of the *SOD1* deletion strain might therefore result from cumulative effects caused by the diminished antioxidative capacity in different compartments implying that a detoxification of ROS close to the site of their generation is crucial for the cell. Our data hence indicate the importance of dually localized Sod1 for growth under conditions of general cellular oxidative stress.

The suitability of yeast as a model for the understanding of the neurological pathology in ALS patients might be limited since ALS patients show highly specific defects in motor neurons that manifest typically only after forty or fifty years of life. Hence, even in affected patients most cells obviously are not or only little affected by the Sod1 mutations. However, yeast cells might represent a valuable model system to analyze the mechanisms by which Sod1 is distributed between mitochondria and the cytosol because our cell fractionation experiments with a yeast Sod1^{G93A} model showed increased amounts of the protein in mitochondria. Also the mammalian Sod1^{G93A} exhibits such an increased accumulation with mitochondria and this was associated with the neurological defects observed in ALS patients and mouse models for ALS [13–15]. It will thus be exciting to explore the mechanisms of distribution of Sod1 between cytosol and mitochondria in future experiments in yeast.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.10.129.

References

[1] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417 (2009) 1–13.
[2] L.Y. Chang, J.W. Slot, H.J. Geuze, J.D. Crapo, Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes, J. Cell Biol. 107 (1988) 2169–2179.
[3] W. Liou, L.Y. Chang, H.J. Geuze, G.J. Strous, J.D. Crapo, J.W. Slot, Distribution of CuZn superoxide dismutase in rat liver, Free Radic. Biol. Med. 14 (1993) 201–207.
[4] M. Urushitani, A. Sik, T. Sakurai, N. Nukina, R. Takahashi, J.P. Julien, Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis, Nat. Neurosci. 9 (2006) 108–118.
[5] A. Okado-Matsumoto, I. Fridovich, Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu, Zn-SOD in mitochondria, J. Biol. Chem. 276 (2001) 38388–38393.
[6] L.A. Sturtz, K. Diekert, L.T. Jensen, R. Lill, V.C. Culotta, A fraction of yeast Cu, Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage, J. Biol. Chem. 276 (2001) 38084–38089.
[7] H. Kawamata, G. Manfredi, Different regulation of wild-type and mutant Cu, Zn superoxide dismutase localization in mammalian mitochondria, Hum. Mol. Genet. 17 (2008) 3303–3317.
[8] S. Reddehase, B. Grumbt, W. Neupert, K. Hell, The disulfide relay system of mitochondria is required for the biogenesis of mitochondrial Ccs1 and Sod1, J. Mol. Biol. 385 (2009) 331–338.
[9] S. Elchuri, T.D. Oberley, W. Qi, R.S. Eisenstein, L. Jackson Roberts, H. Van Remmen, C.J. Epstein, T.T. Huang, CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life, Oncogene 24 (2005) 367–380.
[10] G. Goldsteins, V. Keksa-Goldsteine, T. Ahtoniemi, M. Jaronen, E. Arens, K. Akerman, P.H. Chan, J. Koistinaho, Deleterious role of superoxide dismutase in the mitochondrial intermembrane space, J. Biol. Chem. 283 (2008) 8446–8452.
[11] D.R. Rosen, T. Siddique, D. Patterson, D.A. Figlewicz, P. Sapp, A. Hentati, D. Donaldson, J. Goto, J.P. O'Regan, H.X. Deng, et al., Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, Nature 362 (1993) 59–62.
[12] J.S. Valentine, P.A. Doucette, S. Zittin Potter, Copper–zinc superoxide dismutase and amyotrophic lateral sclerosis, Annu. Rev. Biochem. 74 (2005) 563–593.
[13] J. Liu, C. Lillo, P.A. Jonsson, C. Vande Velde, C.M. Ward, T.M. Miller, J.R. Subramaniam, J.D. Rothstein, S. Marklund, P.M. Andersen, T. Brannstrom, O. Gredal, P.C. Wong, D.S. Williams, D.W. Cleveland, Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria, Neuron 43 (2004) 5–17.
[14] P. Pasinelli, M.E. Belford, N. Lennon, B.J. Bacskai, B.T. Hyman, D. Trotti, R.H. Brown Jr., Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria, Neuron 43 (2004) 19–30.
[15] C. Vande Velde, T.M. Miller, N.R. Cashman, D.W. Cleveland, Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria, Proc. Natl. Acad. Sci. USA 105 (2008) 4022–4027.
[16] A. Ferri, M. Cozzolino, C. Crosio, M. Nencini, A. Casciati, E.B. Gralla, G. Rotilio, J.S. Valentine, M.T. Carri, Familial ALS-superoxide dismutases associate with mitochondria and shift their redox potentials, Proc. Natl. Acad. Sci. USA 103 (2006) 13860–13865.
[17] A.C. Bowling, E.E. Barkowski, D. McKenna-Yasek, P. Sapp, H.R. Horvitz, M.F. Beal, R.H. Brown Jr., Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis, J. Neurochem. 64 (1995) 2366–2369.
[18] L.J. Hayward, J.A. Rodriguez, J.W. Kim, A. Tiwari, J.J. Goto, D.E. Cabelli, J.S. Valentine, R.H. Brown Jr., Decreased metallation and activity in subsets of mutant superoxide dismutases associated with familial amyotrophic lateral sclerosis, J. Biol. Chem. 277 (2002) 15923–15931.
[19] R. Rakhit, A. Chakrabartty, Structure, folding, and misfolding of Cu, Zn superoxide dismutase in amyotrophic lateral sclerosis, Biochim. Biophys. Acta 1762 (2006) 1025–1037.
[20] D. Bergemalm, P.A. Jonsson, K.S. Graffmo, P.M. Andersen, T. Brannstrom, A. Rehnmark, S.L. Marklund, Overloading of stable and exclusion of unstable human superoxide dismutase-1 variants in mitochondria of murine amyotrophic lateral sclerosis models, J. Neurosci. 26 (2006) 4147–4154.
[21] S. Gruschke, K. Grone, M. Heublein, S. Holz, L. Israel, A. Imhof, J.M. Herrmann, M. Ott, Proteins at the polypeptide tunnel exit of the yeast mitochondrial ribosome, J. Biol. Chem. 285 (2010) 19022–19028.
[22] K. Altmann, M. Dürr, B. Westermann, *Saccharomyces cerevisiae* as a model organism to study mitochondrial biology, in: D. Leister, J.M. Herrmann (Eds.), Mitochondria. Practical Protocols, Humana Press, Totowa, New Jersey, 2007, pp. 81–90.
[23] P.R. Castello, D.A. Drechsel, M. Patel, Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain, J. Biol. Chem. 282 (2007) 14186–14193.
[24] D.A. Drechsel, M. Patel, Differential contribution of the mitochondrial respiratory chain complexes to reactive oxygen species production by redox cycling agents implicated in parkinsonism, Toxicol. Sci. 112 (2009) 427–434.
[25] H.M. Cocheme, M.P. Murphy, Complex I is the major site of mitochondrial superoxide production by paraquat, J. Biol. Chem. 283 (2008) 1786–1798.
[26] A.R. Reddi, L.T. Jensen, A. Naranuntarat, L. Rosenfeld, E. Leung, R. Shah, V.C. Culotta, The overlapping roles of manganese and Cu/Zn SOD in oxidative stress protection, Free Radic. Biol. Med. 46 (2009) 154–162.
[27] K.M. O'Brien, R. Dirmeier, M. Engle, R.O. Poyton, Mitochondrial protein oxidation in yeast mutants lacking manganese-(MnSOD) or copper- and zinc-containing superoxide dismutase (CuZnSOD): evidence that MnSOD and CuZnSOD have both unique and overlapping functions in protecting mitochondrial proteins from oxidative damage, J. Biol. Chem. 279 (2004) 51817–51827.