Cellular Memory of Acquired Stress Resistance in *Saccharomyces cerevisiae*

Qiaoning Guan,^{*1,2} Suraiya Haroon,^{*1} Diego González Bravo,[†] Jessica L. Will,^{*} and Audrey P. Gasch^{*,†,3}

^{*}Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706, [†]University of Puerto Rico at Arecibo, Arecibo PR 00614, Puerto Rico, and ³Genome Center of Wisconsin, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT Cellular memory of past experiences has been observed in several organisms and across a variety of experiences, including bacteria “remembering” prior nutritional status and amoeba “learning” to anticipate future environmental conditions. Here, we show that *Saccharomyces cerevisiae* maintains a multifaceted memory of prior stress exposure. We previously demonstrated that yeast cells exposed to a mild dose of salt acquire subsequent tolerance to severe doses of H₂O₂. We set out to characterize the retention of acquired tolerance and in the process uncovered two distinct aspects of cellular memory. First, we found that H₂O₂ resistance persisted for four to five generations after cells were removed from the prior salt treatment and was transmitted to daughter cells that never directly experienced the pretreatment. Maintenance of this memory did not require nascent protein synthesis after the initial salt pretreatment, but rather required long-lived cytosolic catalase Ctt1p that was synthesized during salt exposure and then distributed to daughter cells during subsequent cell divisions. In addition to and separable from the memory of H₂O₂ resistance, these cells also displayed a faster gene-expression response to subsequent stress at >1000 genes, representing transcriptional memory. The faster gene-expression response requires the nuclear pore component Nup42p and serves an important function by facilitating faster reacquisition of H₂O₂ tolerance after a second cycle of salt exposure. Memory of prior stress exposure likely provides a significant advantage to microbial populations living in ever-changing environments.

NATURAL environments are complex and often vary significantly in space and time, posing challenges for the organisms living within them. Single-cell organisms are particularly vulnerable, since variation in external conditions can directly impact internal homeostasis. Therefore, preparing for environmental change after early signs of fluctuation would present a significant advantage for cells growing in the wild. Indeed, many organisms can become tolerant to severe stress after an initial mild pretreatment with the same or a different stressor. This response, termed “acquired stress resistance,” has been observed in microbes such as bacteria and yeast as well as in multicellular organisms including worms, plants, mammals, and even humans (Lu *et al.* 1993; Davies *et al.* 1995; Lewis *et al.* 1995; Lou and Yousef 1997;

Swan and Watson 1999; Chi and Arneborg 2000; Schenk *et al.* 2000; Durrant and Dong 2004; Kandror *et al.* 2004; Scholz *et al.* 2005; Hecker *et al.* 2007; Kensler *et al.* 2007; Matsumoto *et al.* 2007). The conservation of this response suggests that it plays an important role in surviving environmental stress in diverse species.

We previously conducted a systematic analysis of acquired stress resistance in *Saccharomyces cerevisiae* and found that the response is common, but not universal, for all pairs of stress treatments (Berry and Gasch 2008). The level and duration of mild-stress pretreatment varies according to the mild stressor used, but the timing of stress-tolerance acquisition correlates with the gene-expression dynamics during each pretreatment. Consistently, acquired stress resistance is dependent on nascent protein synthesis during the mild-stress exposure, but not during the severe-stress treatment (Berry and Gasch 2008; Lewis *et al.* 2010). In separate work, we identified genes important for acquisition of stress tolerance after different mild-stress pretreatments (Berry *et al.* 2011). Somewhat surprisingly, the mechanism of acquired stress resistance is condition-specific, rather than being dependent on the commonly activated environmental stress response. For

Copyright © 2012 by the Genetics Society of America

doi: 10.1534/genetics.112.143016

Manuscript received June 15, 2012; accepted for publication July 19, 2012

Supporting information is available online at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143016/-DC1>.

¹These authors contributed equally to this work.

²Present address: 2921 Stockton Blvd., BGI@UC Davis, Sacramento, CA 95817.

³Corresponding author: Laboratory of Genetics, 425-g Henry Mall, University of Wisconsin, Madison, WI 53706. E-mail: agasch@wisc.edu.

example, acquisition of H₂O₂ tolerance is commonly observed after different mild-stress pretreatments but occurs through largely distinct gene sets in each case (Kelley and Ideker 2009; Berry *et al.* 2011). This result is explained in part by redundant functions served by different gene sets, but also emerges due to the condition-specific nature of stress defense for each pair of mild- and severe-stress treatments.

One open question is how long acquired stress tolerance persists after cells have been removed from the initial stressor, and whether the acquired stress tolerance can be transmitted to new daughter cells. Cellular memory of past life experiences has been observed in several organisms. For example, yeast and bacteria exposed to prior nutrient signals display altered metabolism and gene expression or chemotaxis patterns, respectively, upon later exposure to specific environmental cues (Koshland 1977; Casadesús and D'Ari 2002; Acar *et al.* 2005; Brickner *et al.* 2007). Adult *Caenorhabditis elegans* that emerge from starvation-induced “dauer” development grow up with higher fecundity and correspondingly altered gene expression (Hall *et al.* 2010). Even amoeba can “learn” to predict environmental fluctuations based on historical events, altering their behavior in anticipation of a recurring pulse of stimulus (Saigusa *et al.* 2008). In some cases, cellular memory can be transmitted across generations, in both single-celled organisms (Casadesús and D'Ari 2002; Ng *et al.* 2003; Acar *et al.* 2005; Ajo-Franklin *et al.* 2007; Brickner *et al.* 2007; Burrill and Silver 2011) and multicellular offspring (Goh *et al.* 2003; Anway *et al.* 2005; Molinier *et al.* 2006; Sigal *et al.* 2006; Crews *et al.* 2007; Boyko *et al.* 2010; Burns and Mery 2010; Carone *et al.* 2010; Ng *et al.* 2010).

Here we show that yeast cells previously exposed to mild stress retain a memory of acquired stress tolerance for many generations after the initial stressor has been removed. We found that the memory exists on two separate levels: persistence of acquired H₂O₂ tolerance and transcriptional memory that promotes faster reacquisition of stress tolerance after a second round of treatments. We present mechanisms of this cellular memory and discuss its functional relevance in natural environments.

Materials and Methods

Strain and growth conditions

All experiments were done in YPD (1% yeast extract, 2% peptone and 2% dextrose) or in synthetic drop-out media lacking leucine and uracil where indicated. Unless otherwise noted, strains used were of the BY4741 background (*Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*). Deletion strains were purchased from Open Biosystems, and gene deletions and correct integration of the *KANMX* cassette were validated by PCR amplification. An independent *ctt1Δ* strain was created in BY4741 by replacing *CTT1* with the *URA3* gene (AGY639). This strain was used to introduce FLAG-tagged *CTT1*, generated by PCR mutagenesis to introduce one copy of the

FLAG epitope (DYKDDDDK) between the fifth and sixth amino acid of *Ctt1p*. The cassette was then integrated into the *ctt1::URA3* locus by homologous recombination and selection on 5-fluoro-orotic acid and verified by sequencing. The strain showed roughly wild-type sensitivity to NaCl and H₂O₂ stress (data not shown). Inducible *FLAG-CTT1* expression was accomplished by cloning the *FLAG-CTT1* fusion described above, along with 629 bp downstream of the *CTT1* coding sequence under control of the *GAL1* promoter in plasmid pRS316-*GAL1*. This plasmid was cotransformed, along with pGEV-LEU (Gao and Pinkham 2000), kindly provided by David Eide, into a *ura⁻* revertant of AGY714 in which *GAL4* had also been deleted for maximal estradiol-dependent induction.

Memory of acquired stress resistance experiments

Cultures were grown in shaker flasks at 30° for at least eight generations to an optical density (OD₆₀₀) of 0.3–0.4. Each culture was split into two cultures: one was treated with 0.7 M NaCl for 60 min. The other culture served as a mock control that was handled identically but received no stress. Cells were then washed once with medium and resuspended in prewarmed, stress-free medium and grown for at least 6 hr.

H₂O₂ tolerance was measured by transferring cells to a 96-well plate and exposing for 2 hr to 11 doses of H₂O₂ plus a no-stress control, ranging from 0 to 6 mM for experiments done in YPD or from 0 to 12 mM for cells grown in synthetic medium (since cells grown in synthetic medium display slightly higher H₂O₂ tolerance). After a 2-hr incubation with shaking, the cells were either plated on YPD plates to assess colony-forming units [using a four-point scale as previously described (Berry and Gasch 2008)], or exposed to LIVE/DEAD stain (Invitrogen, Carlsbad, CA) to measure percentage viability on a Guava 96-well flow cytometer (Millipore, Billerica, MA) and then normalized to viability measured in the unstressed cells. To capture the breadth of H₂O₂ tolerance, we calculated a single acquired-stress survival score for each time point, based on the sum of the viability scores at each dose of H₂O₂ minus the comparable sum for mock-treated cells. Unless otherwise noted, the percentage maximum score relative to the time point immediately after pretreatment is shown.

To induce *FLAG-CTT1* expression in the inducible strain, 1 μM β-estradiol was added to the culture for 3 hr, after which time cells were removed from the culture, washed with fresh medium, and grown in stress-free medium for denoted times.

Mother–daughter cell experiments

S288C-derived yeast strain UCC8613 that carries the loxP-flanked *ADE2* gene was transformed with the plasmid pDL24, which contains a fusion of the Cre gene and estradiol-binding domain driven by the *DSE2* daughter-specific promoter (Lindstrom and Gottschling 2009) (both kindly provided by the Gottschling lab). The memory of acquired stress resistance was measured as described above, except that (i)

the transformed cells were grown in YPD containing 100 $\mu\text{g/ml}$ clonNAT (WERNER BioAgents) to maintain the plasmid and (ii) 1 μM estradiol (Sigma Aldrich, St. Louis) was added to the culture after treatment of primary stress to activate the Cre recombinase, which is expressed only in daughter cells. Cells were plated on SC-ade to score ade⁺ mothers or plated on YPD to count ade⁺ (white) mother cells and ade[−] (red) daughter cells.

Microarray experiments

Cells were grown for at least three doublings in YPD at 30° to early log phase. A sample of the culture was collected as the unstressed reference, and the remaining culture was split into three subcultures. The first subculture immediately received 0.5 mM H₂O₂, and cells were collected for microarray analysis at 10, 20, 30, or 40 min. The second subculture was treated with 0.7 M NaCl for 60 min, washed with medium, returned to 30° YPD medium for 240 min, and then treated with 0.5 mM H₂O₂ as described above. The third subculture served as mock control and was handled identically but received no NaCl treatment before exposure to 0.5 mM H₂O₂. All cells were collected by brief centrifugation and snap-frozen in liquid nitrogen. A similar experiment was performed for paired wild-type and *nup42Δ* cultures and analyzed before stress at 30 min after 0.7 M NaCl treatment and at 10 and 20 min after H₂O₂ treatment.

Total RNA extraction, cDNA synthesis, and labeling were performed as previously described (Gasch 2002) using amino-allyl-dUTP (Ambion, Austin, TX), Superscript RT III (Invitrogen, Carlsbad, CA), and NHS-ester cyanine dyes (Fluoromatrix, Madison, WI). Spotted microarrays used for Figure 4A data were produced in-house using 70-mer oligonucleotides representing each of the yeast ORFs (Qiagen, Valencia, CA). Array hybridization, scanning, and data analysis were performed as described (Berry and Gasch 2008; Alejandro-Orsorio *et al.* 2009). Each time-point sample was compared to the paired unstressed sample to measure fold changes in gene expression. Analysis of the paired wild-type and *nup42Δ* responses was done similarly but hybridized to tiled genomic arrays from Roche Nimblegen as previously described (Huebert *et al.* 2012). All microarray data are available in the National Institutes of Health Gene Expression Omnibus database under accession no. GSE32196.

Genes with significant expression differences in paired pretreated vs. naive samples were identified using the Bioconductor package *limma* (Smyth 2005), and q-values (Storey and Tibshirani 2003) were calculated to assess the false discovery rate (FDR). Data shown in Figure 4 were organized by hierarchical clustering (Eisen *et al.* 1998). Motif analysis was done using the program MEME (Bailey and Elkan 1994), searching 1000 bp upstream of genes from each cluster in Figure 4 using the zero-or-one per sequence model. Examples of the motif were scored in all 1000 bp upstream regions using the program FIMO (Grant *et al.* 2011), and enrichment in the group of genes from cluster 3 was scored using the hypergeometric distribution.

Quantitative RT-PCR and Westerns

Expression of selected genes was measured by real-time quantitative PCR (qPCR) using SYBR Green Jumpstart Taq (Sigma) and an Applied Biosystems 7500 detector (Foster City, CA). The average of technical replicates was normalized to the internal control transcript *ERV25*, which does not show stress-dependent changes in expression. The samples collected after stress addition were compared with the unstressed control. Three biological replicates were performed to assess statistical significance.

Protein was quantified from whole-cell extract prepared from 5 ml of cells collected by centrifugation and immediate freezing. Cell pellets were resuspended in sample buffer, normalizing by cell count. Westerns were performed using primary antibodies against the FLAG tag (Sigma monoclonal FLAG antibody F3165) or Sse3/4p (kindly provided by Betty Craig) and *actin* (Sigma Actin Antibody AC40) as an internal loading control and secondary goat anti-mouse antibody (Licor, Lincoln, NE; 926-32210). Bands were quantified on an Odyssey Infrared Imaging System (Licor), and protein was normalized to *actin* abundance within each lane.

Results

Memory of acquired H₂O₂ resistance after mild NaCl treatment

We first recapitulated that yeast cells acquire H₂O₂ tolerance after a mild NaCl pretreatment. Actively growing cells were treated with a viable dose of 0.7 M NaCl (referred to as the “primary” stress treatment), and at various times an aliquot of culture was removed, exposed to 11 doses of H₂O₂ (referred to as the “secondary” stress) for 2 h, and scored for viability. To capture the full spectrum of acquired H₂O₂ tolerance relative to unstressed cells, a single tolerance score was computed and normalized to the maximum score to represent percentage maximal tolerance (see *Materials and Methods* for details). In agreement with previous results (Berry and Gasch 2008), we found that H₂O₂ tolerance increased during the NaCl pretreatment and reached maximum levels by 60 min (Figure 1B and data not shown).

We next investigated how long the acquired H₂O₂ tolerance persisted after cells were removed from salt. To do this, cells were exposed to 0.7 M NaCl for 60 min, but then removed from the primary stress and allowed to grow in stress-free YPD medium for many generations (tracked by changes in cell density or colony-forming units over time). Remarkably, H₂O₂ tolerance persisted for over 360 min (more than three generations, Figure 1) and remained statistically above background for over four generations after cells were removed from the primary NaCl stress (Supporting Information, Figure S1, and data not shown). Resistance slowly decayed over time, revealing that the increase in H₂O₂ tolerance was due to an epigenetic mechanism rather than to accumulated genetic mutations. Thus, cells retain a memory of prior salt treatment in the form of elevated H₂O₂ tolerance.

Figure 1 Cells retain a memory of acquired stress resistance. Cells were exposed to NaCl for 60 min and then returned to fresh stress-free YPD medium, and H₂O₂ tolerance was scored over time. (A) Cell viability is shown across 10 doses of H₂O₂ (ranging from 0.5 to 5 mM) at various times after removal from NaCl. (B) A single survival score was calculated from all H₂O₂ doses and at each time point (see *Materials and Methods*) and then normalized to the survival score of the mock-treated culture. Resistance relative to the maxi-

mum survival score at 60 min after NaCl treatment is shown (blue line). The percentage of original cells in the culture, as estimated by optical density, is shown by the gray line; the culture underwent between three to four doublings in the course of 360 min. Each plot shows the average and standard deviation of triplicate experiments.

Memory of acquired stress resistance is independent of *de novo* protein synthesis but decays with cell division

To test if active transcription or translation is required to maintain the memory of the acquired H₂O₂ resistance, we added the protein-synthesis inhibitor thiolutin after NaCl treatment, when cells were returned to stress-free medium. A final concentration of 10 μ g/ml thiolutin—a dose at which both transcription and translation are halted (Jimenez *et al.* 1973)—was added to the culture, either immediately after or 120 min after removal of the primary NaCl stress (Figure S1A). In both cases, thiolutin halted the decay of H₂O₂ resistance, indicating that active transcription/protein synthesis is not required to maintain the memory of H₂O₂ tolerance.

Because thiolutin also immediately inhibits cell growth, the above result suggests that the decay of H₂O₂ resistances requires cell division. To further test this, we measured H₂O₂ tolerance in NaCl-treated cells that were removed from stress but subsequently exposed to mating pheromone to arrest cell division. Indeed, the decay of the acquired H₂O₂ resistance was dramatically slowed when cell division was arrested (Figure S1B). Thus, cell division is required for the disappearance of H₂O₂ tolerance within the culture.

Memory of acquired stress resistance is inherited by daughter cells

Two models could explain the decay in H₂O₂ resistance in the cell culture. The first is a “marked” model, in which cells originally exposed to the mild primary stress become marked with H₂O₂ resistance and are slowly diluted out of the dividing culture. In this model, the original cells would retain maximal H₂O₂ resistance but disappear exponentially at the rate of cell division. However, several lines of evidence argue against this model. First, the decay of H₂O₂ resistance in the culture was slower than the measured rate of cell division (Figure 1B), indicating that exponential dilution of marked cells cannot account for the decay. Second, we observed that cells in the culture lost resistance to the highest doses of H₂O₂ earlier than they lost resistance to lower doses (see

Figure 1A). This revealed quantitative loss of H₂O₂ resistance over time in individual cells, rather than dilution of cells permanently marked with high H₂O₂ tolerance.

Instead, we found that the H₂O₂ tolerance was distributed between mother and daughter cells upon cell division, such that new cells inherited H₂O₂ tolerance. We used a reporter construct developed and kindly provided by Lindstrom and Gottschling (2009) to track mother vs. daughter cells in the culture. Using this system, we found that the level and persistence of H₂O₂ resistance in daughter cells was indistinguishable from that in mother cells ($R = 0.99$, Figure S2). Together, these results show that cells inherit H₂O₂ resistance but that resistance diminishes with each cell division.

Mechanism of cellular memory of stress resistance

There are several possible mechanisms of cellular memory in yeast, including propagation of chromatin marks (Brickner 2009; Kundu and Peterson 2009; Kaufman and Rando 2010), inheritance of long-lived memory factors (Acar *et al.* 2005; Zacharioudakis *et al.* 2007; Kundu and Peterson 2010), and feedback in signaling pathways that can maintain a response once cellular memory is activated (Xiong and Ferrell 2003; Acar *et al.* 2005; Mettetal *et al.* 2008). We found no requirement for several chromatin factors implicated in expression memory, including the deacetylase *SIR2*, the *Pho23p* subunit of the *Rpd3*-large histone deacetylase complex, or the histone variant *H2A.z* (Q. Guan and A. P. Gasch, unpublished data). We also found no significant differences in transcript abundance after cells had re-acclimated to stress-free medium, nor evidence of a sequestered pool of nontranslated, stored mRNAs (Aragon *et al.* 2008) (data not shown). These negative results are consistent with the dispensability of nascent protein synthesis in maintaining the memory of H₂O₂ tolerance.

Instead, our results suggested that a long-lived memory factor was induced during NaCl treatment and distributed between dividing mother and daughter cells. We recently identified genes required for acquisition of H₂O₂ tolerance

after pretreatment with several mild stressors (Berry *et al.* 2011). Cytosolic catalase **Ctt1p**, which converts H_2O_2 to oxygen and water, is critical for acquisition of H_2O_2 resistance immediately after mild NaCl treatment (Berry *et al.* 2011). We therefore reasoned that long-lived **Ctt1** protein may be responsible for the memory of H_2O_2 tolerance. We first followed **CTT1** mRNA and **Ctt1p** protein in a strain expressing genomically integrated FLAG-tagged **CTT1** during NaCl treatment and as cells resumed stress-free growth (Figure 2). **CTT1** mRNA is present at low levels before stress (Lipson *et al.* 2009) and highly but transiently induced after NaCl treatment before returning to basal levels by the time cells have been returned to stress-free medium. In contrast, **Ctt1p** protein reached maximum abundance after cells were removed from NaCl and remained at elevated levels in the actively dividing culture for >6 h after cells were removed from NaCl. This time frame correlated with the period of elevated H_2O_2 tolerance in the culture (Figure 1).

To directly test the role of **Ctt1p** in the memory of H_2O_2 resistance, we induced **CTT1** expression in the absence of stress. **CTT1** was deleted from the genome, and, instead, FLAG-tagged **CTT1** was placed under control of the **GAL1** promoter in a strain capable of estradiol-induced transcription (Louvion *et al.* 1993; Gao and Pinkham 2000). **CTT1** expression was transiently induced by 1 μ M estradiol treatment for 3 hr before cells were returned to plain medium. H_2O_2 tolerance increased upon estradiol induction (Figure 3A, red curve), but not after a similar treatment in the empty-vector control (data not shown), confirming that **CTT1** induction is sufficient to increase H_2O_2 tolerance. Importantly, elevated H_2O_2 tolerance was detectable for well over 360 min after cells were removed from estradiol, during which time FLAG-tagged **Ctt1p** remained higher than in

unstressed cells (Figure 3B). Adding NaCl during the estradiol induction did not further increase or prolong H_2O_2 tolerance in the culture (although cells were initially slightly more sensitive to H_2O_2 stress) (Figure 3A, gray curve). Thus, induction of **Ctt1p** is sufficient to explain the persistence of H_2O_2 tolerance after NaCl treatment.

Cells with a memory of acquired stress resistance show faster gene-expression changes upon further stress

Although nascent transcription was not required to maintain acquired H_2O_2 tolerance, we wondered if cellular memory of prior NaCl treatment extended to the transcriptional response to subsequent stress. Several studies have demonstrated transcriptional memory in cells previously exposed to galactose or inositol starvation, if cells are re-exposed to the nutrient shifts at a later time (Acar *et al.* 2005; Brickner *et al.* 2007; Kundu *et al.* 2007; Zacharioudakis *et al.* 2007). We therefore measured genomic expression in cells responding over the course of 60 min to a viable dose of 0.5 mM H_2O_2 . We followed naive cells that had never before been treated with stress and also a culture that had been previously treated with 0.7 M NaCl for 60 min and then grown in stress-free medium for 240 min (two generations) before H_2O_2 treatment. Importantly, we saw no significant expression differences (at an FDR of 0.05) between the two cultures just before the H_2O_2 treatment (Figure 4A).

In contrast, the response to H_2O_2 was significantly different in cells with prior stress exposure (Figure 4A). There were 4341 genes whose expression was altered in naive cells responding to H_2O_2 (FDR < 0.05). Of these, 449 genes showed a statistically significant expression difference (FDR < 0.01) between naive and prestressed cells; relaxing the cutoff to FDR < 0.05 identified 1593 genes with an altered response, amounting to 37% of the H_2O_2 -responsive genes. The affected genes included both induced and repressed genes that fell into distinct clusters. Nearly 90% of these genes showed expression changes during the NaCl pretreatment; however, of the 449 genes affected at the higher confidence level, 51 (11%) showed no significant expression change during NaCl treatment, revealing that the effect on expression extended to H_2O_2 -specific expression changes. This result indicates that prior induction of affected genes was not a prerequisite for the altered gene-expression response in pretreated cells.

One possible mechanism behind the faster genomic response is that prior activation of the signaling system could promote faster reactivation, as shown for the galactose response (Zacharioudakis *et al.* 2007; Acar *et al.* 2008). However, in its simplest form this model cannot explain our results. Although we found significant enrichment among the 449 affected genes for targets of the H_2O_2 -activated transcription factor **Yap1p** [$P = 1.3 \times 10^{-6}$ (Gasch *et al.* 2000)], the “general-stress” factor **Msn2p** [$P = 1.1 \times 10^{-4}$ (Berry *et al.* 2011)], and the **Hog1p** kinase [$P = 4.1 \times 10^{-7}$ (Berry *et al.* 2011)] that responds to osmotic shock, only about one-third of each factor’s targets showed significant

Figure 2 Ctt1 protein persists over time. Levels of **CTT1** mRNA (dark blue line) and FLAG-tagged Ctt1 protein (light blue line) were measured throughout the experiment by quantitative PCR and Western analysis, respectively. Fold-change in FLAG-tagged **CTT1** mRNA was calculated relative to basal levels measured before stress. FLAG-tagged Ctt1p was normalized to an internal actin control before calculating fold-change relative to unstressed cells. Each plot represents the average and standard deviation of at least three biological replicates.

Figure 3 An exogenous pulse of Ctt1p produces a memory of H₂O₂ resistance. A strain in which the endogenous *CTT1* was replaced with plasmid-borne FLAG-tagged *CTT1* driven by the estradiol-regulated promoter was induced with 1 μ M estradiol for 3 hr and then returned to stress-free medium. (A) H₂O₂ tolerance across 11 doses of H₂O₂ (ranging from 1 to 12 mM) was scored for 360 min after cells were removed from estradiol (red curve) and compared to cells harboring the native genomic, FLAG-tagged *CTT1* induced with 0.15 M NaCl (blue curve). This dose of salt was chosen because it produces equivalent Ctt1p compared to estradiol induction. Estradiol-induced cells exposed to 0.7 M NaCl (gray curve) for the last 60 min showed no significant increase in H₂O₂ tolerance. Survival scores were adjusted to the maximum level survived after estradiol induction to represent the percentage maximal survival. (B) FLAG-tagged Ctt1p was measured by quantitative Western analysis, normalized to an internal actin control, under the conditions described in A. The fold-change in expression relative to unstressed cells is shown. Plots represent the average and standard deviation of at least three biological replicates.

expression differences between naive and prestressed cells (even if we relax the cutoff to FDR 0.05). Therefore, the altered gene-expression response cannot be simply due to faster activation of these factors in a manner that affects all of their targets similarly (see *Discussion*). We therefore investigated alternative possibilities.

Nuclear pore component Nup42p is required for the faster gene-expression response

Several studies have shown that a functional nuclear pore is required for transcriptional memory at specific genes due to tethering genes to the nuclear periphery and/or promoting transcriptional looping (Brickner *et al.* 2007; Tan-Wong *et al.* 2009; Ahmed *et al.* 2010; Light *et al.* 2010; Hampsey *et al.* 2011). To investigate the role of nuclear pore components, we followed gene expression in cells lacking Nup42p. Nup42p is required to target the *INO1* gene to the nuclear periphery upon transcriptional induction (Ahmed *et al.* 2010; Light *et al.* 2010); it also has a role in mRNA export after extreme stress (e.g., a 25°–42° heat shock) but not under mild stress (25°–35° heat shock) or unstressed conditions (Stutz *et al.* 1995, 1997; Saavedra *et al.* 1997; Vainberg *et al.* 2000). We found that cells lacking NUP42 behaved like wild-type cells in several assays: they displayed no defect in basal H₂O₂ tolerance and no defect in the acquisition or memory of H₂O₂ tolerance after NaCl pretreatment (Figure S3A). Genomic expression analysis showed that these cells also had little significant difference in basal gene expression before stress (with only nine genes reproducibly altered more than twofold), nor any difference in the genomic response to a single dose of NaCl or H₂O₂ compared to wild-type cells (Figure 4B).

In contrast, *nup42Δ* cells had a major defect in the faster gene-expression response to repeated stress. We followed genomic expression in naive and pretreated *nup42Δ* cells responding to H₂O₂. In contrast to the pretreated wild-type strain, the *nup42Δ* mutant showed little difference in the H₂O₂ response (~1.1-fold on average for the 449 genes affected in wild type) whether or not cells previously experienced NaCl stress (Figure 4B). Instead, both responses looked very similar to the H₂O₂ response of naive wild-type cells. The defect in *nup42Δ* cells was specific to successive stress treatments, since there was no difference in the response to a single dose of H₂O₂ or to the NaCl pretreatment. We confirmed the result using quantitative PCR at three representative genes (*CTT1*, *TSA2*, and *HSP12*; Figure 5 and Figure S4), which showed that the response of pretreated *nup42Δ* cells was superimposable with and statistically indistinguishable from the naive wild-type response ($P > 0.05$, *t*-test).

Nup42p has been implicated in mRNA export under severe-stress exposure, raising the possibility that a general defect in mRNA export prevents a normal expression response. However, several lines of evidence argue against this. First, Western blots showed a defect in production of heat-shock protein Ssa3/4p after a severe shock of 25°–42°, when mRNA export is known to be defective, but not in response to a mild 25°–35° heat shock or 0.7 M NaCl (Figure S3B), arguing against a gross mRNA-export defect under these conditions. Second, the mutant had no defect in acquiring H₂O₂ tolerance after a mild heat shock or 0.7 M NaCl, whereas it did have a defect after a severe 25°–42° shock (Figure S3C). Third, as discussed above, the *nup42Δ*

Figure 4 Cells with prior NaCl exposure have a faster gene-expression response to H₂O₂. Cells were exposed to either 0.7 M NaCl or a mock treatment for 60 min and then grown in stress-free YPD medium for 240 min at which point 0.5 mM H₂O₂ was added to the culture. (A) The heatmap shows log₂ expression changes of 449 genes with significantly different H₂O₂-dependent expression in cells pretreated with NaCl (FDR < 0.01). Each row represents a gene and each column represents a microarray. Time points of the cellular response to 0.7 M NaCl or 0.5 mM H₂O₂ are labeled in minutes; the 0-min sample shows expression just before H₂O₂ treatment in mock- or NaCl-treated cells. Red represents gene induction and green represents gene repression relative to unstressed cells. Each data point is the average of biological replicates. Clusters (labeled 1–6) were manually identified in hierarchically clustered data. (B) The response to H₂O₂ was scored similarly in naive (N) and NaCl-pretreated (P) wild-type and *nup42Δ* cells at 10 and 20 min after treatment. Genes are organized as in A. (C) The average log₂ fold-change in expression of induced and repressed genes in B is shown for wild-type and *nup42Δ* cells. The average response of naive wild-type cells is shown in gray on the *nup42Δ* plot.

cells showed no major expression defect before stress or after single stress treatments, which is not expected from a gross defect in mRNA transport after the initial stress treatment.

To further probe the role of NPC subunits, we measured the transcriptional response of *TSA2* in several other NPC mutants. *Nup59p* is required for mRNA export after severe heat shock but is dispensable for peripheral localization of *INO1* after inositol starvation (Thomsen *et al.* 2008; Ahmed *et al.* 2010; Light *et al.* 2010). We found that a *nup59Δ* had no defect in stress-responsive transcriptional memory at *TSA2*, unlike *nup42Δ* cells (Figure 5); a similar result was found for the related *nup60Δ* mutant (Thomsen *et al.* 2008) (not shown). This strongly argues against a general defect in mRNA export as the cause of the transcriptional difference. In contrast, cells lacking *Nup100p*, which is required for *INO1* transcriptional memory and persistence of its peripheral maintenance after inositol starvation (Light *et al.* 2010), showed no difference in *TSA2* induction in naive and pretreated cells. However, both the NaCl-treated and unstressed mutant cells showed kinetics similar to the pretreated wild-type cells with transcriptional memory, making the result difficult to interpret. It is intriguing to note that data from Ahmed *et al.* (2010) showed slightly increased *INO1* peripheral localization in a *nup100Δ* mutant compared to wild-type cells immediately after the first exposure to inositol starvation (see Discussion).

Cells with memory show faster acquisition of a second round of stress resistance

The faster gene-expression response to subsequent stress could have a very important function in nature: faster reacquisition of stress tolerance upon repeated NaCl exposures. Indeed, we found this to be the case. Cells were treated with 0.7 M NaCl for 120 min, returned to stress-free medium for 240 min, and then exposed again to 0.7 M NaCl;

H₂O₂ tolerance was assessed throughout the experiment (Figure 6A). Cells reached roughly the same level of H₂O₂ tolerance after both NaCl treatments (data not shown); however, during the second cycle of NaCl treatment, cells acquired H₂O₂ resistance faster: cells reached 30% maximum tolerance 10 min faster during the second cycle compared to the first NaCl exposure (Figure 6B). The acceleration of acquired

Figure 5 The effect of NPC subunits on the faster expression response of *TSA2*. The average log₂ fold-change in *TSA2* transcript was scored by quantitative PCR in wild-type, *nup42Δ*, *nup59Δ*, and *nup100Δ* cells as described in Figure 4 and in Materials and Methods. The response of the naive wild type is shown in gray on mutant plots.

H₂O₂ matched the acceleration in gene-expression response. Consistent with the defect in transcriptional memory, the *nup42Δ* mutant did not show faster reacquisition of stress tolerance upon repeated cycles of NaCl ($P > 0.05$, t -tests).

Discussion

Many previous examples of cellular memory are associated with historical fluctuations in nutrient availability (Koshland 1977; Acar *et al.* 2005; Sigal *et al.* 2006; Brickner *et al.* 2007; Crews *et al.* 2007; Burns and Mery 2010; Carone *et al.* 2010; Hall *et al.* 2010; Ng *et al.* 2010). Our main goal here was to characterize yeast memory of osmotic shock. Cells transiently exposed to a viable dose of salt retain a memory of that treatment that extends to at least two

physiological levels: increased H₂O₂ resistance and an altered expression response to subsequent stress. Both aspects of memory are likely inherited by daughter cells that have never directly experienced the NaCl stress. However, memory of H₂O₂ resistance and memory at the gene-expression level are dependent on distinct mechanisms.

In the case of H₂O₂ tolerance, the memory is explained by long-lived Ctt1 protein produced during the NaCl pretreatment and then transmitted to daughter cells with each cell division. The transmission of cellular memory via a long-lived protein is reminiscent of the cellular memory of galactose exposure, which is at least partly dependent on long-lived Gal1p inherited by daughter cells (Acar *et al.* 2005; Zacharioudakis *et al.* 2007; Halley *et al.* 2010; Kundu and Peterson 2010). Presumably, inheritance of Ctt1p allows cells to rapidly detoxify H₂O₂, thereby increasing H₂O₂ survival. The memory of acquired H₂O₂ tolerance is not specific to salt treatment because we observed increased H₂O₂ resistance after starvation (data not shown) and after a mild heat shock (Figure S5), although the mechanism is likely independent of CTT1 [since heat-induced acquisition of H₂O₂ tolerance does not involve CTT1 (Berry *et al.* 2011)]. Burrill and Silver (2011) recently showed that cells can also maintain a memory of DNA damage. Thus, memory of prior environmental stress may be a common phenomenon, but it is likely explained by different mechanisms under different situations.

Separable from the memory of H₂O₂ tolerance, cells with a history of NaCl treatment displayed a faster gene-expression response to H₂O₂, long after cells were removed from the salt. Over 1500 genes showed a faster expression response upon repeated stress, significantly more than the handful of metabolic genes known to demonstrate transcriptional memory. Furthermore, our results show that transcriptional memory can be invoked across distinct stressors. The altered expression response affected both induced and repressed genes and extended to targets of several independently acting regulators, including Hog1p, Msn2p, and Yap1p, indicating that the effect is unlikely due to a single regulatory system. Feedback in previously activated regulatory systems, including the GAL and HOG networks, can produce faster responses to recurring stimuli (Zacharioudakis *et al.* 2007; Acar *et al.* 2008; Mettetal *et al.* 2008). However, this alone is insufficient to explain our results. First, Hog1p is activated during NaCl treatment, but does not regulate gene expression in response to H₂O₂ (J. Clarke and A. P. Gasch, unpublished results). Second, many of the genes with a faster response in cells with memory are not regulated by Hog1p (O'Rourke and Herskowitz 2004), including targets of the H₂O₂-responsive Yap1p. Finally, despite the enrichment of Hog1p and Yap1p targets, only about one-third of each factor's targets were affected; we could find no obvious or known difference in regulation of the affected subsets. Thus, faster activation of these networks cannot fully explain the faster genomic expression response.

Instead, our results suggest a role for nuclear pore components, including Nup42p. Nup42p could perhaps accelerate the

Figure 6 Cells with a memory of stress exposure show faster reacquisition of H₂O₂ tolerance. (A) The experimental schema represents H₂O₂ tolerance in cells that were exposed to 0.7 M NaCl for 60 min (black trace), returned to stress-free YPD medium for 240 min, and then exposed to a second treatment of 0.7 M NaCl (blue trace). (B) The percentage maximal H₂O₂ tolerance is shown, as described in Figure 1B, during the first NaCl treatment (black, as depicted in A) and during the second NaCl treatment (blue) for wild-type (left) and *nup42Δ* (right) cells. To compare the fold-change in H₂O₂ tolerance provoked by each round of NaCl exposure, H₂O₂ tolerance was scaled to the tolerance measured immediately before each NaCl addition (dashed blue line in A). Data represent the average and standard deviation of biological triplicates. Points with statistically significant differences ($P < 0.05$) are indicated with an asterisk.

expression response by facilitating mRNA export or transport of some other molecule; if so, this function must be restricted to successive stress treatments, since the *nup42Δ* mutant had no obvious defects before or after a single dose of stress. *Nup42p* could also facilitate association of the NPC with target genes. Several yeast genes associate with the nuclear pore upon induction (Brickner and Walter 2004; Casolari *et al.* 2004, 2005; Dieppois *et al.* 2006; Schmid *et al.* 2006; Taddei *et al.* 2006; Brickner *et al.* 2007; Sarma *et al.* 2007; Tan-Wong *et al.* 2009), which may promote transcriptional looping and/or couple transcription and mRNA export from the nucleus (Brickner 2009; Hampsey *et al.* 2011). Upon initial inositol starvation, *INO1* translocation to the nuclear periphery is dependent on *Nup42p* (Ahmed *et al.* 2010; Light *et al.* 2010); while initial translocation is independent of *Nup100p* [but perhaps amplified in its absence (Ahmed *et al.* 2010)], *Nup100p* is required for persistent peripheral localization and transcriptional memory of *INO1* after inositol depletion (Light *et al.* 2010). In our system, *nup42Δ* cells pretreated with NaCl behave like unstressed, naive wild-type cells, whereas cells lacking *Nup100p* display *TSA2* induction reminiscent of the wild-type cells' transcriptional memory. While further experiments will be required to dissect this function, these results suggest that gene localization to the NPC could be involved.

In support of this possibility, we identified several sequence motifs previously linked to transcriptional memory upstream of genes with a faster expression response after recurring stresses. Several DNA sequences, known as "DNA zip codes," have been implicated in peripheral gene targeting and transcriptional memory (Ahmed *et al.* 2010; Light *et al.* 2010). One such motif required for initial gene targeting has been characterized upstream of *TSA2*, and we observed a similar sequence upstream of *CTT1* (Figure 7A), both of which show a *Nup42p*-dependent expression effect after multiple stresses. A different zip code sequence, known as the Memory Recruitment Sequence (TCCTTCTTTCC), is required to maintain *INO1* at the pore after stimulus has been removed and is important for transcriptional memory (Light *et al.* 2010). Strikingly, we identified a related motif (Figure 7B) enriched in the group of induced genes with transcriptional memory (cluster 3 from Figure 4A, $P = 6 \times 10^{-4}$). Although future work will be required to dissect the function of this sequence, these results are consistent with the model that association with the nuclear pore is required for the transcriptional memory at a large number of genes.

Faster activation of stress-dependent expression changes has an important physiological outcome: faster acquisition of a second round of H_2O_2 tolerance. The memory of prior stress treatment therefore extends to multiple physiological levels and arises through distinct mechanisms, suggesting its importance in nature. Life in the real world is particularly challenging for single-celled organisms that must maintain internal homeostasis despite an ever-changing environment. As stressful environments likely occur in succession, memory of prior exposure may provide a potent survival strategy in the wild.

Figure 7 Sequences common to genes with faster expression response. (A) The characterized GRS1 zip code upstream of *TSA2* is shown aligned to a similar sequence upstream of the *CTT1* gene. Positions of identity are highlighted in gray. (B) MEME motif identified in the upstream regions of 77 genes induced with a faster response upon recurring stress (cluster 3, Figure 4). Information content (bits), where the height of each letter represents the frequency of the base at that position in the motif, is plotted.

Acknowledgments

We thank Derek Lindstrom, Dan Gottschling, David Eide, and Betty Craig for providing reagents and members of the Gasch Lab for constructive comments. This work was supported by a Beckman Young Investigator award to A.P.G. and National Institutes of Health National Institute of General Medical Sciences grant R01GM083989-01. D.G.B. was supported through the University of Wisconsin-Madison Integrated Biological Sciences Summer Research Program through National Library of Medicine training grant T15LM007359.

Literature Cited

- Acar, M., A. Becskei, and A. van Oudenaarden, 2005 Enhancement of cellular memory by reducing stochastic transitions. *Nature* 435: 228–232.
- Acar, M., J. T. Mettetal, and A. van Oudenaarden, 2008 Stochastic switching as a survival strategy in fluctuating environments. *Nat. Genet.* 40: 471–475.
- Ahmed, S., D. G. Brickner, W. H. Light, I. Cajigas, M. McDonough *et al.*, 2010 DNA zip codes control an ancient mechanism for gene targeting to the nuclear periphery. *Nat. Cell Biol.* 12: 111–118.
- Ajo-Franklin, C. M., D. A. Drubin, J. A. Eskin, E. P. Gee, D. Landgraf *et al.*, 2007 Rational design of memory in eukaryotic cells. *Genes Dev.* 21: 2271–2276.
- Alejandro-Orsorio, A. L., D. J. Huebert, D. T. Porcaro, M. E. Sonntag, S. Nillasithanukroh *et al.*, 2009 The histone deacetylase Rpd3p is required for transient changes in genomic expression in response to stress. *Genome Biol.* 10: R57.
- Anway, M. D., A. S. Cupp, M. Uzumcu, and M. K. Skinner, 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308: 1466–1469.
- Aragon, A. D., A. L. Rodriguez, O. Meirelles, S. Roy, G. S. Davidson *et al.*, 2008 Characterization of differentiated quiescent and nonquiescent cells in yeast stationary-phase cultures. *Mol. Biol. Cell* 19: 1271–1280.

- Bailey, T. L., and C. Elkan, 1994 Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* 2: 28–36.
- Berry, D. B., and A. P. Gasch, 2008 Stress-activated genomic expression changes serve a preparative role for impending stress in yeast. *Mol. Biol. Cell* 19: 4580–4587.
- Berry, D. B., G. Guan, J. Hose, S. Haroon, M. Gebbia *et al.*, 2011 Multiple means to the same end: the genetic basis of acquired stress resistance in yeast. *PLoS Genet.* 7: e1002353.
- Boyko, A., T. Blevins, Y. Yao, A. Golubov, A. Bilichak *et al.*, 2010 Transgenerational adaptation of Arabidopsis to stress requires DNA methylation and the function of Dicer-like proteins. *PLoS ONE* 5: e9514.
- Brickner, D. G., I. Cajigas, Y. Fondufe-Mittendorf, S. Ahmed, P. C. Lee *et al.*, 2007 H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. *PLoS Biol.* 5: e81.
- Brickner, J. H., 2009 Transcriptional memory at the nuclear periphery. *Curr. Opin. Cell Biol.* 21: 127–133.
- Brickner, J. H., and P. Walter, 2004 Gene recruitment of the activated INO1 locus to the nuclear membrane. *PLoS Biol.* 2: e342.
- Burns, J. G., and F. Mery, 2010 Transgenerational memory effect of ageing in *Drosophila*. *J. Evol. Biol.* 23: 678–686.
- Burrill, D. R., and P. A. Silver, 2011 Synthetic circuit identifies subpopulations with sustained memory of DNA damage. *Genes Dev.* 25: 434–439.
- Carone, B. R., L. Fauquier, N. Habib, J. M. Shea, C. E. Hart *et al.*, 2010 Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* 143: 1084–1096.
- Casadesús, J., and R. D'Ari, 2002 Memory in bacteria and phage. *Bioessays* 24: 512–518.
- Casolari, J. M., C. R. Brown, S. Komili, J. West, H. Hieronymus *et al.*, 2004 Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. *Cell* 117: 427–439.
- Casolari, J. M., C. R. Brown, D. A. Drubin, O. J. Rando, and P. A. Silver, 2005 Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. *Genes Dev.* 19: 1188–1198.
- Chi, Z., and N. Arneborg, 2000 *Saccharomyces cerevisiae* strains with different degrees up ethanol tolerance exhibit different adaptive responses response to produce ethanol. *J. Ind. Microbiol. Biotechnol.* 24: 75–78.
- Crews, D., A. C. Gore, T. S. Hsu, N. L. Dangleben, M. Spinetta *et al.*, 2007 Transgenerational epigenetic imprints on mate preference. *Proc. Natl. Acad. Sci. USA* 104: 5942–5946.
- Davies, J. M., C. V. Lowry, and K. J. Davies, 1995 Transient adaptation to oxidative stress in yeast. *Arch. Biochem. Biophys.* 317: 1–6.
- Dieppois, G., N. Iglesias, and F. Stutz, 2006 Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes. *Mol. Cell. Biol.* 26: 7858–7870.
- Durrant, W. E., and X. Dong, 2004 Systemic acquired resistance. *Annu. Rev. Phytopathol.* 42: 185–209.
- Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein, 1998 Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* 95: 14863–14868.
- Gao, C. Y., and J. L. Pinkham, 2000 Tightly regulated, beta-estradiol dose-dependent expression system for yeast. *Biotechniques* 29: 1226–1231.
- Gasch, A. P., 2002 Yeast genomic expression studies using DNA microarrays. *Methods Enzymol.* 350: 393–414.
- Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen *et al.*, 2000 Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* 11: 4241–4257.
- Goh, C. H., H. G. Nam, and Y. S. Park, 2003 Stress memory in plants: a negative regulation of stomatal response and transient induction of rd22 gene to light in abscisic acid-entrained Arabidopsis plants. *Plant J.* 36: 240–255.
- Grant, C. E., T. L. Bailey, and W. S. Noble, 2011 FIMO: scanning for occurrences of a given motif. *Bioinformatics* 27: 1017–1018.
- Hall, S. E., M. Beverly, C. Russ, C. Nusbaum, and P. Sengupta, 2010 A cellular memory of developmental history generates phenotypic diversity in *C. elegans*. *Curr. Biol.* 20: 149–155.
- Halley, J. E., T. Kaplan, A. Y. Wang, M. S. Kobor, and J. Rine, 2010 Roles for H2A.Z and its acetylation in GAL1 transcription and gene induction, but not GAL1-transcriptional memory. *PLoS Biol.* 8: e1000401.
- Hampsey, M., B. N. Singh, A. Ansari, J. P. Laine, and S. Krishnamurthy, 2011 Control of eukaryotic gene expression: gene loops and transcriptional memory. *Adv. Enzyme Regul.* 51: 118–125.
- Hecker, M., J. Pane-Farre, and U. Volker, 2007 SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. *Annu. Rev. Microbiol.* 61: 215–236.
- Huebert, D. J., P. F. Kuan, S. Keles, and A. P. Gasch, 2012 Dynamic changes in nucleosome occupancy are not predictive of gene expression dynamics but are linked to transcription and chromatin regulators. *Mol. Cell. Biol.* 32: 1645–1653.
- Jimenez, A., D. J. Tipper, and J. Davies, 1973 Mode of action of thiolutin, an inhibitor of macromolecular synthesis in *Saccharomyces cerevisiae*. *Antimicrob. Agents Chemother.* 3: 729–738.
- Kandror, O., N. Bretschneider, E. Kreydin, D. Cavalieri, and A. L. Goldberg, 2004 Yeast adapt to near-freezing temperatures by STRE/Msn2,4-dependent induction of trehalose synthesis and certain molecular chaperones. *Mol. Cell* 13: 771–781.
- Kaufman, P. D., and O. J. Rando, 2010 Chromatin as a potential carrier of heritable information. *Curr. Opin. Cell Biol.* 22: 284–290.
- Kelley, R., and T. Ideker, 2009 Genome-wide fitness and expression profiling implicate Mga2 in adaptation to hydrogen peroxide. *PLoS Genet.* 5: e1000488.
- Kensler, T. W., N. Wakabayashi, and S. Biswal, 2007 Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.* 47: 89–116.
- Koshland, D. E., 1977 A response model in a simple sensory system. *Science* 196: 1053–1063.
- Kundu, S., and C. L. Peterson, 2009 Role of chromatin states in transcriptional memory. *Biochim. Biophys. Acta* 1790: 445–455.
- Kundu, S., and C. L. Peterson, 2010 Dominant role for signal transduction in the transcriptional memory of yeast GAL genes. *Mol. Cell. Biol.* 30: 2330–2340.
- Kundu, S., P. J. Horn, and C. L. Peterson, 2007 SWI/SNF is required for transcriptional memory at the yeast GAL gene cluster. *Genes Dev.* 21: 997–1004.
- Lewis, J. A., I. M. Elkon, M. A. McGee, A. J. Higbee, and A. P. Gasch, 2010 Exploiting natural variation in *Saccharomyces cerevisiae* to identify genes for increased ethanol resistance. *Genetics* 186: 1197–1205.
- Lewis, J. G., R. P. Learmonth, and K. Watson, 1995 Induction of heat, freezing and salt tolerance by heat and salt shock in *Saccharomyces cerevisiae*. *Microbiology* 141(Pt. 3): 687–694.
- Light, W. H., D. G. Brickner, V. R. Brand, and J. H. Brickner, 2010 Interaction of a DNA zip code with the nuclear pore complex promotes H2A.Z incorporation and INO1 transcriptional memory. *Mol. Cell* 40: 112–125.
- Lindstrom, D. L., and D. E. Gottschling, 2009 The mother enrichment program: a genetic system for facile replicative life span analysis in *Saccharomyces cerevisiae*. *Genetics* 183: 413–422, 1S1–1S31.
- Lipson, D., T. Raz, A. Kieu, D. R. Jones, E. Giladi *et al.*, 2009 Quantification of the yeast transcriptome by single-molecule sequencing. *Nat. Biotechnol.* 27: 652–658.

- Lou, Y., and A. E. Yousef, 1997 Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Appl. Environ. Microbiol.* 63: 1252–1255.
- Louvion, J. F., B. Havaux-Copf, and D. Picard, 1993 Fusion of GAL4-VP16 to a steroid-binding domain provides a tool for gratuitous induction of galactose-responsive genes in yeast. *Gene* 131: 129–134.
- Lu, D., N. Maulik, I. I. Moraru, D. L. Kreutzer, and D. K. Das, 1993 Molecular adaptation of vascular endothelial cells to oxidative stress. *Am. J. Physiol.* 264: C715–C722.
- Matsumoto, H., N. Hamada, A. Takahashi, Y. Kobayashi, and T. Ohnishi, 2007 Vanguard of paradigm shift in radiation biology: radiation-induced adaptive and bystander responses. *J. Radiat. Res.* 48: 97–106.
- Mettetal, J. T., D. Muzzey, C. Gomez-Urbe, and A. van Oudenaarden, 2008 The frequency dependence of osmo-adaptation in *Saccharomyces cerevisiae*. *Science* 319: 482–484.
- Molinier, J., G. Ries, C. Zipfel, and B. Hohn, 2006 Transgeneration memory of stress in plants. *Nature* 442: 1046–1049.
- Ng, H. H., F. Robert, R. A. Young, and K. Struhl, 2003 Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol. Cell* 11: 709–719.
- Ng, S. F., R. C. Lin, D. R. Laybutt, R. Barres, J. A. Owens *et al.*, 2010 Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. *Nature* 467: 963–966.
- O'Rourke, S. M., and I. Herskowitz, 2004 Unique and redundant roles for HOG MAPK pathway components as revealed by whole-genome expression analysis. *Mol. Biol. Cell* 15: 532–542.
- Saavedra, C. A., C. M. Hammell, C. V. Heath, and C. N. Cole, 1997 Yeast heat shock mRNAs are exported through a distinct pathway defined by Rip1p. *Genes Dev.* 11: 2845–2856.
- Saigusa, T., A. Tero, T. Nakagaki, and Y. Kuramoto, 2008 Amoebae anticipate periodic events. *Phys. Rev. Lett.* 100: 018101.
- Sarma, N. J., T. M. Haley, K. E. Barbara, T. D. Buford, K. A. Willis *et al.*, 2007 Glucose-responsive regulators of gene expression in *Saccharomyces cerevisiae* function at the nuclear periphery via a reverse recruitment mechanism. *Genetics* 175: 1127–1135.
- Schenk, P. M., K. Kazan, I. Wilson, J. P. Anderson, T. Richmond *et al.*, 2000 Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc. Natl. Acad. Sci. USA* 97: 11655–11660.
- Schmid, M., G. Arib, C. Laemmli, J. Nishikawa, T. Durussel *et al.*, 2006 Nup-PI: the nucleopore-promoter interaction of genes in yeast. *Mol. Cell* 21: 379–391.
- Scholz, H., M. Franz, and U. Heberlein, 2005 The hangover gene defines a stress pathway required for ethanol tolerance development. *Nature* 436: 845–847.
- Sigal, A., R. Milo, A. Cohen, N. Geva-Zatorsky, Y. Klein *et al.*, 2006 Variability and memory of protein levels in human cells. *Nature* 444: 643–646.
- Smyth, G. K., 2005 Limma: linear models for microarray data, pp. 397–420 in *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, edited by R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, and W. Huber. Springer, New York.
- Storey, J. D., and R. Tibshirani, 2003 Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. USA* 100: 9440–9445.
- Stutz, F., M. Neville, and M. Rosbash, 1995 Identification of a novel nuclear pore-associated protein as a functional target of the HIV-1 Rev protein in yeast. *Cell* 82: 495–506.
- Stutz, F., J. Kantor, D. Zhang, T. McCarthy, M. Neville *et al.*, 1997 The yeast nucleoporin rip1p contributes to multiple export pathways with no essential role for its FG-repeat region. *Genes Dev.* 11: 2857–2868.
- Swan, T. M., and K. Watson, 1999 Stress tolerance in a yeast lipid mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose. *Can. J. Microbiol.* 45: 472–479.
- Taddei, A., G. Van Houwe, F. Hediger, V. Kalck, F. Cubizolles *et al.*, 2006 Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* 441: 774–778.
- Tan-Wong, S. M., H. D. Wijayatilake, and N. J. Proudfoot, 2009 Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex. *Genes Dev.* 23: 2610–2624.
- Thomsen, R., C. Saguez, T. Nasser, and T. H. Jensen, 2008 General, rapid, and transcription-dependent fragmentation of nucleolar antigens in *S. cerevisiae* mRNA export mutants. *RNA* 14: 706–716.
- Vainberg, I. E., K. Dower, and M. Rosbash, 2000 Nuclear export of heat shock and non-heat-shock mRNA occurs via similar pathways. *Mol. Cell. Biol.* 20: 3996–4005.
- Xiong, W., and J. E. Ferrell Jr., 2003 A positive-feedback-based bistable 'memory module' that governs a cell fate decision. *Nature* 426: 460–465.
- Zacharioudakis, I., T. Gligoris, and D. Tzamarias, 2007 A yeast catabolic enzyme controls transcriptional memory. *Curr. Biol.* 17: 2041–2046.

Communicating editor: M. D. Rose

GENETICS

Supporting Information

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143016/-/DC1>

Cellular Memory of Acquired Stress Resistance in *Saccharomyces cerevisiae*

Qiaoning Guan, Suraiya Haroon, Diego González Bravo, Jessica L. Will, and Audrey P. Gasch

Figure S1 Memory decay is dependent on cell division but not protein synthesis. Cells were exposed to 0.7 M NaCl for 60 min and returned to stress-free YPD medium for growth, and H₂O₂ tolerance was measured throughout the experiment as described in Materials and Methods. (A) Thiolutin was added to the medium immediately after (red squares) or 120 min after (red circles) removal from NaCl treatment, and the relative resistance to H₂O₂ was scored over 480 min. Blue line: H₂O₂ resistance in cells with no thiolutin added, grey line: percentage of original stressed cells in the population. (B) A similar experiment was conducted, except that 10 μ M alpha factor was added 120 min after cells were returned to YPD growth. Relative resistance to H₂O₂ is shown as: red line closed circle (with alpha factor), or blue line closed square (without alpha factor). The percentage of original stressed cells in the culture (inferred based on optical density) is shown as: grey line open circle (with alpha factor), or grey line open triangle (without alpha factor). Black arrow represents time of addition of thiolutin or alpha factor.

Figure S2 Daughter cells inherent acquired stress resistance. Resistance to 1mM H₂O₂ was scored in UCC8613 cells exposed to 0.7M NaCl for 60 min then returned to YPD with 1 μ M estradiol to mark daughter cells as adenine auxotrophs (ade⁻). In this strain, the adenine biosynthesis gene *ADE2* is flanked by LoxP sites, while an estradiol-activated CRE recombinase is expressed via the bud-specific transcription factor Ace2p. Upon addition of estradiol, the bud-specific recombinase excises *ADE2* only in daughter cells. This renders daughter cells permanently auxotrophic for adenine (ade⁻), a phenotype that can be conveniently distinguished by the red coloration of ade⁻ cells. Estradiol-treatment rendered more than 80% of daughter cells ade⁻, while less than 3% of cells spontaneously lost *ADE2* in the absence of estradiol. (A) Cells were exposed to NaCl for 60 min and then resuspended in fresh YPD medium; 1 μ M estradiol was added either immediately or after ~2 generations in stress-free medium (at which point we estimate ~75% of cells had never directly experienced NaCl). Cell viability was scored by plating cells on YPD to measure all cells (blue curve), or on SC-adenine to measure mother cells (grey curve). (B) Cell viability was also scored from YPD plates by counting red (ade⁻) colonies arising from daughter cells and white (ade⁺) colonies generated by mother cells. Data represent the average and standard deviation of at least biological triplicates. Results were indistinguishable if estradiol was added immediately after cell transfer to YPD or after 2 generations of YPD growth (data not shown).

Figure S3 Characterization of the *nup42Δ* mutant. (A) H₂O₂ tolerance was measured as described in Figure 1 in wild type and *nup42Δ* mutant cells before ('b') or at 60 min after treatment with 0.7M NaCl ('A'), and in cells with a memory at 180 min after return to stress-free medium ('M'). The sum viability score across the 11 doses of H₂O₂ is shown, and data represent the average and standard deviation of biological triplicates. In all cases, the mutant was indistinguishable from the wild type cells ($p > 0.1$). (B) Levels of heat-shock factor Ssa3/4p were measured by Western analysis (and normalized to an internal Act1p control in each lane) in cells grown at 25°C and then shifted to either 0.7 M NaCl or fresh medium preheated to 35°C or 42°C for 60 min. Data represent the average of biological duplicates. (C) Sum-viability across 11 doses of H₂O₂ is shown relative to the comparable score in mock-treated cells to represent the level of acquired stress resistance 60 min after 0.7M NaCl, a 25-35°C heat shock, or a 25-42°C heat shock. Together, the data show that the *nup42Δ* mutant behaves like wild type after mild heat or NaCl treatment but not after a severe 25-42°C heat shock.

Figure S4 Transcriptional memory at *CTT1* and *HSP12*. As shown in Figure 5 for wild type and *nup42Δ* cells.

Figure S5 Memory of H₂O₂ resistance after heat shock. H₂O₂ tolerance was scored as described in Figure 1, in cells harboring FLAG-tagged *CTT1* exposed to at 24° – 37°C heat shock (orange curve) and then returned to 30°C degree stress-free medium. The response to 0.7 M NaCl is shown for reference (blue curve).