HIRA, a conserved histone chaperone plays an essential role in low-dose stress response via transcriptional stimulation in fission yeast

Moeko Chujo[1], Yusuke Tarumoto[1], Koichi Miyatake[2], Eisuke Nishida[2], and Fuyuki Ishikawa[1]

From the Departments of [1]Gene Mechanisms and [2]Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

*Running title: *Fission yeast HIRA in stress response*

Address correspondence to: Fuyuki Ishikawa, MD, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan, Tel: +81 75 753 4195; Fax: +81 75 753 4197; E-mail: fishikaw@lif.kyoto-u.ac.jp

Keywords: Stress response; Cross tolerance; Histone chaperone; HIRA; Fission yeast

Background: HIRA is a conserved histone chaperone required for regulation of chromatin structure.
Results: Genes that encode HIRA proteins are responsible for cross tolerance. Specifically, stress-responsive gene expression was most profoundly compromised in HIRA disruptants.
Conclusion: HIRA is involved in cross tolerance via regulation of stress-responsive gene expression.
Significance: This study provides evidence that fission yeast HIRA functions in stress response.

SUMMARY

Cells that have been pre-exposed to mild stress (priming stress) acquire transient resistance to subsequent severe stress even under different combinations of stresses. This phenomenon is called cross tolerance. Although it has been reported that cross tolerance occurs in many organisms, the molecular basis is not clear yet. Here, we identified *slm9*$^+$ as a responsible gene for the cross tolerance in the fission yeast *Schizosaccharomyces pombe*. Slm9 is a homolog of mammalian HIRA histone chaperone. HIRA forms a conserved complex and gene disruption of other HIRA complex components, Hip1, Hip3 and Hip4, also yielded a cross-tolerance-defective phenotype, indicating that the fission yeast HIRA is involved in the cross tolerance as a complex. We also revealed that Slm9 was recruited to the stress-responsive gene loci upon stress treatment in an Atf1-dependent manner. The expression of stress-responsive genes under stress condition was compromised in HIRA disruptants. Consistent with this, Pol II recruitment and nucleosome eviction at these gene loci were impaired in *slm9*Δ cells. Furthermore, we found that the priming stress enhanced the expression of stress-responsive genes in wild-type cells that were exposed to the severe stress. These observations suggest that HIRA functions in stress response through transcriptional regulation.

Cells are equipped with stress response mechanisms at various levels in order to survive and proliferate under ever-changing environmental stresses. Cross tolerance is one of such stress response mechanisms. Cells that have been pre-exposed to mild stress (priming stress) are known to acquire transient resistance to subsequent severe stress. If the two stresses are of the same type, the phenomenon is called acquired tolerance. It is also known that this increased survival happens even under combinations of different types of stresses, such as heat stress and oxidative stress. This phenomenon is called cross tolerance. It has been reported that acquired tolerance and cross tolerance occur in a wide variety of species, including bacteria, plants, yeasts, and mammals (1-8).

Hormesis is a widely accepted term that more comprehensively describes cross tolerance (9,10). This phenomenon represents a biphasic dose response to toxins and stressors, with beneficial effects at low doses and harmful ones at high doses. Recent studies have provided new insights into hormesis as an application in anti-aging research (11,12). Thus, an understanding of the response to low-dose stress is important. However,

generally, it is difficult to detect the response to low-dose stress because the low-dose stress does not induce a significant phenotype. Considering that the response to priming stress is important for survival under subsequent severe stress, the analysis of cross tolerance is expected to lead to further understanding of the response mechanism to low-dose stress.

In the fission yeast *Schizosaccharomyces pombe*, it is well known that a wide range of stresses lead to the activation of stress-activated mitogen-activated protein kinase (MAPK) Spc1/Sty1. The inactivation of this kinase causes hypersensitivities to various stresses (13-16). There are common stress-responsive genes, called core environmental stress response (CESR) genes whose expression is induced more than twofold under at least four of five types of stress conditions examined (17). CESR genes were regulated predominantly by Spc1 via the transcription factor Atf1. It has been proposed that the cross tolerance depends on nascent protein synthesis (7) and requires the induction of CESR genes (17). However, the molecular mechanism of the cross tolerance remains unclear.

Chromatin structure should be highly regulated in many cellular processes, such as DNA replication, repair or transcription. Accumulating evidence has shown that histone chaperones are one of the key proteins involved in those processes (18). Histone chaperones are known to associate with histones and facilitate the assembly and disassembly of nucleosomes. HIRA/HIR is one of the major histone chaperones that are conserved in many eukaryotic organisms (19). Whereas higher eukaryotes have a single HIRA protein (19-22), the fission yeast possesses two HIRA proteins (Slm9 and Hip1) (23,24), same as the budding yeast *Saccharomyces cerevisiae* (Hir1 and Hir2) (25). Fission yeast HIRA proteins stably associate with two other proteins, Hip3 and Hip4, and form a tetrameric complex (HIRA complex) (26,27). Recently, Cabin1 and UBN1 were identified as the human counterparts of Hip3 and Hip4, respectively (28-30), suggesting that the HIRA complex is evolutionarily conserved. HIRA is the histone chaperone for histone H3-H4 and is involved in the replication-independent nucleosome deposition pathway, whereas another histone chaperone CAF-1 is coupled to DNA replication (31-34).

HIRA has been shown to function in transcription as well. HIRA proteins were first identified in the budding yeast as a negative regulator of histone gene expression (25,35). It has been reported that the budding yeast HIR complex interacts with nucleosomes and prevents the remodeling activity of the SWI/SNF complex (36). The ectopic expression of HIRA in human cells also represses the transcription of histones (37). In the fission yeast, HIRA is required for the suppression of Tf2 long terminal repeat (LTR) retrotransposons, normally repressed genes, or cryptic antisense transcripts (38). Consistent with its repressive role in transcription, HIRA also functions in heterochromatin assembly and silencing. In human cells, the formation of senescence-associated heterochromatin foci depends on HIRA (39). Loss of the fission yeast HIRA complex components results in silencing defects at the centromere and mating type loci (27). Recent study has also demonstrated that a complex formed by the histone chaperone Asf1 and HIRA spreads across silenced regions via its association with the chromodomain protein Swi6 to facilitate histone deacetylation and heterochromatin spreading in the fission yeast (40). On the other hand, HIRA can also act as a positive regulator of transcription. The N-terminal and C-terminal halves of chicken HIRA regulate different sets of cell-cycle-related genes positively and negatively, respectively (41). Mutations in the budding yeast *HIR* genes display strong synthetic defects or lethality when combined with mutations in the genes encoding the transcription elongation factor FACT components (42). In higher eukaryotes, HIRA is involved in the incorporation of H3.3 variant histones into transcriptionally active genes (33,43,44). However, it is not clear whether HIRA is involved in transcriptional activation in the fission yeast.

In this study, we found that the fission yeast *slm9*$^{+}$ is responsible for the cross tolerance. The disruption of each component gene of the HIRA complex led to defects in the cross tolerance. In wild-type cells, Slm9 was located at several stress-responsive gene loci under the stress condition and this localization is dependent on Atf1. HIRA disruption caused impaired stress-responsive gene expression, stress-dependent Pol II recruitment, and nucleosome eviction. Moreover, it was suggested that the priming stress facilitates stress-responsive gene expression in wild-type cells under the severe stress. Together, these results highlight the novel function of the fission yeast HIRA in stress

response.

EXPERIMENTAL PROCEDURES

Yeast strains and general techniques– *S. pombe* strains used in this study are listed in Table 1. Growth media and basic techniques for the fission yeast have been described previously (45,46). Cells were grown in the rich medium YES or the synthetic medium SD and supplemented with amino acids as required.

Stress experiments– For the cross tolerance and acquired tolerance experiments, cells were grown in duplicate to the logarithmic phase in YES medium at 32°C. Two cultures each were subjected and not subjected to the priming stress, respectively, for 1 h and centrifuged gently (780 g for 1 min) to remove the medium. Subsequently, both cultures were resuspended in YES medium and severe stress was applied for 1 h. The stress conditions are described below. (P) and (S) indicate priming stress and severe stress, respectively. Oxidative stress: H_2O_2 was added to make a final concentration of 0.1 mM (P) or 25 mM (S). Heat stress: Cells were cultured in a 40°C (P) or 46°C (S) water bath. Osmotic stress: YES medium containing 2.4 M KCl was added to make a final concentration of 0.6 M (P) or 2.4 M (S). After the above stress treatment, the cells were immediately collected by gentle centrifugation (400 g for 2 min) and diluted with YES medium. Five hundred cells per plate were plated onto YES plates and the number of colonies was counted after incubation for 4 days at 32°C. Viability was calculated as percentage of the number of colonies for 500 cells.

For the colony-spotting assay, the cells were grown to the logarithmic phase in YES medium at 32°C. Then, the cells were serially diluted from 5 $\times 10^6$ to 5×10^3 cells/ml (tenfold dilution) and 5 μl of each was spotted onto YES plates. Incubation was carried out for 3-4 days at 32°C. For heat stress, cells that were spotted onto YES plates were incubated for 3-4 days at 37°C, or for 1 h at 47°C followed by incubation for 3-4 days at 32°C. For the other stresses, YES plates containing the following compounds were used: 2 mM H_2O_2, 50 μM menadione, 1.2 M KCl, 2 M sorbitol, and 0.1 M $CaCl_2$.

Chromatin immunoprecipitation– The cells were grown in duplicate to the logarithmic phase in YES medium at 32°C. One aliquot was used as the unstressed control and the other aliquot was exposed to 40°C for 15 min. Subsequently, the cells ($2.5\sim4 \times 10^8$ cells) were cross-linked by adding 1% formaldehyde for 30 min at 25°C and the cross-linking was stopped by treating with 125 mM glycine on ice for 5 min. The cell pellets were washed twice with ice-cold water and twice with lysis buffer 1 (50 mM HEPES–KOH [pH 7.5], 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate). The cell pellets were resuspended in lysis buffer 1 containing 50 mM NaF, 0.1 mM Na_3VO_4, 1 mM PMSF, and 1× Complete (Roche), and broken with zirconia beads using a Multi-Beads Shocker (Yasui Kikai) at 4°C. The lysates were sonicated with Sonifier 250 (Branson) to yield chromatin fragments having an average size of 500 bp. The sonicated lysates were spun at 17,800 g for 15 min at 4°C. The supernatant was immunoprecipitated with mouse anti-Myc antibody (sc-40 Santa Cruz) or mouse anti-RNA polymerase II CTD antibody (05-623 Millipore) for 2 h at 4°C, and this was followed by the addition of magnetic beads (Dynal). After incubation for 1.5 h at 4°C, the beads were washed once with lysis buffer 1 containing 50 mM NaF, 0.1 mM Na_3VO_4, 1 mM PMSF, and 1×Complete (Roche); once with lysis buffer 1 containing 500 mM NaCl and 1 mM PMSF; once with lysis buffer 2 (10 mM Tris–HCl [pH 8.0], 1 mM EDTA, 0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate); and twice with TE (10 mM Tris–HCl [pH 8.0], 1 mM EDTA). The beads were resuspended in TER (TE containing 10 μg/ml RNase) and incubated for 15 min at 37°C. The samples were adjusted to 0.25% SDS and 250 μg/ml proteinase K, and incubated at 37°C overnight. This was followed by another incubation for 6 h at 65°C. The eluted DNA was subjected to phenol/chloroform extraction and precipitated with ethanol. The purified DNA was analyzed by real-time PCR using the StepOnePlus™ Real-Time PCR System and Power SYBR Green PCR master mix (Applied Biosystems). The nucleotide sequences of the primer sets are listed in Table S1.

RT-PCR– Total RNA was isolated as previously described (47) and treated with 0.625 U/g RNA of RNase-free DNase I (TaKaRa) to digest genomic DNA. cDNA samples were synthesized using AMV Reverse Transcriptase (Life Sciences Advanced Technologies, Inc.) and Random Primer (nonadeoxyribonucleotide mixture). The primer sequences are available on request. Real-time PCR was performed using the StepOnePlus™ Real-Time PCR System and Power SYBR Green PCR master mix (Applied

Biosystems). The nucleotide sequences of the primer sets for real-time PCR are listed in Table S1.

Microarray analysis– The cells were grown in quadruplicate at 32°C to the logarithmic phase and an aliquot was collected as the unstressed control. The other three aliquots were exposed to 40°C for 1 h, 25 mM H_2O_2 for 1 h, or 40°C for 1 h followed by 25 mM H_2O_2 for 1 h, respectively. Total RNA was purified as described for RT-PCR. All the 12 RNA samples were analyzed with GeneChip Yeast Genome 2.0 Array (Affymetrix) according to the manufacturer's instructions. After using the RMA algorithm to obtain the summarized probeset-level expression data, the array data were transferred to GeneSpring 7.3 (Agilent Technologies) microarray analysis software for gene ontology (GO) analysis. Standard hypergeometric distribution was used to calculate the p-values for individual GO terms. Significant enrichment of GO was selected using a p-value of <0.05. To avoid the detection of false positives, the Benjamini-Yekutieli correction method was applied to obtain the corrected p-values. The microarray data are available at Gene Expression Omnibus (GEO) under accession number GSE35281.

Preparation of mononucleosomal DNA– The cells were grown in duplicate to the early logarithmic phase in YES medium at 32°C. One aliquot was used as the unstressed control and the other aliquot was exposed to 40°C for 15 min. Subsequently, mononucleosomal DNA was obtained as described previously (48) with some modifications. Cell wall was digested with 1 mg/ml Zymolyase 100T (Seikagaku Corporation) for 40 min at 35°C. Micrococcal nuclease (MNase) digestion was performed with MNase to a final concentration of 133 U/ml. Mononucleosomal DNA fragments were purified from the agarose gel using QIAquick Gel Extraction Kit (Qiagen).

Nucleosome-scanning analysis– The nucleosome-scanning analysis was performed as described previously (49,50). Genomic DNA was obtained from the same protocol as for the preparation of mononucleosomal DNA, without the crosslinking, MNase treatment and gel purification step. Five nanograms of purified mononucleosomal and genomic DNA were analyzed by real-time PCR using the StepOnePlus™ Real-Time PCR System and Power SYBR Green PCR master mix (Applied Biosystems). Ten overlapping primer pairs were set downstream of nucleosome depleted region (NDR) (50,51). The nucleotide sequences of the primer sets were designed by reference to the previous report (50) as listed in Table S1.

RESULTS

Cross tolerance in fission yeast– We first confirmed cross tolerance in the fission yeast. Wild-type (JK317) cells were treated or not treated with mild (priming) stress and subsequently subjected to severe stress, using combinations of heat, oxidative, and osmotic stresses, and cell viability was compared (Fig. 1). In contrast to the case of the budding yeast (7), the combination of mild oxidative stress and severe heat stress induced cross tolerance. Other combinations examined also induced cross tolerance. Overall, we conclude that in the fission yeast, various combinations of stresses generally induce cross tolerance.

Identification of responsible gene of cross-tolerance-defective mutant– In order to identify the factor involved in cross tolerance, we performed a genetic screen for cross-tolerance-defective mutants and isolated several mutants (Tarumoto, Y., Kanoh, J. and Ishikawa, F., submitted for publication). Among them, *7-4* mutant, which showed clearly the cross-tolerance-defective phenotype, was chosen for further analysis (Fig. S1A). *7-4* mutant was backcrossed three times with the parental wild-type strain. Tetrad analysis revealed that the cross-tolerance-defective phenotype of *7-4* mutant was caused by a single mutation. In addition to the cross-tolerance-defective phenotype, we found that *7-4* mutant showed strong sensitivity to heat shock (37°C) and 0.1 M $CaCl_2$ treatment (Fig. S1B). The responsible gene of *7-4* mutant was cloned by complementation of heat and $CaCl_2$ sensitivities with *S. pombe* genomic library (pTN-L1) and subsequent sequencing revealed its identity as *slm9*$^+$. We verified that a single nucleotide deletion occurred at nucleotide 1451 in the ORF of *slm9*$^+$ in *7-4* mutant (Fig. S1C). Slm9 is the homolog of mammalian HIRA in the fission yeast. HIRA is a histone chaperone that is involved in the replication-independent nucleosome deposition pathway and transcriptional control.

HIRA complex is involved in cross tolerance– The fission yeast has two HIRA/HIR proteins, Slm9 and Hip1 (23,24). In order to verify the

function of the fission yeast HIRA in cross tolerance, we constructed *slm9*Δ and *hip1*Δ strains and examined the cross tolerance phenotype. Both *slm9*Δ and *hip1*Δ cells showed the cross-tolerance-defective phenotype under the combination of mild heat stress and severe oxidative stress (Fig. 2A). As Slm9 and Hip1 form a complex with two other proteins, Hip3 and Hip4 (26,27), we also examined these HIRA complex subunit disruptants. We found that both *hip3*Δ and *hip4*Δ cells showed the cross-tolerance-defective phenotype, similar to *slm9*Δ and *hip1*Δ cells (Fig. 2B). These results suggest that the fission yeast HIRA functions as a complex to confer cross tolerance.

To examine the possibility that HIRA disruptants are specifically sensitive to the combination of mild heat stress and severe oxidative stress, the cells were treated with other stress combinations. *slm9*Δ and *hip1*Δ cells also showed the cross-tolerance-defective phenotype when treated with combinations of mild oxidative stress and severe heat stress, and mild osmotic stress and severe oxidative stress (Fig. 2C). These experiments demonstrated that the fission yeast HIRA is involved in cross tolerance regardless of the stress combination. Moreover, the cells were also treated with combinations of same types of stresses, namely, mild oxidative stress and severe oxidative stress (acquired-tolerance-inducible stress). Acquired tolerance was defective in *slm9*Δ and *hip1*Δ cells as well as cross tolerance (Fig. 2D). Hereafter, we used the combinations of mild heat stress and severe oxidative stress as the cross-tolerance-inducible stress.

To determine whether the function of HIRA in stress response is a general feature of histone chaperones, we analyzed other histone chaperone mutants, *pcf1*Δ and *nap1*Δ. Pcf1 is a large subunit of histone chaperone CAF-1 that loads histone H3-H4 onto DNA and is involved in the replication-dependent nucleosome deposition pathway (34,52). Nap1 is involved in the transfer of histone H2A–H2B from the cytoplasm to the nucleus and the deposition of histones onto DNA (53). Cross tolerance occurred in both *pcf1*Δ and *nap1*Δ cells, similar to the case of wild-type cells (Fig. 2E). These results raise an interesting possibility that among histone chaperones, HIRA is specifically involved in cross tolerance.

HIRA functions particularly under low-dose stress– As we examined the cross tolerance phenotype under various combinations of heat, oxidative, and osmotic stresses, we next investigated the viability of cells lacking each subunit of the HIRA complex under the single stress condition (Fig. 3). The HIRA subunit disruptants (*slm9*Δ, *hip1*Δ, *hip3*Δ, and *hip4*Δ) were not so sensitive to osmotic stress (2 M sorbitol and 1.2 M KCl) and oxidative stress caused by 2 mM H_2O_2, whereas *spc1*Δ mutant, which is known to be sensitive to a wide variety of stresses, showed strong sensitivity. On the other hand, the HIRA subunit disruptants showed severe sensitivity to heat shock (37°C, 3 days) and oxidative stress caused by 50 μM menadione. Although the HIRA subunit disruptants showed varied sensitivities to distinct forms of stress, the cross (acquired)-tolerance-defective phenotype of *slm9*Δ and *hip1*Δ cells was observed under different stress combinations (Fig. 2A, C and D). These results further suggest the stress-type-independent function of HIRA in the cross tolerance. It is known that menadione generate intracellular reactive oxygen species and exert weak oxidative stress on the cells (54). It should be noted that the HIRA subunit disruptants showed higher sensitivity to menadione than H_2O_2. Moreover, they were more sensitive to weak and chronic heat shock (37°C, 3 days) than strong and acute heat shock (47°C, 1 h). These observations suggest that HIRA responds to low-dose stress specifically, consistent with its response to the priming stress in the cross tolerance.

HIRA is localized at stress-responsive gene loci upon stress treatment– As the fission yeast HIRA has a function in the cross tolerance, we examined the protein levels of Slm9 and Hip1 under the stress condition. Considering the protein levels of loading control (Cdc2), it appeared that Hip1 was more abundant than Slm9. The protein levels of both Slm9 and Hip1 did not change significantly during the course of the priming stress treatment (Fig. 4A). We also examined Slm9 localization using a strain whose chromosomal copy of $slm9^{+}$ was tagged with the GFP sequence. In cells expressing Slm9-GFP, fluorescent signals were observed in the nuclei, as reported previously (23). This nuclear localization was not altered significantly under the stress condition (Fig. S2A). In addition, the chromatin fractionation assay was performed to determine HIRA localization biochemically (Fig. S2B). Slm9 and Hip1 were both enriched in the chromatin-bound fractions, and the distributions of Slm9 and Hip1 among different fractions did not

change notably under the stress condition (Fig. S2C).

To further investigate whether HIRA is localized at specific chromatic loci upon stress treatment, the chromatin immunoprecipitation (ChIP) assay was performed. Slm9 was enriched at both the promoters and ORFs of CESR genes (*ctt1*$^+$, *gpx1*$^+$, and *hsp9*$^+$) in a stress-dependent manner. However, such physical association of Slm9 was not observed at non-stress responsive loci (*pol1*$^+$ ORF and *dh*) (Fig. 4B). Thus, whereas the protein levels and the chromatin association of HIRA, revealed by Western blotting and chromatin fractionation assay, did not show distinct alteration, HIRA localization at chromatin revealed by the ChIP assay changes under the stress condition.

CESR genes were primarily regulated by Spc1 through its downstream b-ZIP transcription factor Atf1 (17). We hypothesized that specific localization of Slm9 at CESR gene loci is determined by Atf1. Indeed, Slm9 recruitment to CESR gene loci is almost totally dependent on Atf1 (Fig. 4B). This result suggests that Atf1 determines the stress-dependent HIRA recruitment to chromatin.

HIRA is required for stress-responsive gene transcription– Histone chaperones have been surmised to play important roles in transcriptional regulation (18). As the fission yeast HIRA was localized at the stress-responsive gene loci upon stress treatment, we hypothesized that the fission yeast HIRA complex may play a role in the stress response through the transcriptional control of stress-responsive genes. RT-PCR was performed to examine the expression of several CESR genes under the stress conditions in wild-type, *slm9*Δ, and *hip1*Δ cells. *slm9* and *hip1* disruption decreased the expression of many CESR genes (*ctt1*$^+$, *gpx1*$^+$, *gpd1*$^+$, and *tps1*$^+$) under the priming stress compared to wild-type cells (Fig. 5A). There were some exceptions, such as *hsp9*$^+$ and *hsp16*$^+$ that showed increased basal expression in the mutants (Fig. 5A). This basal up-regulation of some CESR genes is consistent with previous reports (26,38). In general, all the genes examined showed smaller differences in transcriptional levels between the non-stress condition and the priming stress condition in the mutant cells compared to the wild-type cells. In contrast, *slm9* and *hip1* disruption increased the expression of those genes under the severe stress condition (Fig. S3). Taken together, the results suggest that HIRA is required for the proper expression of stress-responsive genes. In addition to the gene expression, ChIP assay was carried out to examine the transcriptional kinetics at several gene loci in the wild-type and *slm9*Δ cells under the stress condition. As expected, RNA polymerase II (Pol II) was recruited to both promoters and ORFs of CESR genes (*ctt1*$^+$, *gpx1*$^+$, and *hsp9*$^+$) in the wild-type cells subjected to stress treatment, whereas this recruitment was impaired in *slm9*Δ cells. On the other hand, basal stress-independent Pol II recruitment to non-stress responsive loci (*pol1*$^+$ ORF and *dh*) was unaffected in *slm9*Δ cells (Fig. 5B). This result is consistent with the expression of CESR genes (Fig. 5A). Therefore, our results indicate that HIRA plays an important role in Pol II recruitment and progression, and the transcriptional activation of stress-responsive genes under the low-dose stress conditions.

HIRA particularly regulates stress-responsive genes under stress conditions– To determine whether HIRA specifically regulates stress-responsive genes or not, microarray analysis was carried out on wild-type, *slm9*Δ, and *hip1*Δ cells under four conditions: control, priming stress alone, priming stress followed by severe stress, and severe stress alone. Signal concordance between two arrays was evaluated using Pearson's correlation coefficient (r^2). A strong correlation (r^2>0.998) was noted between *slm9*Δ and *hip1*Δ samples under all conditions (Fig. S4), consistent with the previously reported strong correlation of gene expression between *slm9*Δ and *hip1*Δ cells under the normal condition (38).

We identified genes that exhibited twofold or greater change in the stress-treated samples compared to the control, and categorized them by GO classification. The GO terms that were enriched in induced and repressed genes under all conditions are listed in Table S2 and Table S3, respectively. Among these results, we focused on the priming stress condition because the response to the priming stress would be important for survival under the subsequent severe stress. The principal GO terms that are most significantly associated with the priming-stress-induced genes are selected from Table S2 and shown in Table 2. GO analysis identified "cellular response to stress," "meiosis," and "M phase" as the major enriched biological functions in both wild-type cells and mutants. Whereas the p-values of "meiosis" and "M phase" were similar between the wild-type cells and the mutants, the number of genes

enriched into "cellular response to stress" was much larger in the wild-type cells than in the mutants. In addition, GO terms, including "cellular response to oxidative stress" and "oxidoreductase activity," were only found in the wild-type cells. These results are consistent with the reduced CESR gene expression in the mutants under the priming stress condition (Fig. 5A). On the contrary, the number of genes enriched into "cellular response to stress" was much larger in the mutant cells than in the wild-type cells under the severe stress condition, which was again consistent with the results of RT-PCR (Fig. S3 and Table S2) (See Discussion).

To further confirm the difference in expression between the wild-type cells and the mutants under the priming stress condition, the fold change of gene expression was plotted under the priming stress condition compared to the control condition. The fold change in expression of all genes was smaller in the mutants than in the wild-type cells (Fig. 6A). Similarly, the fold change in expression of CESR genes and genes whose expression was increased more than twofold in the wild-type cells decreased significantly in the mutants (Fig. 6B and C). Moreover, among the priming-stress-induced genes of the wild-type cells, we selected genes that showed twofold or higher change in the wild-type cells compared to each mutant, and performed GO analysis of those genes. GO analysis identified "cellular response to stress" as the most significant term (Table 3). Thus, although HIRA may be required for global gene expression, it is particularly plays an important role in regulating the stress-responsive genes.

HIRA regulates stress-responsive gene expression via nucleosome eviction– To explore the mechanism by which HIRA regulates stress-responsive gene transcription, the nucleosome-scanning analysis of *ctt1*$^+$ region was performed. In wild-type cells, the position of +1 to +3 (relative to the transcription start site (TSS)) nucleosomes, as reflected by MNase sensitivity, were detected as peaks and these peaks were diminished upon heat stress (Fig. 7), as previously reported in H_2O_2-treated cells (50). Although positioned nucleosomes were also observed in *slm9*Δ cells, decreased nucleosome peaks upon stress treatment were not observed in *slm9*Δ cells (Fig. 7). Considering that regulatory regions of *ctt1*$^+$ gene such as TATA box and Atf1 binding site are located in close proximity to the TSS (55) and overlapped with +1 nucleosome position (Fig. 7), it seems that HIRA is required for recruitment of Pol II to these regulatory regions. Furthermore, nucleosomes downstream of TSS are also altered in *slm9*Δ cells (Fig. 7), consistent with the result of ChIP assay detecting impaired Pol II recruitment to ORFs of CESR genes (Fig. 5B). Taken together, these results suggest that HIRA is required for nucleosome eviction to regulate Pol II accessibility and/or progression, and expression of stress-responsive genes.

Priming stress facilitates expression of stress-responsive genes under subsequent severe stress– As HIRA plays a role in the transcriptional control of stress-responsive genes under the stress conditions, we characterized the expression of several CESR genes (*ctt1*$^+$, *gpx1*$^+$, and *hsp9*$^+$) in the wild-type cells during cross tolerance by quantitative RT-PCR. We found that the severe stress alone induced only less than threefold increase in CESR gene expression (Fig. 8A). In contrast, when the cells were treated with the priming stress prior to the severe stress, the expression of those genes increased dramatically (Fig. 8A). Furthermore, the fold change relative to the control condition of CESR gene expression in the wild-type cells under the stress conditions was plotted using microarray data. Similar to the results of quantitative RT-PCR (Fig. 8A), the fold change of CESR gene expression under the severe stress condition showed a dramatic increase when the cells were exposed to the priming stress (Fig. 8B). These findings indicate that the priming stress enhanced stress-responsive gene expression and as a result, the cells acquired resistance to impending stress.

DISCUSSION

We have demonstrated that the fission yeast HIRA complex is involved in cross tolerance. We also found that in the cross tolerance, the expression levels of stress-responsive genes under the severe stress was augmented when the cells were exposed to the priming stress. Although the fission yeast HIRA has been shown to be implicated in gene silencing and the heterochromatin assembly (26,38,40,56), our results showed that the fission yeast HIRA is required for the transcriptional activation of stress-responsive genes under the low-dose stress conditions. Therefore, HIRA would regulate transcription both positively and negatively.

HIRA disruption decreased stress-responsive

gene expression under the priming stress condition (Fig. 5A, Tables 2 and 3). As the expression of stress-responsive genes under the severe stress was enhanced by the priming stress (Fig. 8), the defect of the cross tolerance in the HIRA disruptants may have come from the impaired expression of those genes during the priming stress. Thus, cells may stimulate stress-responsive gene expression under the low-dose stress conditions to deal with stress and to prepare for future stress, consistent with a previous report of the budding yeast (7). In contrast, severe stress alone increased stress-responsive gene expression in *slm9*Δ and *hip1*Δ cells (Fig. S3 and Table S2). Considering that the severe stress alone did not lead to a marked increase of stress-responsive gene expression in the wild-type cells (Fig. 8), we speculate that the induction of those gene expression may be inhibited or occur at only a low level in the wild-type cells under the severe stress conditions. One possible explanation is that the induction of stress-responsive genes for survival would be too late after the high-dose stress, so cells may cease the transcription of those genes under the high-dose stress conditions in order not to consume extra, yet futile, energy. However, such regulation may be compromised in HIRA disruptants and this may lead to the up-regulation of stress-responsive genes. It will be important to study the dose-dependent stress response in detail to test if this hypothesis is plausible or not.

The viability of HIRA disruptants under the severe stress condition was comparable to that of the wild-type cells (Fig. 2A). Furthermore, HIRA disruptants showed high sensitivity to rather low-dose stress (Fig. 3). Thus, HIRA seems to control the transcription of stress-responsive genes specifically under low-dose stress. The accessibility of the transcription machinery is regulated by chromatin assembly and disassembly (57). Slm9 is recruited to the stress-responsive gene loci in the wild-type cells (Fig. 4B), and stress-responsive gene expression and stress-dependent Pol II enrichment are impaired in HIRA disruptants (Fig. 5). Moreover, nucleosome eviction at *ctt1*$^+$ gene region upon stress treatment is hampered by *slm9* disruption (Fig. 7). Taken together, HIRA likely regulates the transcription of stress-responsive genes through the eviction of histones. In addition, the position of nucleosomes was slightly different between wild-type and *slm9*Δ cells (Fig. 7). Although the significance of this displacement is unclear, it may contribute to the accessibility of proteins involved in transcription. Thus, HIRA may also have a role in the regulation of nucleosome positioning. One recent study has shown that histone H3 acetyltransferase Gcn5 facilitates Pol II progression along stress-responsive genes in fission yeast (50). Therefore, both HIRA and Gcn5 should stimulate the eviction of histones at these loci to allow Pol II to progress, and stress-responsive gene expression is induced by stress-activated MAPK Spc1 and its downstream transcription factor Atf1 to respond to stress.

One possible factor that enhances the recruitment of HIRA and Gcn5 may be the 19S ATPase subunits of the proteasome. Mass spectrometric analysis of the fission yeast Slm9 has led to the identification of the 19S ATPase complex as the interacting proteins (27). The 19S ATPase complex is recruited to chromatin in a histone H2B ubiquitylation-dependent manner and plays a nonproteolytic role in Pol II transcriptional elongation in the budding yeast (58-60). Moreover, in the fission yeast, histone H2B ubiquitylation is required for transcriptional elongation and HIRA mutations are synthetically lethal with *htb1-K119R*, the mutation in the conserved ubiquitin acceptor site of histone H2B, indicating the role of histone H2B ubiquitylation in chromatin assembly during transcription (61). Indeed, the overexpression of ubiquitin-conjugating enzyme gene confers enhanced stress tolerance in plants (62) and *RAD6*, which encodes ubiquitin-conjugating enzyme in the budding yeast, is involved in the heat shock induction of bleomycin resistance (cross tolerance) (63). Transcriptional regulation through HIRA recruitment may be required for not only stress response but also other biological responses in general. For instance, a recent study has revealed that Hip3, a component of the HIRA complex, is engaged in the repression of meiosis-specific gene SPCC663.14c expression under the vegetative state (56). Considering that Slm9 recruitment to stress-responsive gene loci depends on Atf1 (Fig. 4B), other factors, such as transcription factor which function together with general factors including HIRA, may determine the specificity of the response. In this way, HIRA may stimulate or repress transcription via mediation of nucleosome states, depending on the situation. Thus, it will be important to reveal the relationship among all these factors as they may cooperatively regulate stress-responsive gene expression.

REFERENCES

1. Hahn, G. M., Ning, S. C., Elizaga, M., Kapp, D. S., and Anderson, R. L. (1989) *Int J Radiat Biol* **56**, 817-825
2. Davies, J. M., Lowry, C. V., and Davies, K. J. (1995) *Arch Biochem Biophys* **317**, 1-6
3. Yonezawa, M., Misonoh, J., and Hosokawa, Y. (1996) *Mutat Res* **358**, 237-243
4. Hengge-Aronis, R., Lange, R., Henneberg, N., and Fischer, D. (1993) *J Bacteriol* **175**, 259-265
5. Santos, B. C., Chevaile, A., Kojima, R., and Gullans, S. R. (1998) *Am J Physiol* **274**, F1054-1061
6. Larkindale, J., and Huang, B. (2004) *J Plant Physiol* **161**, 405-413
7. Berry, D. B., and Gasch, A. P. (2008) *Mol Biol Cell* **19**, 4580-4587
8. Moskalev, A., Shaposhnikov, M., and Turysheva, E. (2009) *Biogerontology* **10**, 3-11
9. Calabrese, E. J., and Baldwin, L. A. (1999) *Toxicol Pathol* **27**, 195-216
10. Calabrese, E. J., and Baldwin, L. A. (2001) *Crit Rev Toxicol* **31**, 353-424
11. Rattan, S. I. (2008) *Ageing Res Rev* **7**, 63-78
12. Gems, D., and Partridge, L. (2008) *Cell Metab* **7**, 200-203
13. Shiozaki, K., and Russell, P. (1995) *Nature* **378**, 739-743
14. Millar, J. B., Buck, V., and Wilkinson, M. G. (1995) *Genes Dev* **9**, 2117-2130
15. Degols, G., Shiozaki, K., and Russell, P. (1996) *Mol Cell Biol* **16**, 2870-2877
16. Degols, G., and Russell, P. (1997) *Mol Cell Biol* **17**, 3356-3363
17. Chen, D., Toone, W. M., Mata, J., Lyne, R., Burns, G., Kivinen, K., Brazma, A., Jones, N., and Bahler, J. (2003) *Mol Biol Cell* **14**, 214-229
18. Avvakumov, N., Nourani, A., and Cote, J. (2011) *Mol Cell* **41**, 502-514
19. Kirov, N., Shtilbans, A., and Rushlow, C. (1998) *Gene* **212**, 323-332
20. Lamour, V., Lecluse, Y., Desmaze, C., Spector, M., Bodescot, M., Aurias, A., Osley, M. A., and Lipinski, M. (1995) *Hum Mol Genet* **4**, 791-799
21. Scamps, C., Lorain, S., Lamour, V., and Lipinski, M. (1996) *Biochim Biophys Acta* **1306**, 5-8
22. Roberts, C., Daw, S. C., Halford, S., and Scambler, P. J. (1997) *Hum Mol Genet* **6**, 237-245
23. Kanoh, J., and Russell, P. (2000) *Genetics* **155**, 623-631
24. Blackwell, C., Martin, K. A., Greenall, A., Pidoux, A., Allshire, R. C., and Whitehall, S. K. (2004) *Mol Cell Biol* **24**, 4309-4320
25. Sherwood, P. W., Tsang, S. V., and Osley, M. A. (1993) *Mol Cell Biol* **13**, 28-38
26. Greenall, A., Williams, E. S., Martin, K. A., Palmer, J. M., Gray, J., Liu, C., and Whitehall, S. K. (2006) *J Biol Chem* **281**, 8732-8739
27. Anderson, H. E., Kagansky, A., Wardle, J., Rappsilber, J., Allshire, R. C., and Whitehall, S. K. (2010) *PLoS One* **5**, e13488
28. Balaji, S., Iyer, L. M., and Aravind, L. (2009) *Mol Biosyst* **5**, 269-275
29. Banumathy, G., Somaiah, N., Zhang, R., Tang, Y., Hoffmann, J., Andrake, M., Ceulemans, H., Schultz, D., Marmorstein, R., and Adams, P. D. (2009) *Mol Cell Biol* **29**, 758-770
30. Rai, T. S., Puri, A., McBryan, T., Hoffman, J., Tang, Y., Pchelintsev, N. A., van Tuyn, J., Marmorstein, R., Schultz, D. C., and Adams, P. D. (2011) *Mol Cell Biol* **31**, 4107-4118
31. Ray-Gallet, D., Quivy, J. P., Scamps, C., Martini, E. M., Lipinski, M., and Almouzni, G. (2002) *Mol Cell* **9**, 1091-1100
32. Green, E. M., Antczak, A. J., Bailey, A. O., Franco, A. A., Wu, K. J., Yates, J. R., 3rd, and Kaufman, P. D. (2005) *Curr Biol* **15**, 2044-2049
33. Tagami, H., Ray-Gallet, D., Almouzni, G., and Nakatani, Y. (2004) *Cell* **116**, 51-61
34. Smith, S., and Stillman, B. (1989) *Cell* **58**, 15-25
35. Spector, M. S., Raff, A., DeSilva, H., Lee, K., and Osley, M. A. (1997) *Mol Cell Biol* **17**, 545-552
36. Prochasson, P., Florens, L., Swanson, S. K., Washburn, M. P., and Workman, J. L. (2005) *Genes Dev* **19**, 2534-2539
37. Nelson, D. M., Ye, X., Hall, C., Santos, H., Ma, T., Kao, G. D., Yen, T. J., Harper, J. W., and Adams, P. D. (2002) *Mol Cell Biol* **22**, 7459-7472
38. Anderson, H. E., Wardle, J., Korkut, S. V., Murton, H. E., Lopez-Maury, L., Bahler, J., and

Whitehall, S. K. (2009) *Mol Cell Biol* **29**, 5158-5167
39. Zhang, R., Poustovoitov, M. V., Ye, X., Santos, H. A., Chen, W., Daganzo, S. M., Erzberger, J. P., Serebriiskii, I. G., Canutescu, A. A., Dunbrack, R. L., Pehrson, J. R., Berger, J. M., Kaufman, P. D., and Adams, P. D. (2005) *Dev Cell* **8**, 19-30
40. Yamane, K., Mizuguchi, T., Cui, B., Zofall, M., Noma, K., and Grewal, S. I. (2011) *Mol Cell* **41**, 56-66
41. Ahmad, A., Kikuchi, H., Takami, Y., and Nakayama, T. (2005) *J Biol Chem* **280**, 32090-32100
42. Formosa, T., Ruone, S., Adams, M. D., Olsen, A. E., Eriksson, P., Yu, Y., Rhoades, A. R., Kaufman, P. D., and Stillman, D. J. (2002) *Genetics* **162**, 1557-1571
43. Ahmad, K., and Henikoff, S. (2002) *Mol Cell* **9**, 1191-1200
44. Mito, Y., Henikoff, J. G., and Henikoff, S. (2005) *Nat Genet* **37**, 1090-1097
45. Moreno, S., Klar, A., and Nurse, P. (1991) *Methods Enzymol* **194**, 795-823
46. Alfa, C., Fantes, P., Hyams, J., McLeod, M., and Warbrick, E. (1993) *Cold Spring Harbor Laboratory Press*
47. Kanoh, J., Sugimoto, A., and Yamamoto, M. (1995) *Mol Biol Cell* **6**, 1185-1195
48. Lantermann, A., Stralfors, A., Fagerstrom-Billai, F., Korber, P., and Ekwall, K. (2009) *Methods* **48**, 218-225
49. Sekinger, E. A., Moqtaderi, Z., and Struhl, K. (2005) *Mol Cell* **18**, 735-748
50. Sanso, M., Vargas-Perez, I., Quintales, L., Antequera, F., Ayte, J., and Hidalgo, E. (2011) *Nucleic Acids Res* **39**, 6369-6379
51. Lantermann, A. B., Straub, T., Stralfors, A., Yuan, G. C., Ekwall, K., and Korber, P. (2010) *Nat Struct Mol Biol* **17**, 251-257
52. Dohke, K., Miyazaki, S., Tanaka, K., Urano, T., Grewal, S. I., and Murakami, Y. (2008) *Genes Cells* **13**, 1027-1043
53. Mosammaparast, N., Ewart, C. S., and Pemberton, L. F. (2002) *EMBO J* **21**, 6527-6538
54. Chen, D., Wilkinson, C. R., Watt, S., Penkett, C. J., Toone, W. M., Jones, N., and Bahler, J. (2008) *Mol Biol Cell* **19**, 308-317
55. Nakagawa, C. W., Yamada, K., and Mutoh, N. (2000) *J Biochem* **127**, 233-238
56. Mizuki, F., Tanaka, A., Hirose, Y., and Ohkuma, Y. (2011) *PLoS One* **6**, e19442
57. Williams, S. K., and Tyler, J. K. (2007) *Curr Opin Genet Dev* **17**, 88-93
58. Ferdous, A., Gonzalez, F., Sun, L., Kodadek, T., and Johnston, S. A. (2001) *Mol Cell* **7**, 981-991
59. Gonzalez, F., Delahodde, A., Kodadek, T., and Johnston, S. A. (2002) *Science* **296**, 548-550
60. Ezhkova, E., and Tansey, W. P. (2004) *Mol Cell* **13**, 435-442
61. Tanny, J. C., Erdjument-Bromage, H., Tempst, P., and Allis, C. D. (2007) *Genes Dev* **21**, 835-847
62. Zhou, G. A., Chang, R. Z., and Qiu, L. J. (2010) *Plant Mol Biol* **72**, 357-367
63. Keszenman, D. J., Candreva, E. C., Sanchez, A. G., and Nunes, E. (2005) *Environ Mol Mutagen* **45**, 36-43

Acknowledgments– We are grateful to Dr. Yukinobu Nakaseko for useful discussion and critical reading of the manuscript. We thank Drs. Fumihiko Sato, Peter Baumann, and Junko Kanoh for warm encouragement and valuable suggestions, and Dr. Taro Nakamura, RIKEN Bioresource Center, for providing the *S. pombe* genomic library (pTN-L1).

FOOTNOTES

*This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (to F.I.). M.C. is a recipient of a fellowship from the Japan Society for the Promotion of Science (JSPS).

[1]To whom correspondence should be addressed: Fuyuki Ishikawa, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan, Tel: +81 75 753 4195; Fax: +81 75 753 4197; E-mail: fishikaw@lif.kyoto-u.ac.jp

[2]The abbreviations used are: CESR, core environmental stress response; GO, gene ontology; MNase, micrococcal nuclease; NDR, nucleosome depleted region; Pol II, RNA polymerase II; TSS, transcription

start site

FIGURE LEGENDS

Figure 1. Various stress combinations induce cross tolerance in the fission yeast.
Viability of wild-type (JK317) cells following exposure to indicated stress is shown. Priming stress was 40°C, 0.6 M KCl, or 0.1 mM H_2O_2 for 1 h and severe stress was 46°C, 2.4 M KCl, or 25 mM H_2O_2 for 1 h. Results are means of at least four independent experiments and error bars represent standard error. Significant difference between viabilities with or without priming stress was determined by the Student's *t*-test (* $P<0.05$, ** $P<0.01$).

Figure 2. HIRA complex is required for cross tolerance.
The viability of the indicated cells following exposure to cross (acquired)-tolerance-inducible stress is shown. Results are means of at least three independent experiments and error bars represent standard error. (A, B, E) Cells were subjected to 40°C for 1 h (priming stress) and 25 mM H_2O_2 for another 1 h (severe stress). (C) Oxidative and heat stresses: cells were subjected to 0.1 mM H_2O_2 for 1 h (priming stress) and 46°C for another 1 h (severe stress). Osmotic and oxidative stresses: cells were subjected to 0.6 M KCl for 1 h (priming stress) and 25 mM H_2O_2 for another 1 h (severe stress). (D) Cells were subjected to 0.1 mM H_2O_2 for 1 h (priming stress) and 25 mM H_2O_2 for another 1 h (severe stress).

Figure 3. HIRA complex mutants are especially sensitive to low-dose stress.
Tenfold serial dilutions of wild-type (JK317), *slm9*Δ (MC3749), *hip1*Δ (MC3725), *hip3*Δ (MC3773), *hip4*Δ (MC3799), and *spc1*Δ (MC3801) cells were spotted onto YES plates or YES plates containing H_2O_2, menadione, KCl, or sorbitol. For heat stress, the spotted YES plates were subjected to the indicated heat stress dose.

Figure 4. HIRA is recruited to stress-responsive gene loci upon stress treatment.
(A) *slm9-12myc* (MC3793) and *hip1-12myc* (MC3795) cells were exposed to 40°C for the indicated times. Whole cell extracts were prepared and analyzed by Western blotting with anti-Myc and anti-Cdc2 (control) antibodies. (B) *slm9-12myc* (MC3793) and *slm9-12myc atf1*Δ (MC4219) cells were exposed or not exposed to 40°C for 15 min. ChIP assay using anti-Myc antibody was performed. Purified DNA was analyzed by real-time PCR using primer sets for the promoter (prom) and coding (ORF) regions of stress-responsive genes (*ctt1*$^+$, *gpx1*$^+$, and *hsp9*$^+$), non-stress-responsive gene (*pol1*$^+$), and heterochromatic locus (outer repeats of centromere, *dh*). Values shown were normalized to *cdc2*$^+$ promoter. Results are means of three independent experiments and error bars represent standard error.

Figure 5. HIRA is required for stress-responsive gene expression and Pol II recruitment.
(A) Wild-type (JK317), *slm9*Δ (MC3749), and *hip1*Δ (MC3725) cells were exposed or not exposed to 40°C for 1 h. Total RNA was analyzed by RT-PCR using primer sets for stress-responsive genes (*ctt1*$^+$, *gpx1*$^+$, *gpd1*$^+$, *tps1*$^+$, *hsp9*$^+$, and *hsp16*$^+$). *act1*$^+$ is shown as loading control. (B) Wild-type (JK317) and *slm9*Δ (MC3749) cells were exposed or not exposed to 40°C for 15 min. ChIP assay using anti-Pol II antibodies was performed. Purified DNA was analyzed in the same way as described in Fig. 4B. Results are means of three independent experiments and error bars represent standard error.

Figure 6. HIRA disruption mainly affects stress-responsive gene expression.
(A, B, C) Fold change of gene expression under priming stress (40°C for 1 h) condition compared to no stress condition is plotted for wild-type (JK317), *slm9*Δ (MC3749), and *hip1*Δ (MC3725) cells. Horizontal bars represent means of fold change and mean values are shown below the abscissa axis. Fold change in expression of all genes (A), CESR genes (B), and genes whose expression was increased more than twofold in wild-type cells (C) is shown. Significant difference in expression between wild-type and mutant cells was determined by the Student's *t*-test (* $P<0.05$, ** $P<0.01$).

Figure 7. HIRA is required for nucleosome eviction at stress-responsive gene locus.

Wild-type (JK317) and *slm9*Δ (MC3749) cells were exposed or not exposed to 40°C for 15 min. Mononucleosomal DNA was isolated from the cells and nucleosome-scanning analysis was performed. Real-time PCR was carried out using 10 overlapping primer sets along *ctt1*$^+$ gene. Nucleosomal DNA enrichment is defined by the ratio of the amplified products with mononucleosomal DNA to genomic DNA. Results are means of three independent experiments and error bars represent standard error. Inferred locations of nucleosomes (light gray ovals) with respect to ORF (white rectangle) and TSS (black arrow) are shown. TATA box (-30 to -23) and Atf1 binding site (-57 to -50) are also represented as black rectangle and dark gray rectangle, respectively (55).

Figure 8. Priming stress enhances stress-responsive gene expression under severe stress.
(A) Wild-type (JK317) cells were either not exposed to stress (no stress) or exposed to various stresses: 40°C for 1 h (priming stress alone), 25 mM H_2O_2 for 1 h (severe stress alone), or 40°C for 1 h followed by 25 mM H_2O_2 for 1 h (priming stress followed by severe stress). Total RNA was analyzed by quantitative RT-PCR using primer sets for stress-responsive genes (*ctt1*$^+$, *gpx1*$^+$, and *hsp9*$^+$) and non-stress-responsive gene (*ade6*$^+$). Values shown were normalized to *act1*$^+$ expression. Results are means of five independent experiments and error bars represent standard error. (B) Fold change of CESR gene expression under three stress conditions compared to no stress condition is plotted for wild-type (JK317) cells. The three stress conditions are as follows: priming stress alone (40°C for 1 h, 1st), severe stress alone (25 mM H_2O_2 for 1 h, 2nd), and priming stress followed by severe stress (40°C for 1 h followed by 25 mM H_2O_2 for 1 h, 1st+2nd). Horizontal bars represent means of fold change and mean values are shown below the abscissa axis.

Table 1 *S. pombe* strains used in this study

Strain	Genotype
JK316	*h*$^{+}$ *leu1-32 ura4-D18*
JK317	*h*$^{-}$ *leu1-32 ura4-D18*
YT2272	*h*$^{-}$ *leu1-32 ura4-D18 spc1* ::*kanMX6*
MC3725	*h*$^{-}$ *leu1-32 ura4-D18 hip1* ::*ura4*$^{+}$
MC3749	*h*$^{-}$ *leu1-32 ura4-D18 slm9* ::*ura4*$^{+}$
MC3768	*h*$^{-}$ *leu1-32 ura4-D18 pcf1* ::*kanMX6*
MC3773	*h*$^{-}$ *leu1-32 ura4-D18 hip3* ::*ura4*$^{+}$
MC3793	*h*$^{-}$ *leu1-32 ura4-D18 slm9-12myc* (*ura4*$^{+}$)
MC3795	*h*$^{-}$ *leu1-32 ura4-D18 hip1-12myc* (*ura4*$^{+}$)
MC3797	*h*$^{-}$ *leu1-32 ura4-D18 slm9-GFP* (*ura4*$^{+}$)
MC3799	*h*$^{-}$ *leu1-32 ura4-D18 hip4* ::*ura4*$^{+}$
MC3801	*h*$^{-}$ *leu1-32 ura4-D18 spc1* ::*ura4*$^{+}$
MC3849	*h*$^{-}$ *leu1-32 ura4-D18 nap1* ::*ura4*$^{+}$
MC4219	*h*$^{-}$ *leu1-32 ura4-D18 slm9-12myc* (*ura4*$^{+}$) *atf1* ::*hphMX6*

Table 2 Principal gene ontology terms enriched in priming-stress-induced genes

GO term	Number of induced genes in	% of induced genes in term	Number of total genes in term	% of total genes in term	p-value
WT					
cellular response to stress	115	72.8	694	14.1	9.97E-38
meiosis	42	26.6	353	7.2	7.26E-05
cellular response to oxidative stres	16	10.1	60	1.2	7.26E-05
oxidoreductase activity	37	23.4	275	5.6	1.38E-04
M phase	42	26.6	518	10.5	2.70E-02
slm9 **Δ**					
cellular response to stress	80	69.0	694	14.1	2.20E-26
meiosis	43	37.1	353	7.2	1.02E-06
M phase	43	37.1	518	10.5	3.22E-04
hip1 **Δ**					
cellular response to stress	65	68.4	694	14.1	4.33E-19
meiosis	35	36.8	353	7.2	1.38E-04
M phase	35	36.8	518	10.5	1.25E-02

Table 3 Gene ontology terms enriched in genes that showed WT fold change/mutant fold change≧2 under priming stress condition

GO term	Number of selected genes in	% of selected genes in term	Number of total genes in term	% of total genes in term	p-value
WT fold change/*slm9*Δ fold change≧2					
cellular response to stress	47	92.2	694	14.1	1.83E-19
cellular response to stimulus	47	92.2	730	14.9	1.83E-19
response to stress	47	92.2	733	14.9	1.42E-18
response to stimulus	47	92.2	819	16.7	1.68E-18
oxidoreductase activity	15	29.4	275	5.6	3.11E-03
oxidoreductase activity, acting on CH-OH group of donors	2	3.9	63	1.3	3.40E-03
response to oxidative stress	8	15.7	69	1.4	5.84E-03
oxidation reduction	14	27.5	243	4.9	9.38E-03
oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accept	2	3.9	58	1.2	1.17E-02
cellular response to oxidative stress	7	13.7	60	1.2	1.33E-02
WT fold change/*hip1*Δ fold change≧2					
cellular response to stress	52	74.3	694	14.1	3.25E-23
cellular response to stimulus	52	74.3	730	14.9	3.25E-23
response to stress	52	74.3	733	14.9	3.17E-22
response to stimulus	52	74.3	819	16.7	5.27E-22
oxidoreductase activity	16	22.9	275	5.6	1.36E-03
oxidoreductase activity, acting on CH-OH group of donors	2	2.9	63	1.3	4.51E-03
oxidation reduction	15	21.4	243	4.9	4.51E-03
response to oxidative stress	8	11.4	69	1.4	7.70E-03
oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accept	2	2.9	58	1.2	1.68E-02
cellular response to oxidative stress	7	10.0	60	1.2	1.91E-02

Figure 1

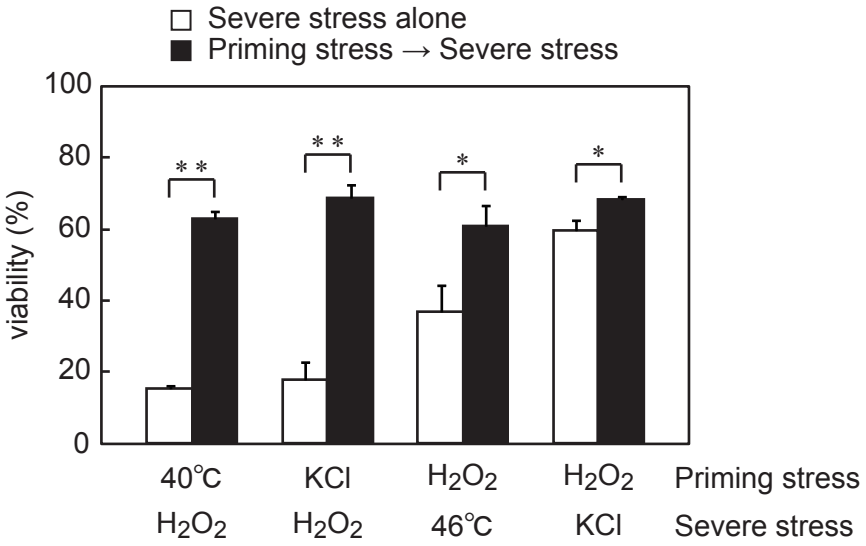

Severe stress alone
Priming stress → Severe stress
viability (%)
100
80
60
40
20
0
* *
* *
*
*
40℃
H2O2
KCl
H2O2
H2O2
46℃
H2O2
KCl
Priming stress
Severe stress

Figure 2

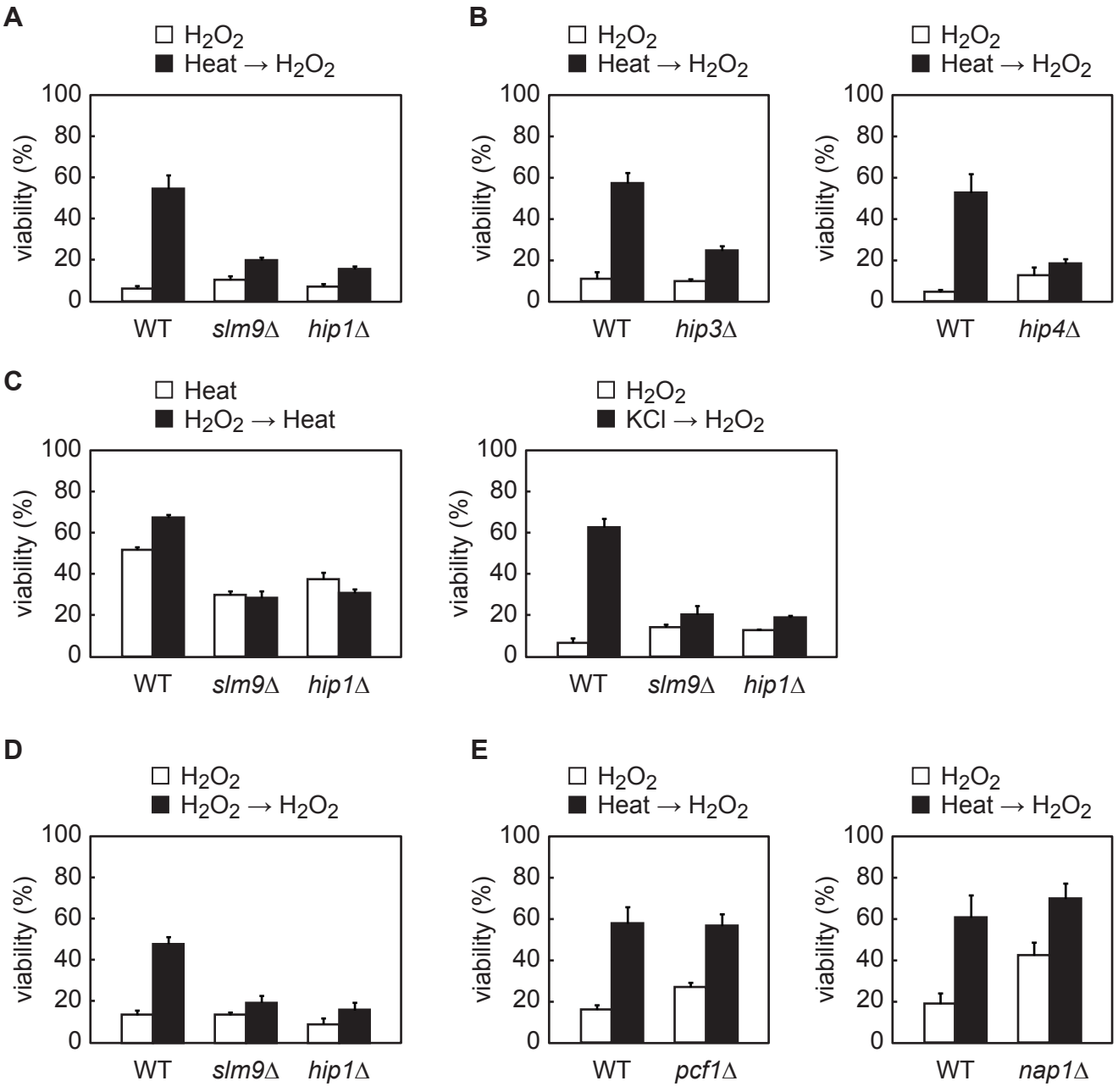

A
□ H_2O_2
■ Heat → H_2O_2
viability (%)
100
80
60
40
20
0
WT
*slm9*Δ
*hip1*Δ
B
□ H_2O_2
■ Heat → H_2O_2
viability (%)
WT
*hip3*Δ
□ H_2O_2
■ Heat → H_2O_2
viability (%)
WT
*hip4*Δ
C
□ Heat
■ H_2O_2 → Heat
viability (%)
WT
*slm9*Δ
*hip1*Δ
□ H_2O_2
■ KCl → H_2O_2
viability (%)
WT
*slm9*Δ
*hip1*Δ
D
□ H_2O_2
■ H_2O_2 → H_2O_2
viability (%)
WT
*slm9*Δ
*hip1*Δ
E
□ H_2O_2
■ Heat → H_2O_2
viability (%)
WT
*pcf1*Δ
□ H_2O_2
■ Heat → H_2O_2
viability (%)
WT
*nap1*Δ

Figure 3

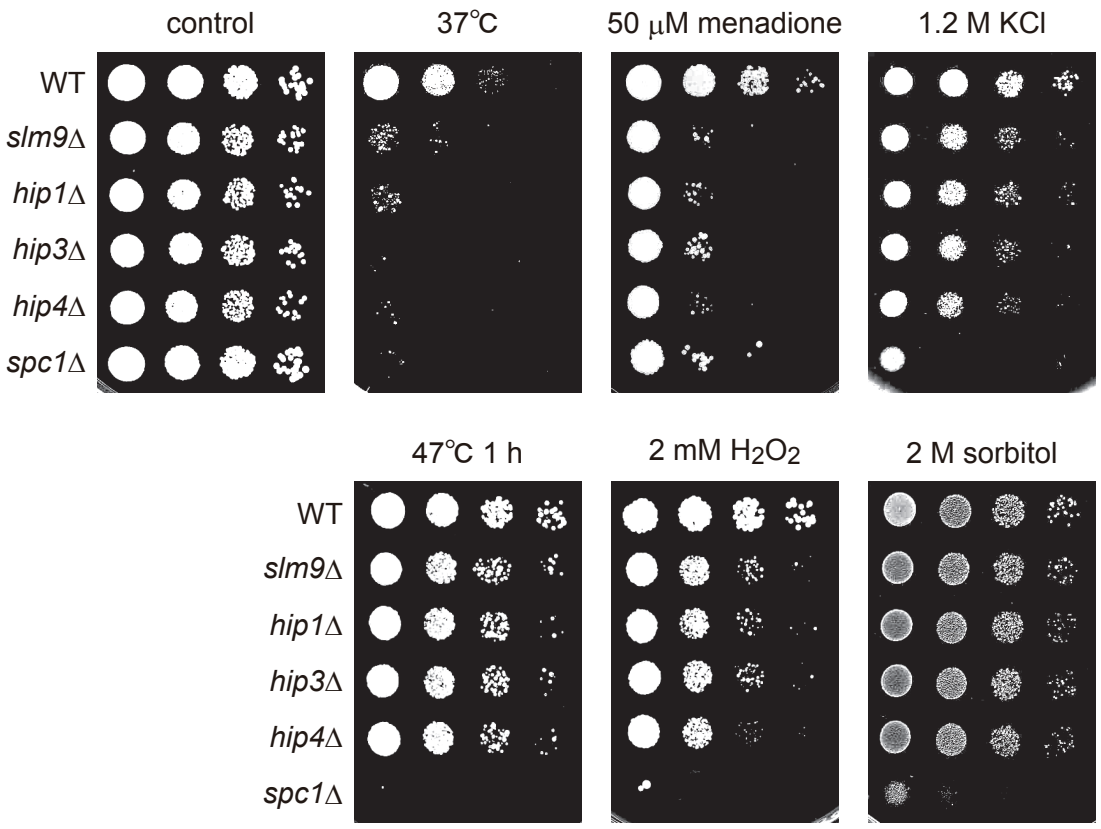

control
37°C
50 μM menadione
1.2 M KCl
WT
slm9Δ
hip1Δ
hip3Δ
hip4Δ
spc1Δ
47°C 1 h
2 mM H2O2
2 M sorbitol
WT
slm9Δ
hip1Δ
hip3Δ
hip4Δ
spc1Δ

Figure 4

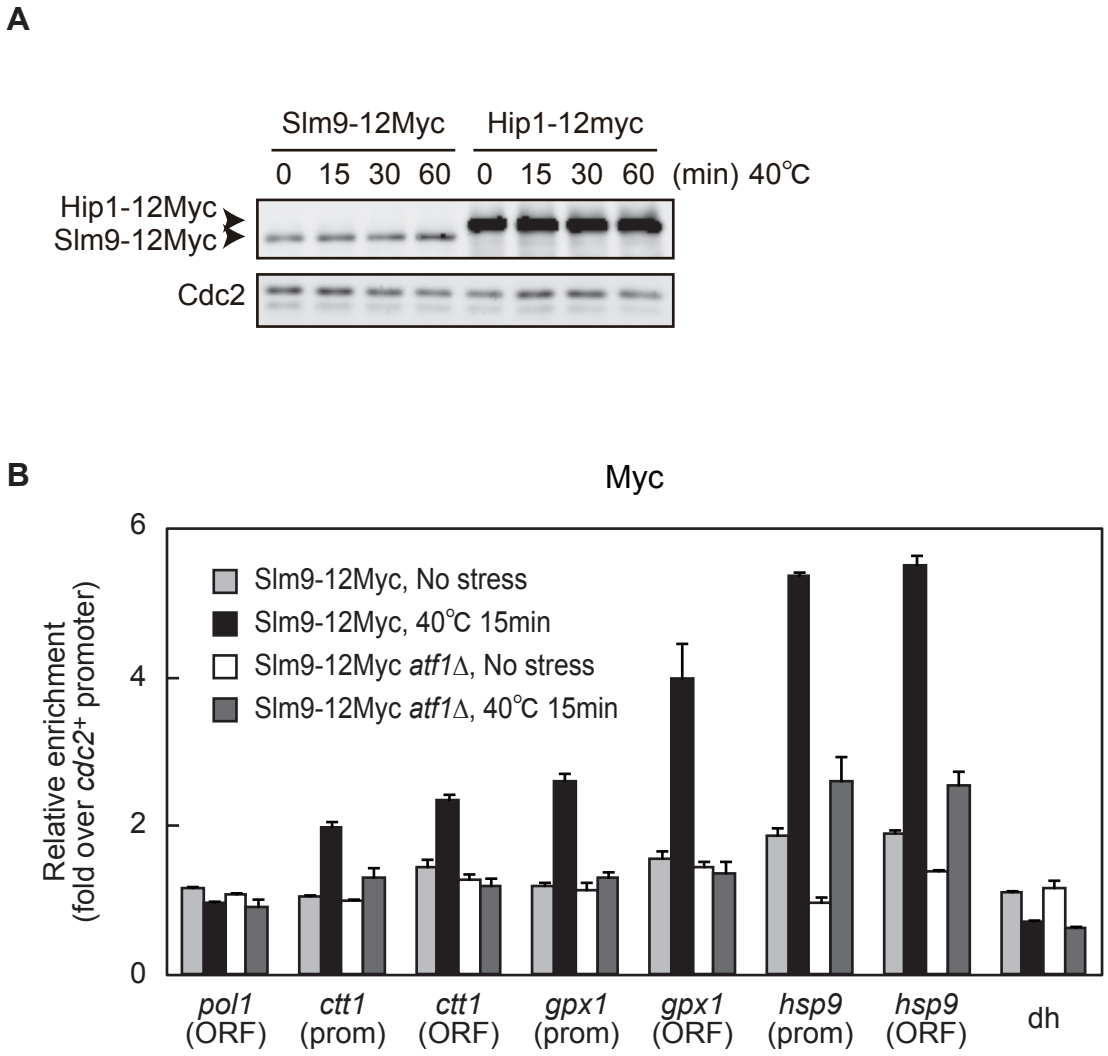

A
Slm9-12Myc
Hip1-12myc
0 15 30 60 0 15 30 60 (min) 40℃
Hip1-12Myc
Slm9-12Myc
Cdc2
B
Myc
Relative enrichment
(fold over cdc2+ promoter)
Slm9-12Myc, No stress
Slm9-12Myc, 40℃ 15min
Slm9-12Myc atf1Δ, No stress
Slm9-12Myc atf1Δ, 40℃ 15min
pol1 (ORF)
ctt1 (prom)
ctt1 (ORF)
gpx1 (prom)
gpx1 (ORF)
hsp9 (prom)
hsp9 (ORF)
dh

Figure 5

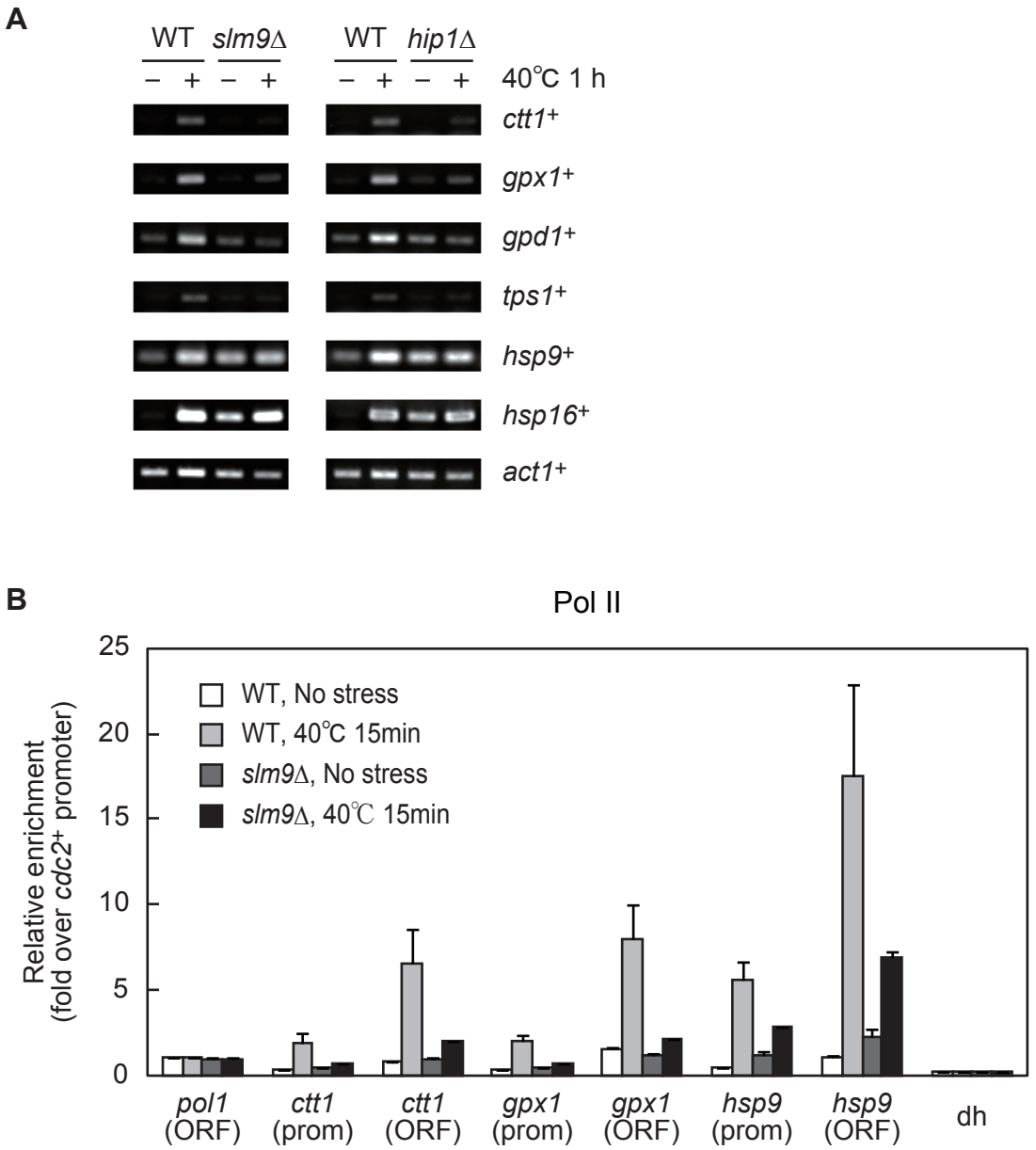

A
WT
slm9Δ
WT
hip1Δ
− + − +
− + − +
40℃ 1 h
ctt1+
gpx1+
gpd1+
tps1+
hsp9+
hsp16+
act1+
B
Pol II
Relative enrichment
(fold over cdc2+ promoter)
25
20
15
10
5
0
WT, No stress
WT, 40℃ 15min
slm9Δ, No stress
slm9Δ, 40℃ 15min
pol1
(ORF)
ctt1
(prom)
ctt1
(ORF)
gpx1
(prom)
gpx1
(ORF)
hsp9
(prom)
hsp9
(ORF)
dh

Figure 6

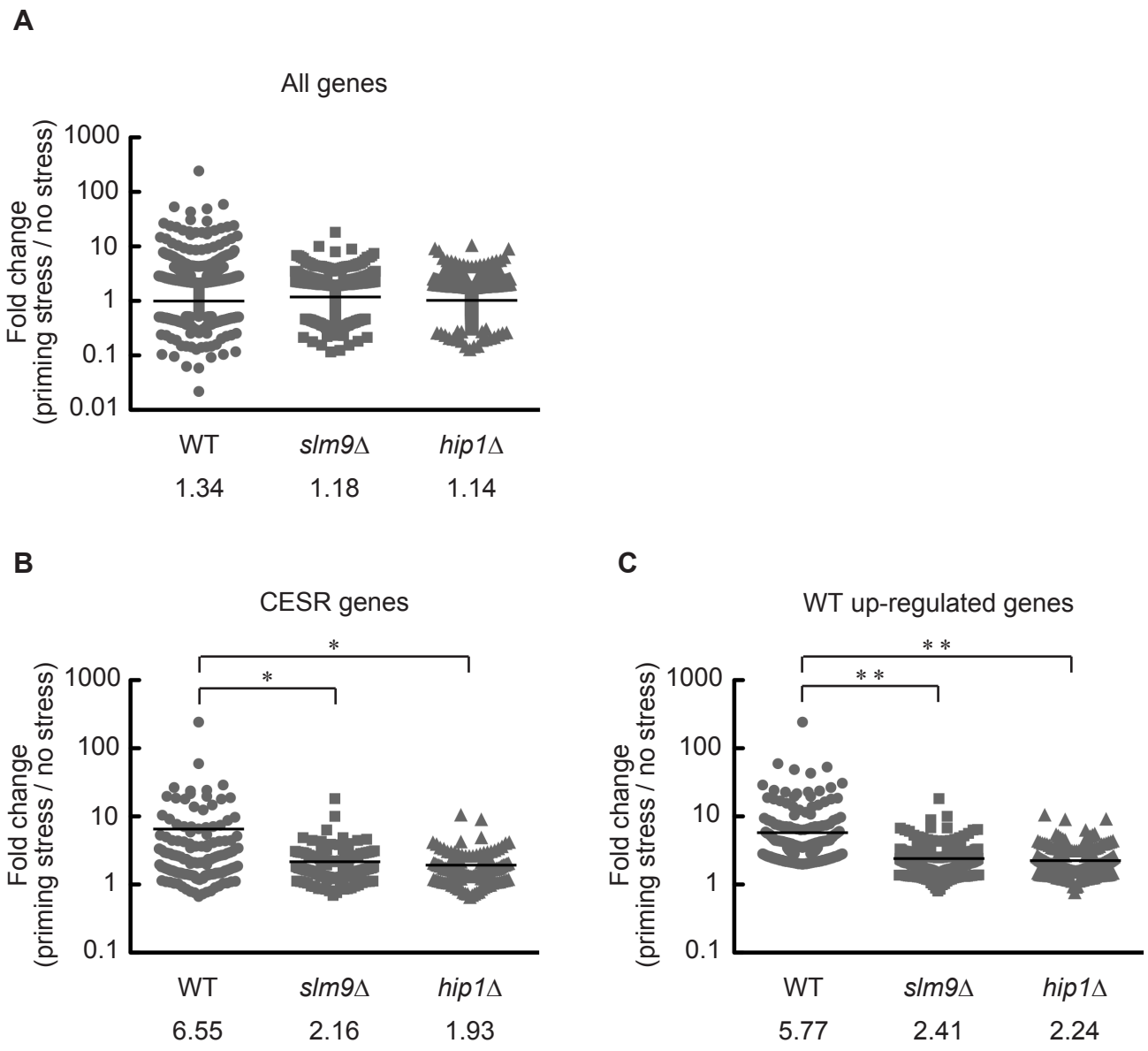

A
All genes
Fold change (priming stress / no stress)
1000
100
10
1
0.1
0.01
WT
slm9Δ
hip1Δ
1.34
1.18
1.14
B
CESR genes
*
*
6.55
2.16
1.93
C
WT up-regulated genes
**
**
5.77
2.41
2.24

Figure 7

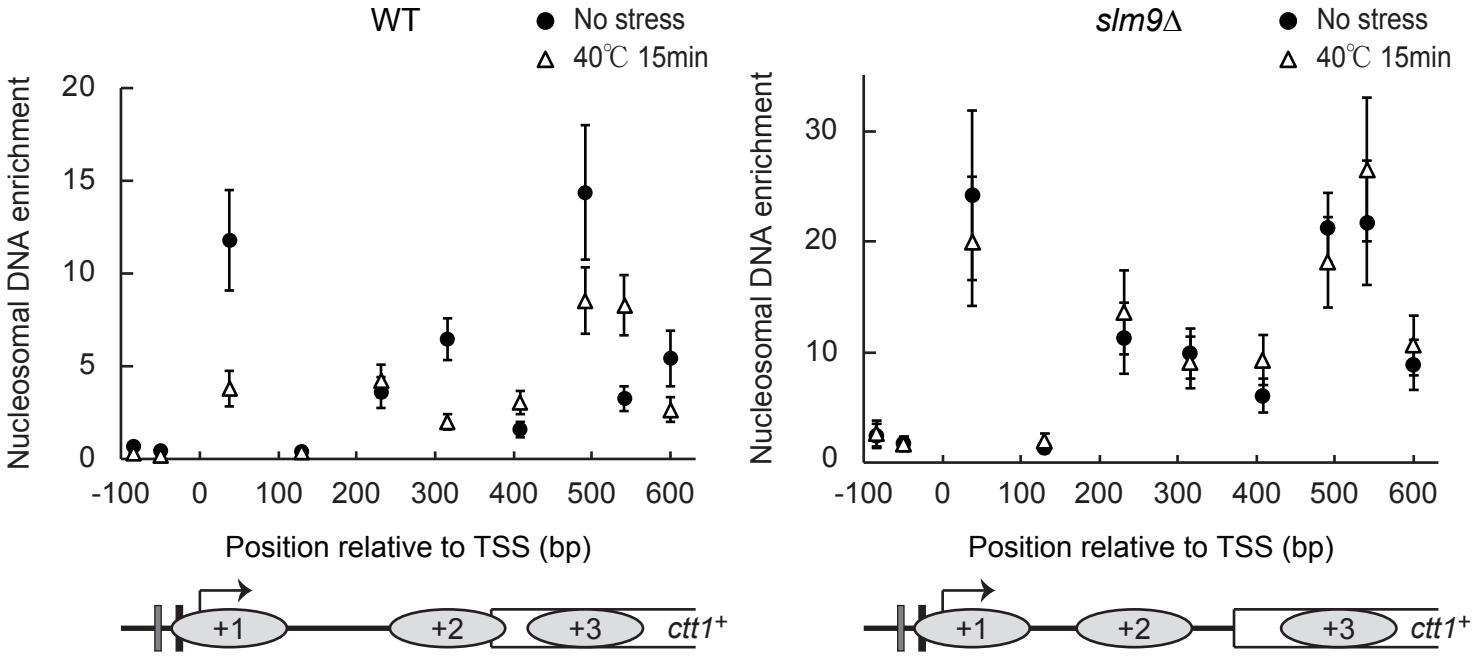
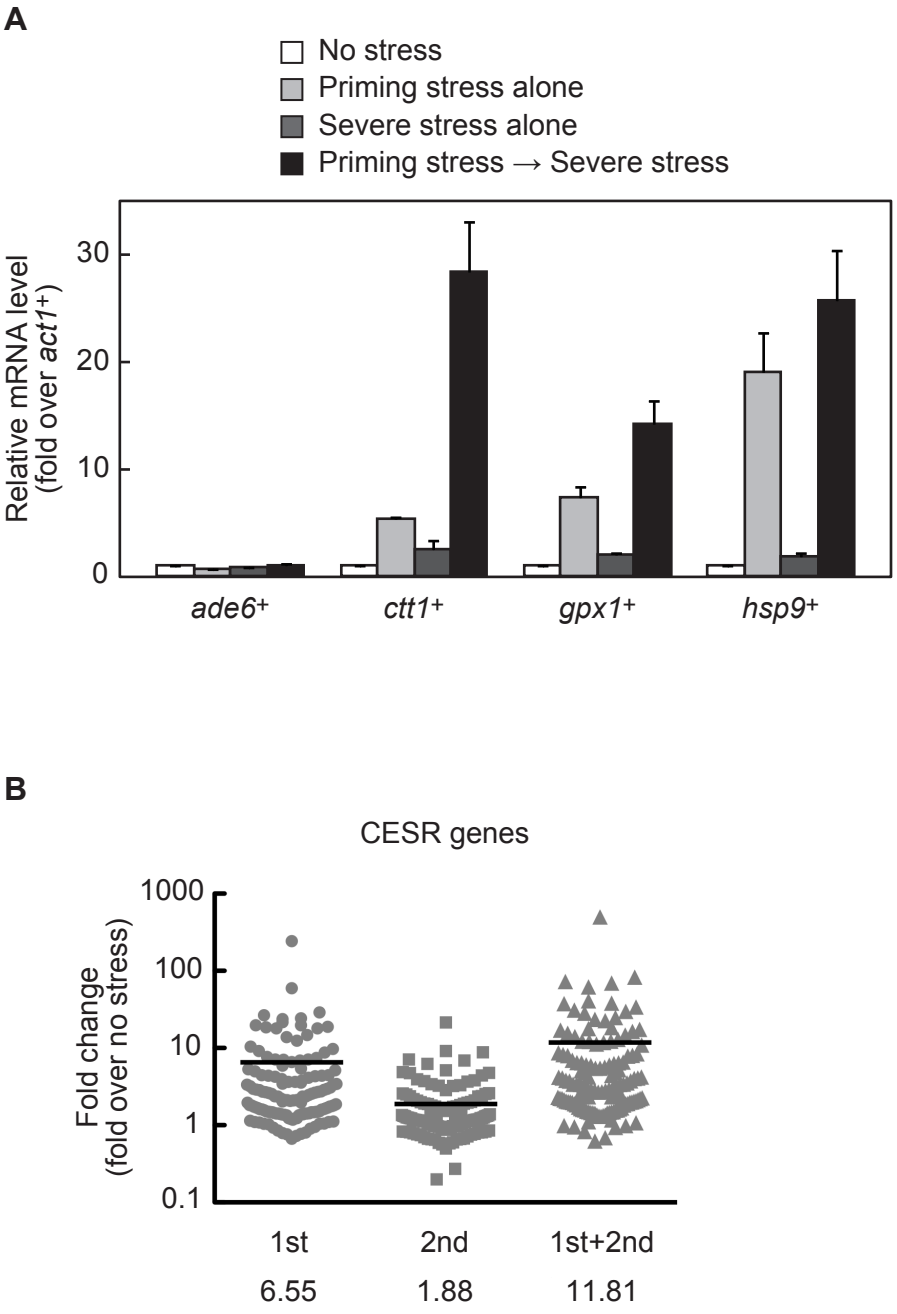

WT
No stress
40℃ 15min
Nucleosomal DNA enrichment
20
15
10
5
0
-100
0
100
200
300
400
500
600
Position relative to TSS (bp)
+1
+2
+3
ctt1+
slm9Δ
No stress
40℃ 15min
Nucleosomal DNA enrichment
30
20
10
0
-100
0
100
200
300
400
500
600
Position relative to TSS (bp)
+1
+2
+3
ctt1+

Figure 8


A
No stress
Priming stress alone
Severe stress alone
Priming stress → Severe stress
Relative mRNA level
(fold over act1+)
30
20
10
0
ade6+
ctt1+
gpx1+
hsp9+
B
CESR genes
Fold change
(fold over no stress)
1000
100
10
1
0.1
1st
2nd
1st+2nd
6.55
1.88
11.81

Supplemental Information

HIRA, a conserved histone chaperone plays an essential role in low-dose stress response via transcriptional stimulation in fission yeast

Moeko Chujo[1], Yusuke Tarumoto[1], Koichi Miyatake[2], Eisuke Nishida[2], and Fuyuki Ishikawa[1]

From the Departments of [1]Gene Mechanisms and [2]Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

Figure S1

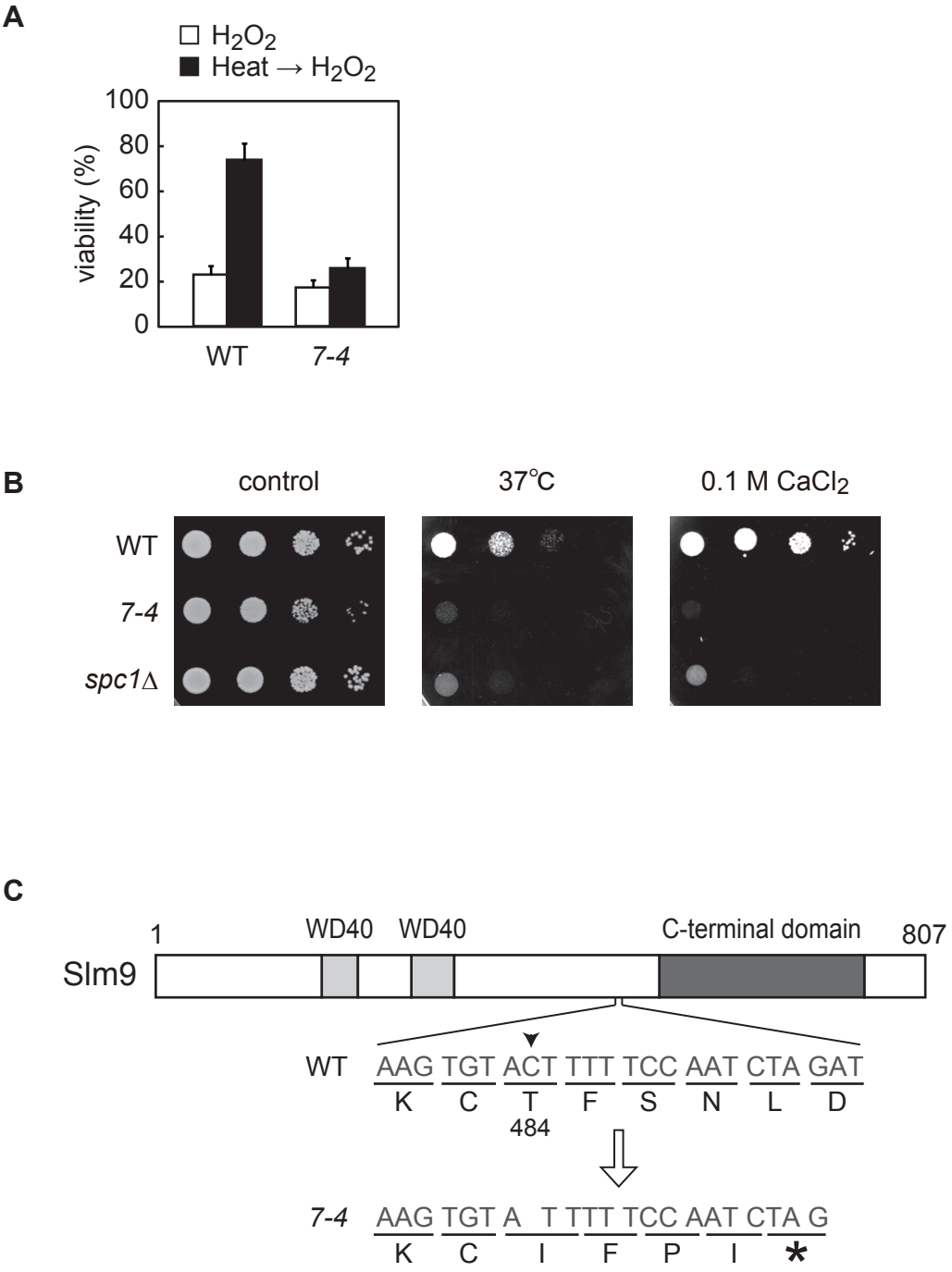


Figure S1. Characterization of cross-tolerance-defective mutant, *7-4*.
(A) Viability of wild-type (JK317) and *7-4* cells following exposure to 40°C for 1 h (priming stress) and 25 mM H_2O_2 for 1 h (severe stress) is shown. Results are means of six independent experiments and error bars represent standard error. (B) Tenfold serial dilutions of wild-type (JK317), *7-4*, and *spc1*Δ (YT2272) cells were spotted onto YES plates or YES plates containing $CaCl_2$. For heat stress, the spotted YES plates were incubated at 37°C. (C) Schematic structure of Slm9 protein. Slm9 has WD40 motifs (light grey) and a C-terminal domain (dark grey) that is well-conserved among HIRA family proteins from many eukaryotes (19,24). A single nucleotide deletion occurred at nucleotide 1451 (arrowhead) in *7-4* mutant, causing the reading frame shift.

Figure S2

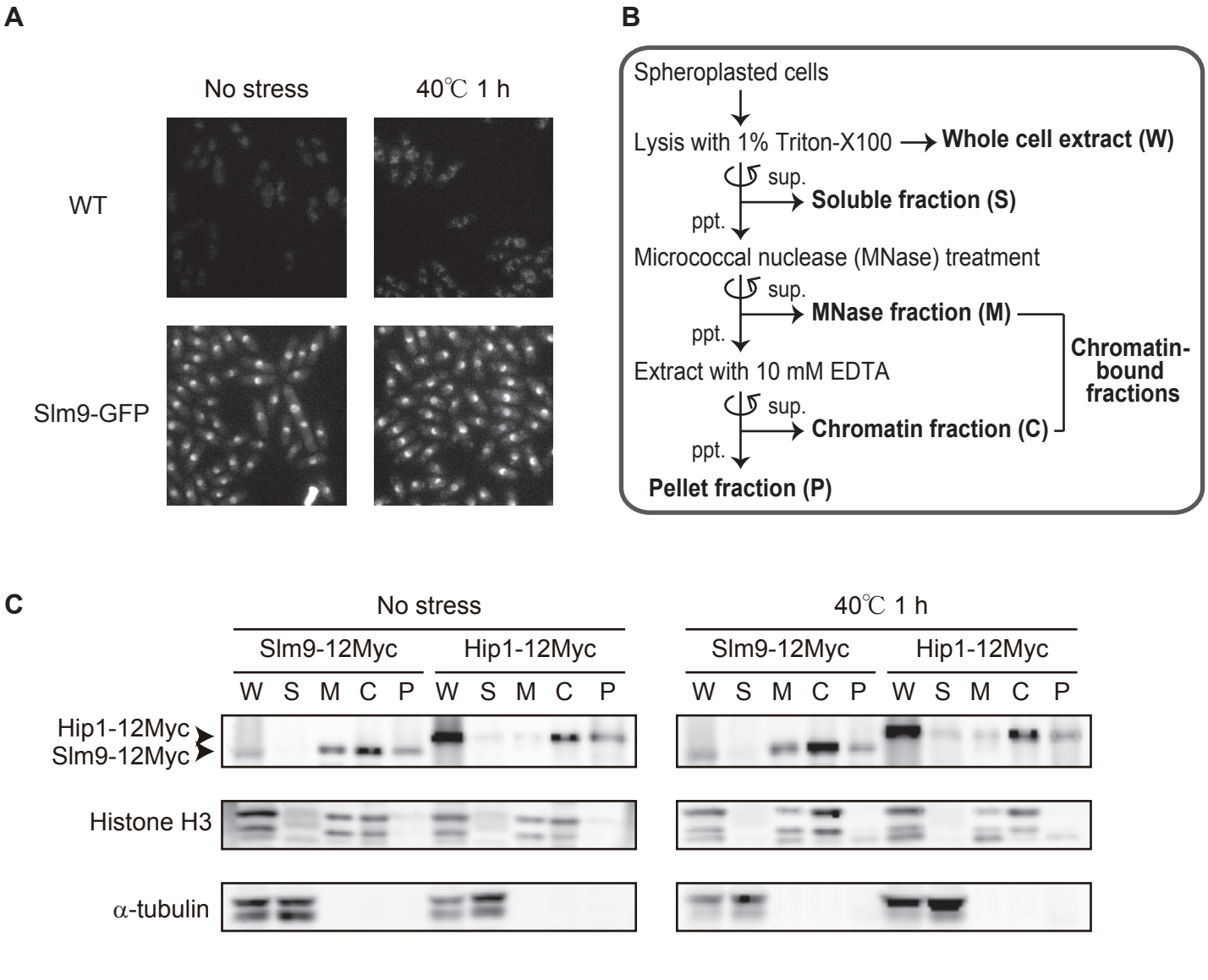


Figure S2. Nuclear localization and chromatin association of HIRA are unaffected by stress treatment.

(A) Wild-type (JK317) and *slm9-GFP* (MC3797) cells were grown in YES medium at 32°C and exposed or not exposed to 40°C for 1 h. Cells were washed with PBS and living cells in PBS were observed without fixation under a DeltaVision microscope (Applied Precision). (B) Procedure for the chromatin fractionation assay used in this study. (C) *slm9-12myc* (MC3793) and *hip1-12myc* (MC3795) cells were exposed or not exposed to 40°C for 1 h. Chromatin fractionation assay was performed as shown in (B). Proteins from each fraction were analyzed by Western blotting with anti-Myc, anti-histone H3, and anti-α-tubulin antibodies. The soluble fraction contains nucleoplasmic and cytoplasmic proteins, and the MNase fraction and the chromatin fraction contain chromatin-bound proteins. Histone H3 and α-tubulin were used as controls for the chromatin-bound fractions and the soluble fraction, respectively. W, whole cell extract; S, soluble fraction; M, MNase fraction; C, chromatin fraction; P, pellet fraction.

Figure S3

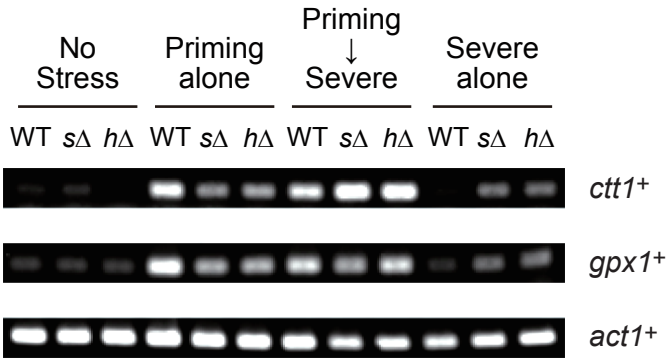


Figure S3. CESR gene expression is augmented under severe stress in HIRA disruptants.
Wild-type (JK317), *slm9*Δ (MC3749), and *hip1*Δ (MC3725) cells were either not exposed to stress (no stress) or exposed to various stresses: 40°C for 1 h (priming stress alone), 40°C for 1 h followed by 25 mM H_2O_2 for 1 h (priming stress followed by severe stress), or 25 mM H_2O_2 for 1 h (severe stress alone). Total RNA was analyzed by RT-PCR using primer sets for stress-responsive genes (*ctt1*$^+$ and *gpx1*$^+$). *act1*$^+$ is shown as loading control. *s*Δ, *slm9*Δ; *h*Δ, *hip1*Δ.

Figure S4

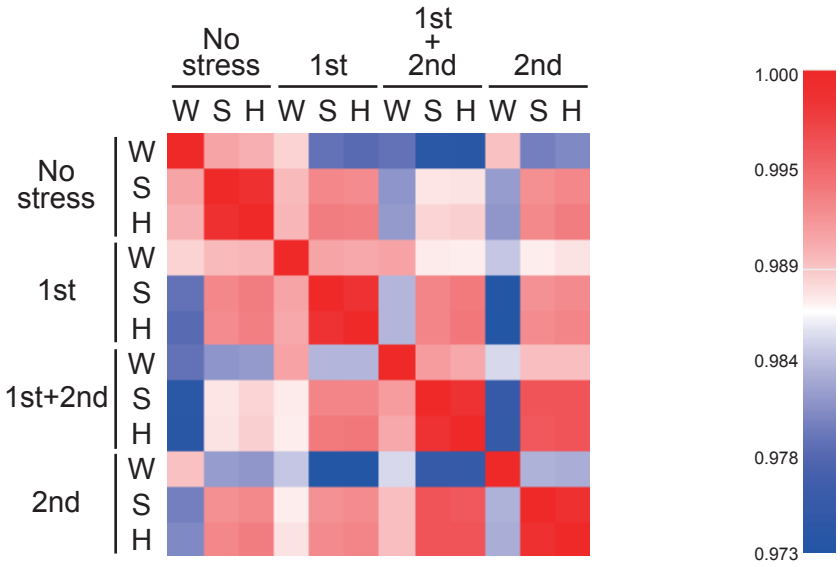


Figure S4. Gene expression in *slm9*Δ and *hip1*Δ cells shows strong correlation.
Microarray analysis was performed for wild-type (JK317), *slm9*Δ (MC3749), and *hip1*Δ (MC3725) cells under four conditions: control (No stress), priming stress alone (40°C for 1 h, 1st), priming stress followed by severe stress (40°C for 1 h followed by 25 mM H_2O_2 for 1 h, 1st+2nd), and severe stress alone (25 mM H_2O_2 for 1 h, 2nd). Pearson's correlation coefficient (r^2) was calculated for each pair of arrays and shown as a heat map. W, wild-type; S, *slm9*Δ; H, *hip1*Δ.

Table S1 Primers for real-time PCR

Locus	Forward primer	Reverse primer
act1 (ORF)	5'-AGCGTGGTTATACTTTCTCTACT-3'	5'-GGAGGAAGATTGAGCAGCAG-3'
ade6 (ORF)	5'-GAAAGATGCTGCCGTCATTTTAG-3'	5'-GCTGCGGTACGAGCATAAGTAAC-3'
cdc2 (promoter)	5'-ACATGAAGCGCTAGCCCTAAGTT-3'	5'-TCAACTAGCGATAGACTAGTGGAAACGCAGAGGA-3'
pol1 (ORF)	5'-AAGACGGTCTGCAAGAAGAATCTC-3'	5'-GCTCAAAAACCAATCCACCTTT-3'
ctt1 (promoter)	5'-CGCTAATAATGATGCTCTTTGGC-3'	5'-CAATAGGAAAACTCTTACCAACGC-3'
ctt1 (ORF)	5'-AAGAACGTTGCCGGTCACTT-3'	5'-TGGCGTTCACGTACAGGAGAT-3'
gpx1 (promoter)	5'-TGTTGTAACTAACGCAAACTACTTAATCG-3'	5'-GGCTGAGACTCATACTTAAACAGTATAGGTATC-3'
gpx1 (ORF)	5'-AAGATCAATGTTAATGGCGACAAC-3'	5'-GATGACTTGACCTTGACGATTGAC-3'
hsp9 (promoter)	5'-CGAATGGTGCGAAGAAAAGG-3'	5'-GCTCGCTATCCAATCAGACAAA-3'
hsp9 (ORF)	5'-AAGGTCGCCTCTGCTTTTACC-3'	5'-AGCGTTGAGCCTTGTCATGAG-3'
dh	5'-AACAAAGCGACAATAGCAGTC-3'	5'-TAGCTTGTTGACATAATGAAGACCAA-3'
ctt1-1	5'-GATGCTCTTTGGCTCACTAAGC-3'	5'-TGTAGAATTACCAACGTCATATTTGC-3'
ctt1-2	5'-TGGAATCTCGGCCATTTG-3'	5'-TATATTCAAGCAACTTGCAATTG-3'
ctt1-3	5'-AATTGCAAGTTGCTTGAATATACAGC-3'	5'-GAACAAGGGAATTACAATCACACAC-3'
ctt1-4	5'-TGTGTGTGATTGTAATTCCCTTG-3'	5'-AAAAAGACAGTCAAAAAATTGTGTT-3'
ctt1-5	5'-GACTGTCTTTTTTTTTCCCCTCC-3'	5'-GAGCAAATGATTTTAAACTAGCTTGTC-3'
ctt1-6	5'-AGCTAGTTTAAAATCATTTGCTCG-3'	5'-TTCGCTGATGTTTGAATCCTTAG-3'
ctt1-7	5'-GGATTCAAACATCAGCGAAATG-3'	5'-TTACCCACACGAGCCGCA-3'
ctt1-8	5'-CTGCGGCTCGTGTGGGTA-3'	5'-CAGGAATACGCTCGCGATC-3'
ctt1-9	5'-CATTTAATCGACGTCTTTCAACAC-3'	5'-CGGTGCATTCGAATTCACC-3'
ctt1-10	5'-CATGCAAAGGGTTCCGGT-3'	5'-TAGGGGTTTTCTTACCAACCTTAG-3'

Table S2 Gene ontology terms enriched in stress-induced genes

Stress condition	GO term	Number of induced genes in	% of induced genes in term	Number of total genes in term	% of total genes in term	p-value
priming stress alone	**WT**					
	cellular response to stress	115	72.8	694	14.1	9.97E-38
	cellular response to stimulus	115	72.8	730	14.9	1.24E-37
	response to stress	118	74.7	733	14.9	2.56E-35
	response to stimulus	118	74.7	819	16.7	2.90E-33
	response to oxidative stress	17	10.8	69	1.4	3.08E-05
	meiosis	42	26.6	353	7.2	7.26E-05
	cellular response to oxidative stress	16	10.1	60	1.2	7.26E-05
	M phase of meiotic cell cycle	42	26.6	353	7.2	7.26E-05
	meiotic cell cycle	42	26.6	355	7.2	7.49E-05
	oxidoreductase activity	37	23.4	275	5.6	1.38E-04
	cellular response to chemical stimulus	16	10.1	119	2.4	2.10E-03
	oxidation reduction	34	21.5	243	4.9	2.81E-03
	oxidoreductase activity, acting on CH-OH group of donors	3	1.9	63	1.3	3.22E-03
	oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accepto	2	1.3	58	1.2	5.38E-03
	M phase	42	26.6	518	10.5	2.70E-02
	slm9 **Δ**					
	response to stress	83	71.6	733	14.9	2.20E-26
	cellular response to stress	80	69.0	694	14.1	2.20E-26
	cellular response to stimulus	80	69.0	730	14.9	1.15E-24
	response to stimulus	83	71.6	819	16.7	8.79E-23
	meiosis	43	37.1	353	7.2	1.02E-06
	M phase of meiotic cell cycle	43	37.1	353	7.2	1.02E-06
	meiotic cell cycle	43	37.1	355	7.2	1.06E-06
	M phase	43	37.1	518	10.5	3.22E-04
	cell cycle phase	43	37.1	550	11.2	8.97E-04
	cell cycle process	43	37.1	594	12.1	2.00E-03
	hip1 **Δ**					
	response to stress	68	71.6	733	14.9	4.33E-19
	cellular response to stress	65	68.4	694	14.1	4.33E-19
	cellular response to stimulus	65	68.4	730	14.9	1.21E-17
	response to stimulus	68	71.6	819	16.7	1.60E-16
	meiosis	35	36.8	353	7.2	1.38E-04
	M phase of meiotic cell cycle	35	36.8	353	7.2	1.38E-04
	meiotic cell cycle	35	36.8	355	7.2	1.38E-04
	M phase	35	36.8	518	10.5	1.25E-02
	cell cycle phase	35	36.8	550	11.2	2.45E-02
	cell cycle process	35	36.8	594	12.1	3.66E-02
priming stress and severe stress	**WT**					
	response to stress	172	77.5	733	14.9	0.00E+00
	cellular response to stress	170	76.6	694	14.1	0.00E+00
	response to stimulus	172	77.5	819	16.7	0.00E+00
	cellular response to stimulus	170	76.6	730	14.9	0.00E+00
	oxidoreductase activity	51	23.0	275	5.6	3.40E-07
	response to oxidative stress	22	9.9	69	1.4	3.70E-07
	cellular response to oxidative stress	21	9.5	60	1.2	3.20E-06
	oxidation reduction	45	20.3	243	4.9	7.97E-04
	oxidoreductase activity, acting on CH-OH group of donors	3	1.4	63	1.3	5.55E-03
	cellular response to chemical stimulus	21	9.5	119	2.4	9.44E-03
	response to chemical stimulus	22	9.9	196	4.0	2.75E-02
	slm9 **Δ**					
	response to stress	153	90.5	733	14.9	0.00E+00
	cellular response to stress	152	89.9	694	14.1	0.00E+00
	response to stimulus	153	90.5	819	16.7	0.00E+00
	cellular response to stimulus	152	89.9	730	14.9	0.00E+00
	oxidoreductase activity	50	29.6	275	5.6	5.09E-10
	oxidoreductase activity, acting on CH-OH group of donors	6	3.6	63	1.3	8.76E-08
	oxidation reduction	45	26.6	243	4.9	3.82E-07
	oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accepto	5	3.0	58	1.2	6.79E-07
	response to oxidative stress	16	9.5	69	1.4	5.42E-04
	cellular response to oxidative stress	16	9.5	60	1.2	1.60E-03
	carbohydrate catabolic process	1	0.6	76	1.5	2.12E-03
	hip1 **Δ**					
	response to stress	145	89.0	733	14.9	0.00E+00
	cellular response to stress	143	87.7	694	14.1	0.00E+00
	response to stimulus	145	89.0	819	16.7	0.00E+00
	cellular response to stimulus	143	87.7	730	14.9	0.00E+00
	oxidoreductase activity	51	31.3	275	5.6	3.91E-12
	oxidation reduction	46	28.2	243	4.9	7.55E-09
	oxidoreductase activity, acting on CH-OH group of donors	6	3.7	63	1.3	1.58E-08
	oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accepto	5	3.1	58	1.2	1.66E-07
	response to oxidative stress	16	9.8	69	1.4	1.67E-04
	carbohydrate catabolic process	1	0.6	76	1.5	7.36E-04
	cellular response to oxidative stress	15	9.2	60	1.2	2.83E-03
severe stress alone	**WT**					
	cellular response to stress	35	97.2	694	14.1	1.28E-11
	response to stress	36	100.0	733	14.9	3.71E-11
	cellular response to stimulus	35	97.2	730	14.9	3.71E-11
	response to stimulus	36	100.0	819	16.7	4.27E-10
	slm9 **Δ**					
	cellular response to stress	85	77.3	694	14.1	1.97E-36
	response to stress	85	77.3	733	14.9	7.23E-35
	cellular response to stimulus	85	77.3	730	14.9	7.23E-35
	response to stimulus	85	77.3	819	16.7	8.68E-33
	oxidoreductase activity	30	27.3	275	5.6	5.15E-09
	oxidoreductase activity, acting on CH-OH group of donors	4	3.6	63	1.3	3.87E-07

oxidation reduction	27	24.5	243	4.9	2.68E-06
oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accepto	4	3.6	58	1.2	1.04E-05
response to oxidative stress	11	10.0	69	1.4	5.01E-04
cellular response to oxidative stress	11	10.0	60	1.2	7.83E-04
meiosis	25	22.7	353	7.2	2.69E-03
M phase of meiotic cell cycle	25	22.7	353	7.2	2.69E-03
meiotic cell cycle	25	22.7	355	7.2	2.82E-03
cellular carbohydrate metabolic process	4	3.6	216	4.4	3.05E-02
***hip1* Δ**					
cellular response to stress	78	80.4	694	14.1	1.22E-35
response to stress	78	80.4	733	14.9	3.08E-34
cellular response to stimulus	78	80.4	730	14.9	3.08E-34
response to stimulus	78	80.4	819	16.7	1.34E-31
oxidoreductase activity	27	27.8	275	5.6	1.01E-08
oxidoreductase activity, acting on CH-OH group of donors	4	4.1	63	1.3	5.41E-06
oxidation reduction	24	24.7	243	4.9	9.43E-06
response to oxidative stress	11	11.3	69	1.4	9.77E-05
oxidoreductase activity, acting on CH-OH group of donors, NAD or NADP as accepto	4	4.1	58	1.2	1.09E-04
cellular response to oxidative stress	11	11.3	60	1.2	1.45E-04
cellular carbohydrate metabolic process	4	4.1	216	4.4	4.37E-03
carbohydrate metabolic process	8	8.2	226	4.6	7.45E-03
monosaccharide metabolic process	2	2.1	68	1.4	1.93E-02
glucose metabolic process	2	2.1	42	0.9	2.73E-02
alcohol metabolic process	3	3.1	145	3.0	2.73E-02

Table S3 Gene ontology terms enriched in stress-repressed genes

Stress condition	GO term	Number of repressed genes in	% of repressed genes in term	Number of total genes in term	% of total genes in term	p-value
priming stress alone	**WT**					
	extracellular region	15	33.3	48	1.0	2.69E-07
	cell wall	13	28.9	58	1.2	2.69E-07
	external encapsulating structure	13	28.9	58	1.2	2.69E-07
	cell surface	14	31.1	111	2.3	4.54E-07
	cell periphery	25	55.6	390	7.9	9.94E-06
	fungal-type cell wall	10	22.2	36	0.7	4.46E-05
	organic acid biosynthetic process	10	22.2	134	2.7	1.23E-03
	carboxylic acid biosynthetic process	10	22.2	134	2.7	1.23E-03
	plasma membrane	14	31.1	234	4.8	2.39E-03
	cellular nitrogen compound biosynthetic process	10	22.2	237	4.8	2.68E-03
	small molecule biosynthetic process	10	22.2	307	6.2	2.68E-03
	cellular amino acid biosynthetic process	10	22.2	104	2.1	1.42E-02
	amine biosynthetic process	10	22.2	112	2.3	3.09E-02
	hydrolase activity, hydrolyzing O-glycosyl compounds	4	8.9	48	1.0	3.11E-02
	***slm9*Δ**					
	cell wall	10	38.5	58	1.2	4.39E-06
	cell surface	10	38.5	111	2.3	4.39E-06
	external encapsulating structure	10	38.5	58	1.2	4.39E-06
	extracellular region	11	42.3	48	1.0	5.18E-06
	fungal-type cell wall	8	30.8	36	0.7	5.65E-05
	cellular amino acid and derivative metabolic process	6	23.1	223	4.5	1.69E-04
	amine metabolic process	6	23.1	235	4.8	3.34E-04
	cellular amine metabolic process	6	23.1	224	4.6	6.93E-04
	organic acid metabolic process	6	23.1	282	5.7	7.58E-04
	cellular amino acid metabolic process	6	23.1	206	4.2	7.58E-04
	carboxylic acid metabolic process	6	23.1	282	5.7	7.58E-04
	oxoacid metabolic process	6	23.1	282	5.7	7.58E-04
	cellular ketone metabolic process	6	23.1	294	6.0	1.35E-03
	cytokinetic cell separation	6	23.1	31	0.6	1.57E-03
	organic acid biosynthetic process	5	19.2	134	2.7	1.04E-02
	carboxylic acid biosynthetic process	5	19.2	134	2.7	1.04E-02
	pyrimidine nucleoside metabolic process	1	3.8	10	0.2	1.68E-02
	beta-glucosidase activity	2	7.7	10	0.2	1.68E-02
	cell periphery	10	38.5	390	7.9	1.93E-02
	hydrolase activity, hydrolyzing O-glycosyl compounds	5	19.2	48	1.0	2.30E-02
	cytokinetic process	6	23.1	108	2.2	3.08E-02
	glucosidase activity	2	7.7	23	0.5	3.63E-02
	***hip1*Δ**					
	cell wall	11	37.9	58	1.2	6.39E-07
	cell surface	16	55.2	111	2.3	6.39E-07
	external encapsulating structure	11	37.9	58	1.2	6.39E-07
	extracellular region	11	37.9	48	1.0	1.00E-05
	cellular amino acid and derivative metabolic process	7	24.1	223	4.5	8.88E-05
	fungal-type cell wall	8	27.6	36	0.7	8.88E-05
	organic acid metabolic process	7	24.1	282	5.7	1.31E-04
	amine metabolic process	7	24.1	235	4.8	1.31E-04
	carboxylic acid metabolic process	7	24.1	282	5.7	1.31E-04
	oxoacid metabolic process	7	24.1	282	5.7	1.31E-04
	cellular ketone metabolic process	7	24.1	294	6.0	2.42E-04
	cellular amine metabolic process	7	24.1	224	4.6	2.42E-04
	cellular amino acid metabolic process	7	24.1	206	4.2	3.06E-04
	cytokinetic cell separation	6	20.7	31	0.6	2.41E-03
	organic acid biosynthetic process	6	20.7	134	2.7	3.68E-03
	carboxylic acid biosynthetic process	6	20.7	134	2.7	3.68E-03
	hydrolase activity, hydrolyzing O-glycosyl compounds	5	17.2	48	1.0	4.40E-03
	small molecule metabolic process	8	27.6	924	18.8	9.12E-03
	cell periphery	11	37.9	390	7.9	1.57E-02
	hydrolase activity, acting on glycosyl bonds	8	27.6	58	1.2	1.58E-02
	pyrimidine nucleoside metabolic process	1	3.4	10	0.2	1.80E-02
	beta-glucosidase activity	2	6.9	10	0.2	1.80E-02
	external side of plasma membrane	7	24.1	46	0.9	2.27E-02
	cell wall organization or biogenesis	6	20.7	122	2.5	2.62E-02
	cellular amino acid biosynthetic process	6	20.7	104	2.1	3.18E-02
	glucosidase activity	2	6.9	23	0.5	4.06E-02
	small molecule biosynthetic process	6	20.7	307	6.2	4.06E-02
priming stress and severe stress	**WT**					
	endoplasmic reticulum	103	33.3	589	12.0	6.39E-04
	cell division	59	19.1	306	6.2	1.82E-03
	cytokinetic process	12	3.9	108	2.2	2.13E-03
	rRNA processing	37	12.0	169	3.4	2.55E-03
	rRNA metabolic process	37	12.0	170	3.5	2.55E-03
	cellular component biogenesis at cellular level	46	14.9	338	6.9	2.55E-03
	ribosome biogenesis	46	14.9	235	4.8	3.20E-03
	cellular component organization or biogenesis at cellular level	96	31.1	1346	27.4	3.37E-03
	cytokinetic cell separation	12	3.9	31	0.6	3.61E-03
	cell wall organization or biogenesis	16	5.2	122	2.5	3.78E-03
	cellular component organization or biogenesis	97	31.4	1426	29.0	5.15E-03
	cellular cell wall organization or biogenesis	16	5.2	120	2.4	5.56E-03
	mitotic cell cycle	35	11.3	259	5.3	6.04E-03
	cytokinesis	18	5.8	134	2.7	7.33E-03
	protein glycosylation	6	1.9	68	1.4	7.55E-03
	glycoprotein biosynthetic process	6	1.9	68	1.4	7.55E-03
	macromolecule glycosylation	6	1.9	68	1.4	7.55E-03
	glycosylation	6	1.9	68	1.4	7.55E-03
	cellular component biogenesis	46	14.9	610	12.4	7.94E-03
	cell periphery	7	2.3	390	7.9	8.25E-03
	mitosis	34	11.0	182	3.7	1.04E-02
	glycoprotein metabolic process	6	1.9	70	1.4	1.04E-02
	M phase of mitotic cell cycle	34	11.0	183	3.7	1.14E-02
	nuclear division	34	11.0	184	3.7	1.25E-02
	intrinsic to membrane	133	43.0	925	18.8	1.26E-02

	ribonucleoprotein complex biogenesis	46	14.9	259	5.3	1.54E-02
	organelle fission	34	11.0	188	3.8	1.92E-02
	cell wall	7	2.3	58	1.2	2.03E-02
	external encapsulating structure	7	2.3	58	1.2	2.03E-02
	nucleolus	56	18.1	320	6.5	2.28E-02
	Golgi apparatus	58	18.8	349	7.1	2.28E-02
	cellular cell wall organization	16	5.2	75	1.5	2.28E-02
	cell wall organization	16	5.2	75	1.5	2.28E-02
	integral to membrane	133	43.0	909	18.5	2.34E-02
	ncRNA processing	37	12.0	246	5.0	2.54E-02
	external encapsulating structure organization	16	5.2	76	1.5	2.54E-02
	cell surface	18	5.8	111	2.3	2.64E-02
	nuclear lumen	56	18.1	547	11.1	3.10E-02
	cell septum	3	1.0	239	4.9	4.73E-02
	***slm9* Δ**					
	rRNA processing	29	19.6	169	3.4	7.55E-06
	rRNA metabolic process	29	19.6	170	3.5	7.55E-06
	ribosome biogenesis	35	23.6	235	4.8	1.68E-05
	ncRNA processing	29	19.6	246	5.0	4.81E-05
	cell division	40	27.0	306	6.2	8.80E-05
	ribonucleoprotein complex biogenesis	35	23.6	259	5.3	1.39E-04
	nucleolus	42	28.4	320	6.5	2.43E-04
	cellular component biogenesis at cellular level	35	23.6	338	6.9	3.95E-04
	cytokinetic process	9	6.1	108	2.2	4.45E-04
	ncRNA metabolic process	29	19.6	286	5.8	4.45E-04
	cellular component biogenesis	35	23.6	610	12.4	4.64E-04
	nuclear lumen	42	28.4	547	11.1	9.72E-04
	cytokinesis	14	9.5	134	2.7	1.19E-03
	extracellular region	13	8.8	48	1.0	3.29E-03
	cell wall	12	8.1	58	1.2	5.30E-03
	external encapsulating structure	12	8.1	58	1.2	5.30E-03
	cytokinetic cell separation	9	6.1	31	0.6	6.88E-03
	cell periphery	12	8.1	390	7.9	9.74E-03
	cell surface	14	9.5	111	2.3	2.20E-02
	fungal-type cell wall	9	6.1	36	0.7	2.56E-02
	cellular component organization or biogenesis	36	24.3	1426	29.0	3.41E-02
	organelle lumen	42	28.4	717	14.6	3.42E-02
	intracellular organelle lumen	42	28.4	717	14.6	3.42E-02
	endoplasmic reticulum	56	37.8	589	12.0	4.65E-02
	***hip1* Δ**					
	cell surface	18	13.6	111	2.3	1.68E-04
	cell division	36	27.3	306	6.2	1.68E-04
	cell wall	12	9.1	58	1.2	6.28E-04
	external encapsulating structure	12	9.1	58	1.2	6.28E-04
	cell periphery	28	21.2	390	7.9	6.28E-04
	rRNA processing	23	17.4	169	3.4	1.37E-03
	rRNA metabolic process	23	17.4	170	3.5	1.37E-03
	cytokinetic process	8	6.1	108	2.2	1.37E-03
	ncRNA processing	23	17.4	246	5.0	1.37E-03
	cytokinesis	12	9.1	134	2.7	2.43E-03
	endoplasmic reticulum	56	42.4	589	12.0	2.43E-03
	extracellular region	12	9.1	48	1.0	6.10E-03
	ribosome biogenesis	27	20.5	235	4.8	6.10E-03
	cell wall organization or biogenesis	11	8.3	122	2.5	6.10E-03
	ncRNA metabolic process	23	17.4	286	5.8	6.54E-03
	plasma membrane	19	14.4	234	4.8	1.38E-02
	cellular cell wall organization or biogenesis	11	8.3	120	2.4	1.39E-02
	cellular component biogenesis at cellular level	27	20.5	338	6.9	1.42E-02
	cytokinetic cell separation	8	6.1	31	0.6	1.71E-02
	cellular cell wall organization	11	8.3	75	1.5	2.23E-02
	cell wall organization	11	8.3	75	1.5	2.23E-02
	ribonucleoprotein complex biogenesis	27	20.5	259	5.3	2.46E-02
	external encapsulating structure organization	11	8.3	76	1.5	2.46E-02
	fungal-type cell wall	8	6.1	36	0.7	4.78E-02
	cellular component biogenesis	27	20.5	610	12.4	5.24E-02
severe stress alone	**WT**					
	DNA integration	11	4.1	11	0.2	5.76E-08
	transposition	11	4.1	12	0.2	3.17E-07
	RNA-dependent DNA replication	11	4.1	15	0.3	6.03E-07
	RNA-directed DNA polymerase activity	11	4.1	13	0.3	9.46E-07
	cell cycle	61	22.8	727	14.8	6.77E-06
	aspartic-type endopeptidase activity	11	4.1	16	0.3	2.34E-05
	aspartic-type peptidase activity	11	4.1	16	0.3	2.34E-05
	UDP-glycosyltransferase activity	6	2.2	27	0.5	2.96E-05
	cell cycle process	31	11.6	594	12.1	2.96E-05
	cell cycle phase	31	11.6	550	11.2	4.90E-05
	chromosome, centromeric region	19	7.1	94	1.9	5.35E-05
	cell division	44	16.5	306	6.2	8.87E-05
	microtubule cytoskeleton	33	12.4	176	3.6	1.11E-04
	transferase activity, transferring phosphorus-containing groups	50	18.7	301	6.1	5.61E-04
	cell septum	44	16.5	239	4.9	5.61E-04
	DNA polymerase activity	11	4.1	30	0.6	6.70E-04
	interphase	5	1.9	60	1.2	6.70E-04
	DNA binding	58	21.7	361	7.3	7.20E-04
	biological regulation	39	14.6	1299	26.4	1.18E-03
	mitotic cell cycle	31	11.6	259	5.3	1.73E-03
	kinetochore	12	4.5	65	1.3	1.95E-03
	signaling	24	9.0	383	7.8	1.95E-03
	cell wall organization or biogenesis	25	9.4	122	2.5	1.95E-03
	cellular component organization or biogenesis	54	20.2	1426	29.0	2.12E-03
	cellular component organization or biogenesis at cellular level	53	19.9	1346	27.4	2.12E-03
	spindle	19	7.1	137	2.8	2.18E-03
	M phase	28	10.5	518	10.5	2.53E-03
	protein kinase activity	26	9.7	118	2.4	2.53E-03
	protein phosphorylation	28	10.5	125	2.5	2.53E-03

regulation of cell size	1	0.4	30	0.6	2.65E-03
cellular cell wall organization or biogenesis	25	9.4	120	2.4	3.19E-03
signal transduction	24	9.0	362	7.4	4.06E-03
signaling process	24	9.0	363	7.4	4.21E-03
signal transmission	24	9.0	363	7.4	4.21E-03
regulation of nitrogen compound metabolic process	1	0.4	537	10.9	4.42E-03
regulation of cellular process	36	13.5	1053	21.4	4.55E-03
mitosis	28	10.5	182	3.7	4.97E-03
regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1	0.4	531	10.8	4.97E-03
chromosome	25	9.4	409	8.3	4.97E-03
M phase of mitotic cell cycle	28	10.5	183	3.7	5.20E-03
condensed chromosome, centromeric region	13	4.9	66	1.3	5.20E-03
fungal-type cell wall organization or biogenesis	11	4.1	72	1.5	5.20E-03
barrier septum	41	15.4	230	4.7	5.52E-03
nuclear division	28	10.5	184	3.7	5.53E-03
protein serine/threonine kinase activity	25	9.4	113	2.3	6.56E-03
chromosomal part	19	7.1	391	8.0	7.88E-03
cytoskeletal part	31	11.6	242	4.9	7.91E-03
organelle fission	28	10.5	188	3.8	8.32E-03
cytoskeleton	36	13.5	267	5.4	8.35E-03
DNA replication	20	7.5	130	2.6	9.45E-03
regulation of biological process	39	14.6	1134	23.1	9.91E-03
phosphotransferase activity, alcohol group as acceptor	28	10.5	169	3.4	1.22E-02
regulation of cellular metabolic process	1	0.4	661	13.5	1.40E-02
fungal-type cell wall biogenesis	11	4.1	60	1.2	1.67E-02
ATP binding	69	25.8	483	9.8	1.75E-02
nucleotidyltransferase activity	18	6.7	93	1.9	1.80E-02
adenyl ribonucleotide binding	69	25.8	485	9.9	1.94E-02
interphase of mitotic cell cycle	5	1.9	55	1.1	2.01E-02
cellular component organization	45	16.9	1196	24.3	2.36E-02
cellular cell wall organization	15	5.6	75	1.5	2.39E-02
cell wall organization	15	5.6	75	1.5	2.39E-02
ATP-dependent helicase activity	15	5.6	76	1.5	2.76E-02
external encapsulating structure organization	15	5.6	76	1.5	2.76E-02
purine NTP-dependent helicase activity	15	5.6	76	1.5	2.76E-02
transferase activity	76	28.5	659	13.4	2.98E-02
cell wall biogenesis	12	4.5	70	1.4	2.98E-02
nucleic acid binding	76	28.5	792	16.1	2.99E-02
ATP-dependent DNA helicase activity	7	2.6	34	0.7	2.99E-02
chromosome segregation	25	9.4	187	3.8	3.05E-02
condensed chromosome	13	4.9	91	1.9	3.25E-02
regulation of metabolic process	3	1.1	767	15.6	3.39E-02
nucleoside binding	70	26.2	526	10.7	3.77E-02
microtubule	15	5.6	59	1.2	3.86E-02
transferase activity, transferring hexosyl groups	6	2.2	72	1.5	3.86E-02
condensed chromosome kinetochore	8	3.0	60	1.2	4.62E-02
DNA helicase activity	7	2.6	36	0.7	4.70E-02
regulation of DNA-dependent DNA replication initiation	1	0.4	36	0.7	4.70E-02
DNA-dependent ATPase activity	7	2.6	54	1.1	4.77E-02
hydrolase activity, hydrolyzing O-glycosyl compounds	3	1.1	48	1.0	4.89E-02
***slm9*Δ**					
ribosome biogenesis	40	48.2	235	4.8	7.37E-11
cellular component biogenesis at cellular level	40	48.2	338	6.9	4.25E-10
ribonucleoprotein complex biogenesis	40	48.2	259	5.3	9.56E-10
ncRNA processing	29	34.9	246	5.0	2.30E-09
rRNA processing	29	34.9	169	3.4	3.02E-09
rRNA metabolic process	29	34.9	170	3.5	3.02E-09
cellular component biogenesis	40	48.2	610	12.4	2.73E-08
ncRNA metabolic process	29	34.9	286	5.8	1.93E-07
cellular component organization or biogenesis	60	72.3	1426	29.0	1.37E-06
nucleolus	42	50.6	320	6.5	1.64E-06
nuclear lumen	42	50.6	547	11.1	5.50E-06
cellular component organization or biogenesis at cellular level	60	72.3	1346	27.4	7.23E-06
cell division	29	34.9	306	6.2	3.49E-04
RNA processing	30	36.1	441	9.0	2.12E-03
nuclear part	42	50.6	1084	22.1	3.41E-03
organelle lumen	42	50.6	717	14.6	2.04E-02
intracellular organelle lumen	42	50.6	717	14.6	2.04E-02
RNA metabolic process	30	36.1	786	16.0	2.30E-02
mitosis	20	24.1	182	3.7	2.31E-02
M phase of mitotic cell cycle	20	24.1	183	3.7	2.40E-02
nuclear division	20	24.1	184	3.7	2.50E-02
cell septum	2	2.4	239	4.9	3.14E-02
organelle fission	20	24.1	188	3.8	3.21E-02
membrane-enclosed lumen	42	50.6	740	15.1	3.77E-02
***hip1*Δ**					
rRNA processing	21	40.4	169	3.4	9.28E-05
rRNA metabolic process	21	40.4	170	3.5	9.28E-05
ncRNA processing	21	40.4	246	5.0	9.28E-05
ribosome biogenesis	27	51.9	235	4.8	9.28E-05
cellular component biogenesis at cellular level	27	51.9	338	6.9	9.28E-05
ribonucleoprotein complex biogenesis	27	51.9	259	5.3	7.00E-04
ncRNA metabolic process	21	40.4	286	5.8	1.65E-03
cellular component biogenesis	27	51.9	610	12.4	7.76E-03
cell division	22	42.3	306	6.2	1.56E-02
cell wall	4	7.7	58	1.2	2.50E-02
external encapsulating structure	4	7.7	58	1.2	2.50E-02
cellular component organization or biogenesis	27	51.9	1426	29.0	4.80E-02

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Chromatin fractionation assay

The chromatin fractionation assay was performed as described previously (S1,S2) with some modifications. Cells (5×10^8 cells) were harvested, suspended in ice-cold STOP buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaN_3), and placed on ice for 5 min. The cell suspension was spun at 800 g for 1 min at 4°C, and the resulting cell pellet was resuspended in PEMS (100 mM PIPES [pH 6.9], 1 mM EGTA, 1 mM $MgSO_4$, 1 M sorbitol) containing 1 mg/ml Lysing enzymes (Sigma) and 1 mg/ml Zymolyase 100T (Seikagaku Corporation). The suspension was incubated at 37°C until almost all the cells were spheroplasted (60-90 min). The cell suspension was spun at 2,300 g for 1 min at 4°C, and the resulting cell pellet was washed three times with ice-cold wash buffer (1 M sorbitol, 25 mM MOPS [pH 7.2]). Then, the cells were resuspended in HBS buffer (25 mM MOPS [pH 7.2], 15 mM $MgCl_2$, 15 mM EGTA, 0.4 M sorbitol) containing 1 mM PMSF, 1×Complete (Roche), and 1% Triton X-100, and placed on ice for 5 min. The resulting whole-cell extract was spun at 20,400 g for 15 min at 4°C to obtain the supernatant (soluble fraction) and the pellet. The pellet was washed three times and resuspended in ice-cold digestion buffer (25 mM MOPS [pH 7.2], 15 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.4 M sorbitol) containing 1×Complete (Roche), and the suspension was incubated with 1 U/μl MNase for 2 min at 37°C. After centrifugation at 20,400 g for 5 min at 4°C, the pellet was digested once more with MNase as described above and the supernatants were combined (MNase fraction). The pellet was washed once with ice-cold digestion buffer and resuspended in ice-cold extraction buffer (25 mM MOPS [pH 7.2], 10 mM EDTA, 0.4 M sorbitol) containing 1× Complete (Roche), and placed on ice for 5 min. Then, the suspension was spun at 20,400 g for 15 min at 4°C to obtain the supernatant (chromatin fraction) and the pellet. The pellet was washed two times with extraction buffer (pellet fraction). Proteins from each fraction were separated by SDS-PAGE and detected by Western blotting.

SUPPLEMENTAL REFERENCES

S1. Ogawa, Y., Takahashi, T., and Masukata, H. (1999) *Mol Cell Biol* **19**, 7228-7236

S2. Sadaie, M., Kawaguchi, R., Ohtani, Y., Arisaka, F., Tanaka, K., Shirahige, K., and Nakayama, J. (2008) *Mol Cell Biol* **28**, 6973-6988