Induction of endoplasmic reticulum stress by human cytosolic superoxide dismutase 1 in yeast cells

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ABSTRACT

Accumulation of unfolded proteins in the endoplasmic reticulum (ER) leads to ER stress, which triggers a transcriptome shift known as the unfolded protein response (UPR) commonly in eukaryotic species. Nevertheless, in some neurodegenerative diseases, the UPR is somehow induced by aggregation of cytosolic pathogenic proteins. To address the UPR induction mechanism by human superoxide dismutase 1 (hSOD1), the aggregation of which causes amyotrophic lateral sclerosis, here we used yeast *Saccharomyces cerevisiae* as a simple model organism. Expression of hSOD1 or its mutant increased UPR levels in *S. cerevisiae* cells carrying a knockout mutation of the *STE24* gene, which encodes an ER-located membrane-integral protein that digests cytosolic proteins aberrantly translocated to the ER. Our findings cumulatively indicate that SOD1 induces ER stress and UPR by disturbing protein and lipid-membrane homeostasis in and on the ER.

KEYWORDS

Neurodegenerative diseases, Unfolded protein response, *Saccharomyces cerevisiae*

INTRODCTION

The endoplasmic reticulum (ER) is a membrane-enclosed cellular compartment in which secretory and transmembrane proteins are folded and modified. Proteins carrying a hydrophobic ER translocation signal, namely, ER client proteins, are transported to the ER through the Sec61 translocon. After being correctly folded, these proteins exit the ER and are exported to the cell surface or other cellular compartments via the Golgi apparatus. Moreover, a wide variety of lipid molecules are biosynthesized on the ER membrane. Dysfunction of the ER is called ER stress and is frequently accompanied by the accumulation of unfolded proteins in the ER. In response to ER stress, eukaryotic species commonly provoke unfolded protein response (UPR).

The molecular mechanism that triggers UPR was initially uncovered through frontier studies using the yeast *Saccharomyces cerevisiae* as a model organism (Ishiwata-Kimata and Kimata, 2023). Ire1 is an ER-resident transmembrane protein that acts as an ER-stress sensor. In response to ER stress, Ire1 self-associates and is activated as an endoribonuclease to promote the splicing of *HAC1* mRNA. While the unspliced form of *HAC1* mRNA is poorly translated, the spliced form of *HAC1* mRNA is effectively translated into the nuclear transcription factor Hac1, which induces various genes, including those encoding proteins that function in and/or for the ER.

The intracellular signaling pathways for UPR are more complicated in mammalian cells (Mori, 2009). Similar to yeasts, ER-stressed mammalian cells perform the cytoprotective UPR to induce genes for alleviation of ER stress. However, it is also widely accepted that in mammalian cells, chronical ER stress induce apoptosis through multiple signaling pathways in which Ire1 is partly involved (Chen and Brandizzi, 2013; Hetz and Papa, 2018). Although this phenomenon, namely the proapoptotic UPR, contributes to the elimination of irreversibly damaged cells and thus may be physiologically beneficial, it also causes aggravation of symptoms in various human pathogenic conditions (Hetz and Papa, 2018; Walter et al., 2025).

Aberrant ER client proteins that accumulate in the ER induce UPR through multiple mechanisms. The luminal domain of Ire1 directly recognizes unfolded proteins accumulated in the ER lumen, leading to the self-association and activation of Ire1 (Kimata et al., 2007; Promlek et al., 2011; Gardner and Walter, 2011). On the other hand, the transmembrane domain of Ire1 is responsible for its activation in response to membrane lipid abnormalities, also known as lipid bilayer stress (LBS) (Halbleib et al., 2017). We previously reported that a misfolded transmembrane protein model that aggregates on the ER membrane activates Ire1 by inducing LBS in *S. cerevisiae* cells

(Phuong et al., 2023). However, through undetermined mechanisms, misfolded cytosolic or nuclear proteins also occasionally induce UPR, for instance, in some neurodegenerative diseases (Kim et al., 2022).

ER-associated protein degradation (ERAD) is also an evolutionally conserved cellular strategy for coping with misfolded proteins that accumulate in the ER (Wu and Rapoport, 2018). When not properly folded, ER client proteins are retrogradely translocated to the cytosol and are degraded by the proteasome. This event, known as ERAD, is tightly linked to UPR (Hwang and Qi, 2018). Even without external stress stimuli, UPR is induced in ERAD-deficient mutant cells, probably because ERAD contributes to the clearance of endogenously generated misfolded proteins that accumulate in the ER.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by selective death of motor neurons, which results in progressive muscle atrophy. Superoxide dismutase 1 (SOD1) is a cytosolic protein, mutations of which, such as the glycine93-to-alanine (G93A) missense mutation, are known to be a risk factor for developing ALS. It is today widely accepted that a prominent cause of ALS is the accumulation of conformationally aberrant wild-type or mutant SOD1 in motor neurons (Hayashi et al., 2016; Wang et al., 2024). When abnormally accumulated, SOD1 triggers UPR, which may induce apoptosis and lead to disease progression.

How does SOD1 accumulation damage the ER and induce UPR? According to the previous reports by others (Nishitoh et al., 2008; Tsuburaya et al., 2018), mutant SOD1 is associated with an ERAD component, Derlin-1, resulting in the inhibition of ERAD. As an alternative scenario, mutant SOD1 is reported to be partly carried to the secretory pathway, which starts from the ER, in animal cells, although it does not carry an apparent ER-translocation signal (Urushitani et al., 2008). It may also be possible that cytosolic accumulation of SOD1 affects ER membrane integrity to induce LBS.

S. cerevisiae has historically and widely been used as a valuable tool to explore biological phenomena observed in other eukaryotic species. Many cellular processes related to neurodegenerative diseases have been reproduced in *S. cerevisiae* for deep understanding of the pathogenic mechanisms (Liguori et al., 2021; Epremyan et al., 2023). We previously reported that human hSOD1 (hSOD1) is slightly translocated into the ER through the Sec61 translocon in *S. cerevisiae* carrying a knockout mutation of *STE24*, which encodes an ER-located membrane-integral protease (Hosomi et al., 2023). In this study, we thus investigated the relationship between UPR and SOD1 by heterogeneously expressing hSOD1 in *S. cerevisiae* cells.

MATERIALS AND METHODS

S. cerevisiae strains and plasmids

S. cerevisiae strains and plasmids used in this study are listed in Tables 1 and 2.

BY4741 (alias Y00000; *MAT***a** *leu2Δ0 ura3Δ0 his3Δ1 met15Δ0*) is a laboratory standard *S. cerevisiae* strain (Brachmann et al., 1998). Y06920 is an *STE24* knockout (*ste24Δ*) derivative of BY4741, in which the genomic *STE24* coding sequence (CDS) was replaced to the *KanMX4* selectable marker (Giaever and Nislow, 2014). BY4741 and Y06920 were obtained from EUROSCARF (http://www.euroscarf.de/). In this study, we also used an *ire1Δ* strain KMY1516 (*MATα leu2-3,112::*3XUPRE-GFP*::LEU2 ura3-52 his3Δ200 trp1Δ901 lys2-801::*3XUPRE-lacZ*::LYS2 ire1::TRP1*; Kimata et al., 2004) and its derivatives. In our previous study (Promlek et al., 2011), the *STE24*-knockout DNA module (5'-untranslated region (5'-UTR) of *STE24*–*KanMX4*–3'-UTR of *STE24*) was PCR-amplified from the genome DNA of Y06920 and was used for transformation of KMY1516 to obtain its *ste24Δ* derivative, KMY1516-ste24Δ.

The YCp-type plasmids pRS313, pRS315, and pRS316 contain the *HIS3*, *LEU2*, and *URA3* selectable markers, respectively (Sikorski and Hieter, 1989). The yeast YEp-type plasmid pRS426 contains the *URA3* selectable marker (Christianson et al., 1992).

We previously inserted the *IRE1* gene (5'-UTR–CDS–3'-UTR of *IRE1*) into pRS313 to obtain the plasmid pRS313-IRE1 (Kimata et al., 2004). A partial deletion mutation (ΔIII; deletion of amino-acid residues 253–272) and a point mutation (corresponding to the amino-acid replacement of valine535 to arginine (V535R)) were introduced into the *IRE1* gene on pRS313-IRE1 using the overlap PCR and *in vivo* gap-repair techniques, as described in Kimata et al. (2004).

As described in Hosomi et al. (2023), here we employed a C-terminally short peptide (epitope)-tagged version of hSOD1. A DNA fragment composed of the *S. cerevisiae SOD1* promoter, hSOD1 CDS, and *S. cerevisiae ACT1* terminator was previously inserted into pRS315, and the resulting plasmid was named pRS315-ScSOD1pro-HsSOD1-V5-3xsequon (Hosomi et al., 2023). In the present study, this DNA fragment was transferred to pRS426 to yield the plasmid pRS426-hSOD1. The mutant form of pRS426-hSOD1, namely pRS426-hSOD1-G93A, were similarly constructed from a mutant form of pRS315-ScSOD1pro-HsSOD1-V5-3xsequon carrying the G93A mutation on its hSOD1-coding sequence.

In this study, pRS426-hSOD1 and pRS426-hSOD1-G93A were used to express wild-type and G93A SOD1, respectively, and pRS316 was used as the empty vector control.

S. cerevisiae culturing and gene manipulation

Synthetic dextrose (SD) medium contained 2% glucose, 0.66% Difco yeast nitrogen base without amino acids (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and appropriate auxotrophic requirements. Cells were cultured at 30 °C by aerobically shaking in SD medium, and exponentially growing cells were harvested.

Transformation of *S. cerevisiae* cells were performed as described in Adams et al. (1997).

β-Galactosidase assay

In accordance with Adams et al. (1997), cells harvested from 1mL of cultures were suspended in 1.0 mL of Z buffer (100 mM sodium phosphate buffer (pH 7.0) containing 10 mM KCl, 1 mM Mg_2SO_4, and 0.27 v/v% β-mercaptoethanol). After the addition of 50 μL of chloroform and 20 μL of 0.1% sodium dodecyl sulfate to the cell suspensions, the cells were disrupted by vortex agitation (top-speed, 10 s). The β-galactosidase reaction was performed by adding 200 μL of the substrate solution (4 mg/mL *o*-nitrophenyl-β-galactoside in Z buffer) to 800 μL of the resulting cell lysates, which were further incubated at 28 °C for appropriate durations. The reaction was terminated by adding 500 μL of 500 mM Na_2CO_3 to the reaction mixtures, which were then clarified by brief centrifugation and monitored for optical density at 420 nm (OD_{420}).

The β-galactosidase activity was calculated by the following formula:
(OD_{420} of the final reaction mixture (concentration of the reaction product *o*-nitrophenol))
/(OD_{600} of the initial cell suspensions (cell density) X Reaction time (min))

RNA analysis

In accordance with Le et al. (2021), total RNA was extracted using the hot phenol method and subjected to the poly T-primed reverse transcription. The resulting cDNA samples were subjected to PCR using a primer set to amplify the *HAC1* species (TACAGGGATTTCCAGAGCACG (forward; hybridized to the *HAC1* exon 1) and TGAAGTGATGAAGAAATCATTCAATTC (reverse; hybridized to the *HAC1* exon 2), as described in Le et al. (2021). Since, through the RT-PCR, the unspliced and spliced *HAC1* mRNA variants yielded different-sized products, they were separated by 2% agarose electrophoresis. The fluorescence band intensity of the ethidium bromide (EtBr)-stained gels was quantified to calculate *HAC1* mRNA splicing efficiency using the following formula: 100 × [band intensity of spliced form/(band intensity of spliced form + band

intensity of unspliced form)]. XL-DNA ladder 100 (Apro Science, Tokushima, Japan) was used as a molecular weight marker for the agarose-gel electrophoresis.

Statistical analysis

The results from three independent clones of the same genotype are expressed as averages and standard deviations. Student's two-tailed unpaired t-tests were performed using Microsoft Excel to obtain p-values.

RESULTS

As described in the Materials and Methods section, here we constructed and used multi-copy YEp plasmids for the strong expression of wild-type and G93A mutant hSOD1. This is because, in our preliminary study, YCp-based low-copy plasmids carrying the same hSOD1-expression modules did not appear to induce UPR under the conditions employed in this study (data not shown).

In the experiment shown in Fig. 1A, we assessed the cellular UPR levels by monitoring the *HAC1*-mRNA splicing. Transformation of wild-type *IRE1+* cells with the hSOD1 expression plasmids was unlikely to induce the *HAC1*-mRNA splicing (Fig. 1A; Lanes 1, 2, and 3). We previously reported that the *ste24Δ* mutation weakly induces UPR even in the absence of external stress stimuli (Promlek et al., 2011). In agreement with this insight, the *HAC1*-mRNA splicing was stimulated, albeit only slightly, by the *ste24Δ* mutation, even without the hSOD1-expression plasmids (Fig. 1A; compare lane 4 to 1). Moreover and importantly, when cells carried the *ste24Δ* mutation, the *HAC1*-mRNA splicing was further induced by the wild-type or G93A hSOD1-expression plasmid (Fig. 1A; Lanes 4, 5, and 6).

This observation was confirmed by using another UPR monitoring method. The UPR element (UPRE) is a short nucleotide sequence to which Hac1 binds for downstream gene induction of UPR. Because the *ire1Δ* strain KMY1516 carried a fusion of the UPRE-containing promoter sequence and bacterial *lacZ* CDS, namely the UPRE-lacZ reporter, on its genome, the UPR level was monitored by measuring cellular β-galactosidase activity (Promlek et al., 2011). We previously noted that the expression level of Ire1 from the low-copy YCp-type *IRE1* plasmid pRS313-IRE1 is not largely different from that from the chromosomal *IRE1* gene (Kimata et al., 2007). Therefore, we hereafter used the *ire1Δ* strain KMY1516 carrying pRS313-IRE1 as an *IRE1+* strain.

The results from the UPRE-lacZ reporter assay shown in Fig. 1B and C is appeared to be consistent with those from the *HAC1* mRNA-splicing assay shown in Fig 1A. The UPRE-lacZ reporter did not seem to be induced by the hSOD1-expression plasmids in wild-type *IRE1+* cells (Fig. 1B). The induction of UPR by the *ste24Δ* mutation was also demonstrated by the UPRE-lacZ reporter assay (compare the leftmost columns of Fig. 1B and C). Fig. 1C shows the increase in the UPR levels by the hSOD1-expression plasmids in *ste24Δ* cells. According to our observations shown in Fig. 1A and C, wild-type SOD1 and G93A SOD1 induced similar levels of UPR.

As described in the Introduction section, we previously proposed that Ire1 senses two different types of ER stress via distinct mechanisms (Tran et al., 2019; Phuong et al.,

2023). The luminal domain of Ire1 directly captures unfolded proteins accumulated in the ER lumen to recognize proteotoxic stress, whereas the transmembrane domain of Ire1 is responsible for the recognition of LBS (Kimata et al., 2007; Gardner et al., 2011; Promlek et al., 2011; Halbleib et al., 2017). Activation of Ire1 by ER proteotoxic stress, which, for example, is induced by the N-glycosylation inhibiting antibiotic tunicamycin, is deduced to be selectively compromised by a partial deletion mutation in the luminal domain of Ire1, namely, the ΔIII mutation. On the other hand, activation of Ire1 by LBS, which, for example, is induced by depletion of the essential membrane-lipid component inositol, is deduced to be selectively compromised by a point mutation in the transmembrane domain of Ire1, namely, the V535R mutation. In order to address the mechanism of UPR induction by hSOD1, here we employed these two mutants of the *IRE1* gene. Fig. 2 shows that both the ΔIII and V535R mutations attenuated UPR levels in *ste24Δ* cells expressing hSOD1. Therefore, we presume that hSOD1 induces ER stress and UPR via both ER proteotoxic stress and LBS.

DISCUSSION

UPR is believed to be a cellular response triggered by abnormal conditions in the ER. Accumulation of mutant ER-client proteins carrying the ER translocation signal is a prominent cause of ER stress that induces UPR (Promlek et al., 2011; Phuong et al., 2023). However, as described in the Introduction section, it is also possible that accumulation of aberrant cytosolic proteins leads to the induction of ER stress and UPR under some neurodegenerative disease conditions including ALS. To address this issue, here we investigated what occurs when hSOD1, which is a prominent cause of ALS (Hayashi et al., 2016; Wang et al., 2024), is expressed in *S. cerevisiae* cells. As shown in Fig. 1, here we demonstrated the UPR induction by hSOD1 not in wild-type but in *ste24Δ* mutant cells.

Ste24 is a membrane-integral metalloprotease conserved in eukaryotic species. According to the structure of Ste24, its substrates are likely positioned at the cytosolic surface of the membrane (Goblirsch and Wiener, 2020). Although *STE24* was initially identified as a gene involved in the biogenesis of the mating pheromone **a**-factor in *S. cerevisiae*, it is today accepted that Ste24 has other functions in various organisms (Goblirsch and Wiener, 2020). A prominent role of Ste24 is to digest proteins that clog the Sec61 translocon (Ast et al., 2016). On the other hand, we previously proposed that Ste24 prevents translocation of some proteins that do not carry the ER translocation signal in *S. cerevisiae* cells (Hosomi et al., 2020).

We and others previously proposed that hSOD1 is partially translocated to the ER in mammalian cells and *S. cerevisiae ste24Δ* mutant cells (Urushitani et al., 2008; Hosomi et al., 2023), while hSOD1 is deduced to be a cytosolic protein. Therefore, it is highly possible that the partial localization of hSOD1 in the ER leads to ER proteotoxic stress, which activates Ire1. Conversely, our observation that hSOD1 induces UPR in *S. cerevisiae ste24Δ* cells provides a supporting evidence for our previous argument that some cytosolic proteins, including hSOD1, are partially translocated to the ER in the absence of Ste24 (Hosomi et al., 2020; Hosomi et al., 2023).

Nevertheless, here we also demonstrated that the UPR levels in hSOD1-expressing *ste24Δ* cells were mitigated not only by the ΔIII but also by the V535R mutation of Ire1. This observation suggests that in addition to ER proteotoxic stress, LBS contributes to the UPR induction under this condition. We thus speculate that it is also possible that hSOD1 abundantly accumulated in the cytosol damages the ER membrane, for instance, through aberrant interaction between hSOD1 and the ER membrane, which may be inhibited by Ste24.

Taken together, we propose a scenario presented in Fig. 3. ER stress, which activates Ire1 and triggers the UPR, is induced by hSOD1 in two different ways. First, hSOD1 is partially mistransported to the ER, leading to proteotoxic stress. Ire1 may directly detect ER-localized hSOD1. Second, hSOD attacks the cytosolic surface of the ER membrane. When highly accumulated and aggregated in the cytosol, hSOD1 may physically push the ER membrane. We also speculate that Ste24 inhibits these two events by digesting hSOD1 molecules approaching the ER.

It should also be noted that there remain unanswered questions. Mutations in the hSOD1 gene, such as G93A, are known to be risk factors for the familial ALS, although wild-type hSOD1 is also involved in the onset of ALS (Hayashi et al., 2016; Wang et al., 2024). Therefore, it is highly possible that G93A hSOD1 is more toxic than wild-type hSOD1. Consistently, we previously reported that G93A hSOD1 accumulates more abundantly than wild-type hSOD1 in the ER of *S. cerevisiae ste24Δ* cells (Hosomi et al., 2023). However, here we showed that wild-type hSOD1 and G93A hSOD1 similarly induced UPR in *S. cerevisiae ste24Δ* cells. The differences and similarities between wild-type hSOD1 and its G93A mutant should be addressed in future studies. Besides, as an alternative scenario for the induction of UPR in ALS, hSOD1 associates with the ERAD machinery from the cytosolic side to compromise ERAD (Nishitoh et al., 2008; Tsuburaya et al., 2018). It should also be addressed in the future how hSOD1 affects the ERAD machinery in *S. cerevisiae* cells.

Funding

This research was funded by the Japan Society for the Promotion of Science (JSPS) KAKENHI (23H02174 to YK).

Conflict of interest

The authors declare no conflict of interest.

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Table 1 Plasmids used in this study

Name	Description	Source
pRS313	YCp-type plasmid carrying the *HIS3* selectable marker	Sikorski and Hieter, 1989
pRS316	YCp-type plasmid carrying the *URA3* selectable marker	Sikorski and Hieter, 1989
pRS426	YEp-type plasmid carrying the *URA3* selectable marker	Christianson et al., 1992
pRS313-IRE1	A derivative of pRS313 carrying the *IRE1* gene	Kimata et al., 2004
pRS315-ScSOD1pro-HsSOD1-V5-3xsequon	Wild-type hSOD1-expression plasmid	Hosomi et al., 2023
pRS315-ScSOD1pro-HsSOD1-V5-3xsequon(G93A)	The same as pRS426-hSOD1 except that the hSOD1 gene contains the G93A mutation	Hosomi et al., 2023
pRS426-hSOD1	The same as pRS315-ScSOD1pro-HsSOD1-V5-3xsequon except that the plasmid backbone is pRS426 instead of pRS315	This study
pRS426-hSOD1-G93A	The same as pRS426-hSOD1 except that the hSOD1 gene contains the G93A mutation	This study

The plasmids pRS426-hSOD1, and pRS426-hSOD1-G93A are unavailable as purified plasmid DNA because they were produced in *S. cerevisiae* cells using the *in vivo* homologous recombination technique.

Table 2 Strains used in this study

Name	Brief genotype	Description	Source
BY4741	*IRE1+*	*MAT***a** *leu2Δ0 ura3Δ0 his3Δ1 met15Δ0*	EUROSCARF
Y06920	*IRE1+ ste24Δ*	*ste24::kanMX4* derivative of BY4741	EUROSCARF
KMY1516	*ire1Δ*	*MATα leu2-3,112::*3XUPRE-GFP*::LEU2 ura3-52 his3Δ200 trp1Δ901 lys2-801::*3XUPRE-lacZ*::LYS2 ire1::TRP1*	Kimata et al., 2004
KMY1516 [pRS313-IRE1]	*IRE1+*	KMY1516 transformed with pRS313-IRE1	This study
KMY1516 [pRS313-IRE1-ΔIII]	*IRE1-*ΔIII	KMY1516 transformed with pRS313-IRE1-ΔIII	This study
KMY1516 [pRS313-IRE1-V535R]	*IRE1-*V535R	KMY1516 transformed with pRS313-IRE1-V535R	This study
KMY1516-ste24Δ	*ire1Δ ste24Δ*	*ste24::kanMX4* derivative of KMY1516	Promlek et al., 2011
KMY1516-ste24Δ [pRS313-IRE1]	*IRE1+ ste24Δ*	KMY1516-ste24Δ transformed with pRS313-IRE1	This study
KMY1516-ste24Δ [pRS313-IRE1-ΔIII]	*IRE1-*ΔIII *ste24Δ*	KMY1516-ste24Δ transformed with pRS313-IRE1-ΔIII	This study
KMY1516-ste24Δ [pRS313-IRE1-V535R]	*IRE1-*V535R *ste24Δ*	KMY1516-ste24Δ transformed with pRS313-IRE1-V535R	This study

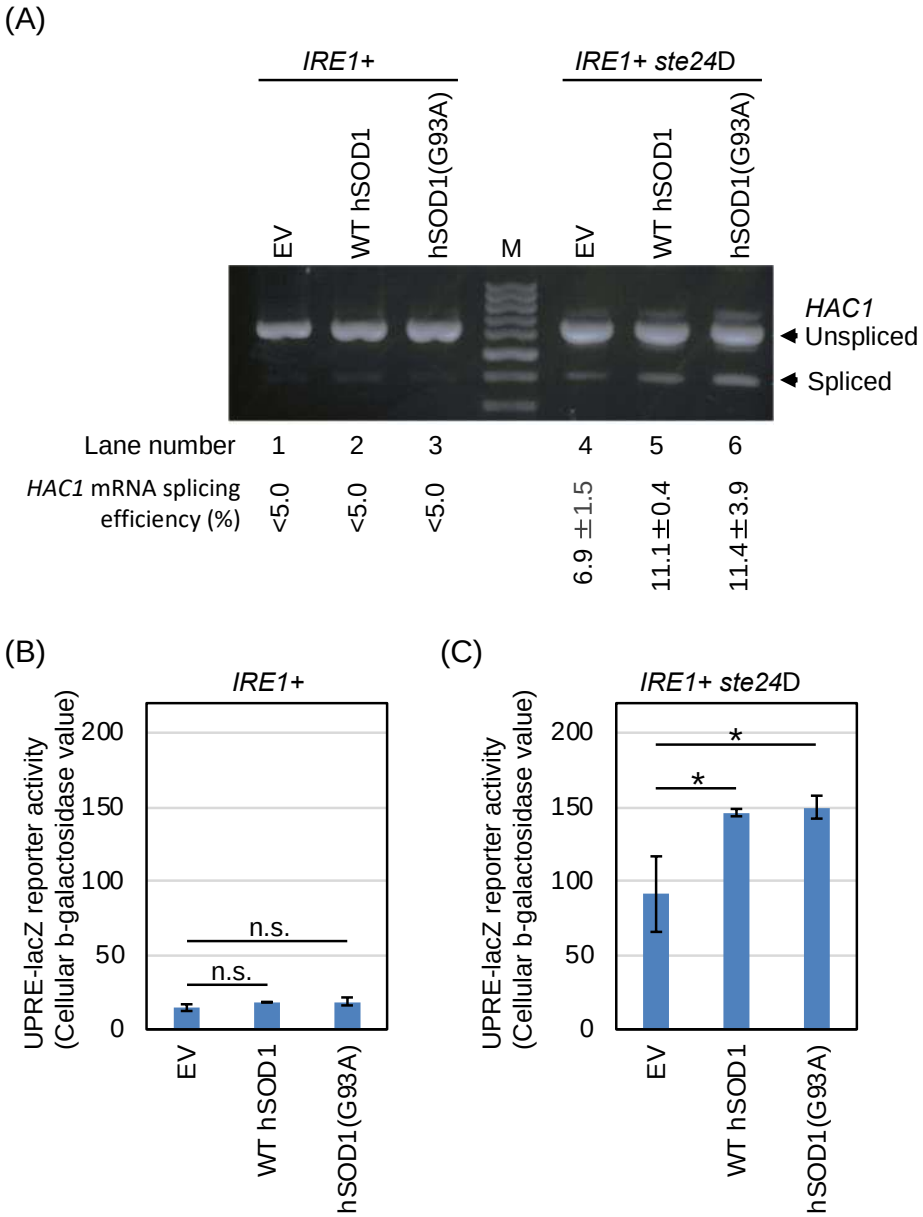

Fig. 1 Induction of the UPR by hSOD1 in *S. cerevisiae* cells carrying the *ste24Δ* mutation
After transformation with the hSOD1-expression plasmid, pRS426-hSOD1 (wild-type (WT) hSOD1) or pRS426-hSOD1-G93A (hSOD1(G93A)), or the empty plasmid vector (EV), cells were subjected to the indicated assays. (A) To monitor the *HAC1*-mRNA splicing, transformants obtained from BY4741 (*IRE1+*) and Y06920 (*IRE1+ste24Δ*) were subjected to total RNA extraction and RT-PCR, the products of which were electrophoretically run on 2% agarose containing EtBr. M: Molecular weight marker (XL-DNA ladder 100). (B) Transformants obtained from KMY1516[pRS313-IRE1] (*IRE1+*) and KMY1516-ste24Δ[pRS313-IRE1] (*IRE1+ste24Δ*) were monitored for cellular β-galactosidase activity. n.s.: Not significant. *: $p<0.05$.

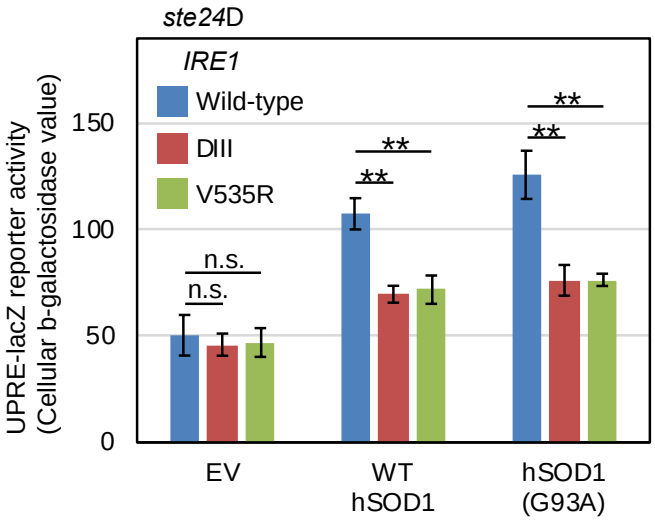

Fig. 2 Mitigation of the hSOD1-induced UPR by *IRE1* mutations

KMY1516[pRS313-IRE1] (Wild-type IRE1) was modified to carry the indicated mutations on the *IRE1* gene or remained unmutagenized, followed by transformation with the hSOD1-expression plasmid, pRS426-hSOD1 (wild-type (WT) hSOD1) or pRS426-hSOD1-G93A (hSOD1(G93A)), or the empty plasmid vector (EV). The transformants were then monitored for cellular β-galactosidase activity. n.s.: Not significant. **: $p<0.01$.

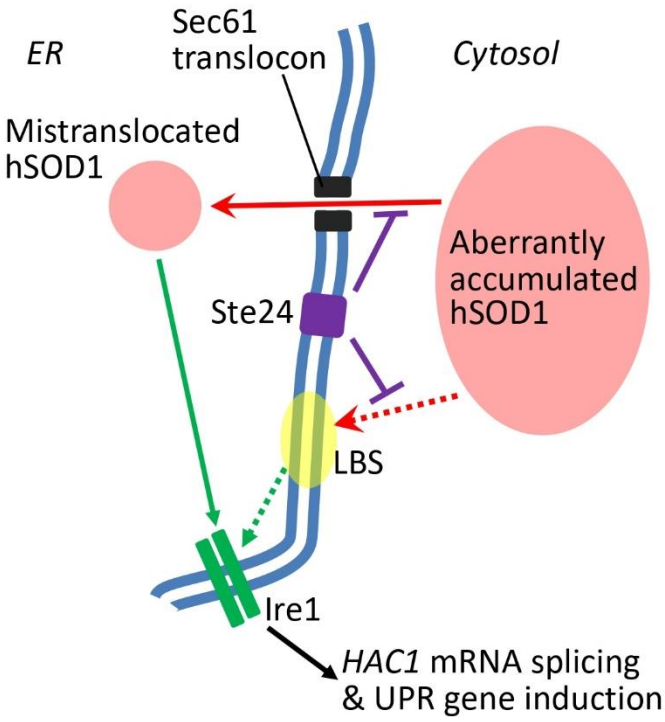

Fig. 3 Possible induction mechanism of UPR by hSOD1 in *S. cerevisiae* cells
Cytosolically accumulated hSOD1 is partially translocated to the ER (red solid arrow) and recognized as unfolded proteins by Ire1 (green solid arrow). Moreover, hSOD1 induces LBS (red dotted arrow), which also activates Ire1 (green dotted arrow).