Different Mechanisms Confer Gradual Control and Memory at Nutrient- and Stress-Regulated Genes in Yeast

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Cells respond to environmental stimuli by fine-tuned regulation of gene expression. Here we investigated the dose-dependent modulation of gene expression at high temporal resolution in response to nutrient and stress signals in yeast. The GAL1 activity in cell populations is modulated in a well-defined range of galactose concentrations, correlating with a dynamic change of histone remodeling and RNA polymerase II (RNAPII) association. This behavior is the result of a heterogeneous induction delay caused by decreasing inducer concentrations across the population. Chromatin remodeling appears to be the basis for the dynamic GAL1 expression, because mutants with impaired histone dynamics show severely truncated dose-response profiles. In contrast, the GRE2 promoter operates like a rapid off/on switch in response to increasing osmotic stress, with almost constant expression rates and exclusively temporal regulation of histone remodeling and RNAPII occupancy. The Gal3 inducer and the Hog1 mitogen-activated protein (MAP) kinase seem to determine the different dose-response strategies at the two promoters. Accordingly, GAL1 becomes highly sensitive and dose independent if previously stimulated because of residual Gal3 levels, whereas GRE2 expression diminishes upon repeated stimulation due to acquired stress resistance. Our analysis reveals important differences in the way dynamic signals create dose-sensitive gene expression outputs.

Cells continuously adapt their protein composition to changing environmental conditions. The regulation of gene expression is one of the fundamental mechanisms to adjust the global protein repertoire of the cell in order to maintain cell function and integrity in response to environmental challenges. Budding yeast is a powerful model to unravel the modes of transcriptional adaptation at the levels both of specific genes and of the whole organism (1, 2). Additionally, the basic structure of the signaling cascades responding to environmental perturbations is conserved from yeast to humans. It implies the alteration of core kinase activities, which modulate the expression of defense genes through a range of specific transcription factors. Extensive knowledge which precisely describes the molecular machinery and its global impact on gene expression in response to many types of stress has accumulated (3–7). However, the vast majority of these studies are performed with harsh environmental insults and therefore saturating stimulation. As a consequence, only very limited information or approaches are available to understand how cells adapt their gene expression programs to small or gradual changes in their environment.

It is assumed that cells have acquired mechanisms that ensure a transcriptional response which is finely adjusted according to the strength of the stress or stimulation. However, the nature of the signaling molecules which confer gradual transcription outputs remains to be determined in most cases. Fine-tuning of gene expression responses can occur with different purposes, and the generation of a graded response can be achieved at different stages along the signal transduction path. For example, a linear response to mating pheromone has been described for the yeast mating mitogen-activated protein (MAP) kinase cascade (8). Additionally, specific transcriptional activators such as yeast Msn2 or Crz1 and mammalian NF-κB transmit linear signals to their cognate promoters by modulating their nuclear accumulation (9–12).

The same signal transduction pathway might have to distinguish related signals that originated from different stressors. This has been very recently described for yeast Msn2, a transcription factor responding to general stress and capable of filtering different stress inputs to generate graded gene expression outputs (13). Furthermore, among the often numerous genes activated in response to a given stress, the cell has to impose different sensitivities to guarantee an equilibrated adaptive response. Here, chromatin structure has been implied in modulating the threshold of gene activation in the yeast phosphate response (14), and different natural promoters and cis regulatory elements confer characteristic dose-sensitive expression profiles upon osmotic and oxidative stress (15).

Here we investigated the mechanisms that confer a gradual and dose-sensitive gene expression for two types of environmental cues: (i) the availability of a specific carbon and energy source and (ii) cytotoxic stress. We used two very well defined model genes, the nutrient-regulated GAL1 and stress-regulated GRE2 genes.

The expression of the yeast GAL genes is specifically upregulated by the presence of galactose in the growth medium via the transcriptional activator Gal4 (16). Gal4 is already bound at its cis regulatory elements and is capable of binding small activation domains (17, 18). Upon growth in glucose,
FIG 1 Comparison of transcriptional memory at the GAL1 and GRE2 genes. (A) GAL1 expression is efficiently sensitized upon subsequent galactose induction. A GAL1-lucCP+ reporter gene was used in live-cell luciferase assays to determine the expression rates in raffinose-containing minimal medium after supplementation with the indicated concentrations of galactose. Naive cells (− memory) were stimulated just once with galactose, while transcriptional memory (+ memory) was achieved by a previous galactose induction, as explained in Materials and Methods. (B) The stress-induced GRE2 expression decreases and is not sensitized by repeated stimulation. A GRE2-lucCP+ reporter gene was used in live-cell luciferase assays to determine the expression rates in glucose-containing minimal medium after supplementation with NaCl. Cells were pretreated with 0.7 M NaCl (+ memory) or not pretreated (− memory) before the induction with the indicated NaCl doses. (C) Effect of Gal3 levels on the dose response of GAL1. A GAL1-lucCP+ reporter gene was used in live-cell luciferase
limiting and galactose-containing medium, GAL gene expression is induced with the help of the GAL3 inducer. Upon stimulation, GAL3 binds galactose and ATP, interrupts Gal80 inhibition of Gal4, and permits transcriptional activation (19–21). Gal4 additionally recruits chromatin-modifying complexes and mediator (22–27) in order to efficiently induce GAL gene expression.

**GRE2** is a prototypical gene involved in the hypersomatic and oxidative stress defense, which includes the stimulated gene expression encompassing hundreds of different cellular functions (5, 28). Its promoter is bound by the specific transcription factor Sko1 in a complex with the general coresspressor Cyc8-Tup1 under normal noninducing growth conditions (29). Upon hypersomatic stress, transcriptional activation of GRE2 is rapidly achieved by the association of Sko1 with the stress-activated MAP kinase Hog1, which switches Sko1 from repression to activation by multiple phosphorylation and the additional recruitment of chromatin modifiers and the mediator complex (30). As a result, GRE2 gene expression is very fast and transiently activated, as commonly observed for transcriptional stress responses in yeast.

Additionally, the cell’s history can modulate the transcriptional response at specific genes. Transcriptional memory has been described for several inducible yeast genes, including GAL1. Here, a previous galactose induction facilitates the transcriptional response to the second galactose exposure. Different mechanisms have been proposed to establish transcriptional memory at the GAL genes, including the tethering of actively transcribed GAL1 to the nuclear envelope via the histone variant Htz1, prolonged chromatoin remodeling via Swi/Snf, or the inheritance of signaling compounds such as the Gal1 and Gal3 inducers (31–34).

The general architectures of the GAL1 and GRE2 regulons are very similar and involve a switch of a promoter-bound transcription factor from an inactive (or repressed) state to an active state by the direct association with a specifically activated inducer. Here, we identify important differences in how both systems regulate and identify the molecules which modulate the characteristics of yeast wild-type strains BY4741 and W303-1A. Multicopy integration plasmid pRS406-GAL1-lucFluc was built by swapping MET25 with the GAL1 promoter in pRS406-MET25-Fluc and integrating into strain MMY162-2C. For constitutive or induced overexpression of GAL3 under the control of the TDI3 or GAL1 promoter, the entire GAL3 gene was integrated in the Gateway destination vectors pAG416GPD-cbdB and pAG416GAL1-cbdB (39). For constitutive overexpression of ENA1 under the control of the PMA1 promoter, the plasmid pRS699-ENA1 (a gift from J. M. Mulet, Valencia, Spain) was used.

**Live-cell luciferase assays.** Yeast strains containing the indicated luciferase fusion genes were grown at 28°C in synthetic dextrose (SD) or synthetic raffinose (SRaf) medium lacking histidine (0.67% yeast nitrogen base, 2% glucose, 50 mM succinic acid [pH 5.5], 0.1 g/liter leucine, 0.1 g/liter methionine, 0.025 g/liter uracil) to exponential growth phase. Culture aliquots were then incubated with 0.5 mM luciferin (Sigma) on a roller at 28°C for 90 min. The cells were then transferred in 100-μl aliquots in white 96-well plates (Nunc) with or without the indicated concentrations of NaN, menadione, or galactose supplied from appropriate stock solutions. The light emission was then continuously recorded in a GloMax microplate luminometer (Promega) in three biological replicates. Data were processed with Microsoft Excel software. For representation of the relative light units of each reporter gene, we normalized the raw data for the number of cells in each assay. The maximal synthesis rate (V_{max}) and the maximal luciferase activity (A_{max}) were calculated as described previously (38).

**ChIP.** Chromatin immunoprecipitation (ChIP) was performed essentially as described previously (41). For the immunoprecipitation of HA fusion proteins, a mouse monoclonal anti-HA antibody (12CA5; Roche) was used in combination with Dynabeads protein A (Invitrogen). For the immunoprecipitation of TAP fusion proteins, pan-mouse IgG Dynabeads (Invitrogen) were used. For the immunoprecipitation of histone H3, a polyclonal anti-H3 antibody (ab1791; Abcam) was used in combination with Dynabeads protein A (Invitrogen). Quantitative PCR (qPCR) analyses at the indicated chromosomal loci were performed in real time using assays to determine the expression rates in raffinose-containing minimal medium after supplementation with the indicated concentrations of galactose. Cells were grown in synthetic glucose medium before induction was started in synthetic raffinose medium containing the indicated galactose concentrations. Δgal3 mutants containing plasmid-carried GAL3 under constitutive control (GPD-GAL3) or the empty vector (Δgal3) were compared with wild-type cells containing the empty vector (ΔGAL3). Constitutive overexpression of GAL3 leads to GAL induction by raffinose; therefore, an additional control in glucose-containing SD medium is included in the last panel. (D) The increase of GRE2 expression upon repeated NaCl induction depends on a functional ENA gene cluster. The indicated strains (DBY746) were compared for GRE2-lucCP expression under conditions identical to those for panel B. (E) Transient activation of GRE2 depends on the Ena1 levels. Yeast wild-type cells (BY4741) were assayed for GRE2-lucCP expression in the presence of constitutive ENA1 overexpression (PMA1-His1-ENA1) or the empty plasmid (wt) upon exposure to 0.4 M NaCl. Cells were pretreated (+ memory) or not (− memory) with 0.7 M NaCl for all panels, the mean values for three independent biological replicas are shown for each galactose or NaCl concentration (standard deviation, <15%).

**Dose-Sensitive Gene Regulation in Yeast**

**MATERIALS AND METHODS**

**Yeast strains.** The Saccharomyces cerevisiae strains used in this study were wild-type (wt) BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) and strains carrying the allelic alleles gal5::KanMX4, gen5::KanMX4, snf2::KanMX4, gal11::KanMX4, and htz1::KanMX4 (35). Yeast strains expressing chromosomally tagged TAP fusion proteins were BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) with GALA-TAP-HisMX and GAL3-TAP-His3MX (36). Yeast strains expressing chromosomally tagged hemagglutinin (HA) fusion proteins were W303-1A (MATa leu2-3,112 trpl-1 1 ade1-100 ura3-1 his3-11,15) with 3×HA-HOG1, 3×HA-SKO1 (30), and 3×HA-RPB3 (37). Yeast strains expressing firefly luciferase were MMY116-2C (MATa leu2-3,112 trpl-1 can1-100 ura3-1 his3-11,15) without GAL1-Fluc and MMY116-2C with GAL1-Fluc integrated in the URA3 locus (a gift from A. Mazo-Vargas). DBY746 (MATa ura3-52 trpl-1 289 his3Δ1 leu2-3,112) and its ena4−::LEU2 derivative were used to study the effect of the ENA gene dose on the GRE2 transcriptional memory.

**Plasmid constructions.** Single-copy reporter fusions with a destabilized luciferase gene (lucCP) were constructed as described previously (38). The upstream regulatory sequences of GRE2 (nucleotides −940 to −7), GAL1 (nucleotides −450 to −10) (38), CTT1 (nucleotides −983 to −10), SOD2 (nucleotides −977 to −16) (15), ALD6 (nucleotides −785 to −2), and HOR2 (nucleotides −948 to −33) (this study) were used. An integrative version of the GRE2-lucCP reporter fusion was constructed by insertion of the lucCP− gene into the pAG306GAL1-cbdB Gateway destination vector (39), which was integrated into the URA3 locus of yeast wild-type strain W303-1A. An integrative version of the GRE2-lucCP reporter fusion was constructed by insertion of the lucCP− gene before the KanMX marker in the pUG6 plasmid. The LucCP− KanMX-containing cassette was PCR amplified and fused to the GRE2 promoter in the genomes of yeast wild-type strains BY4741 and W303-1A. Multi-copy integration plasmid pRS406-GAL1-lucFluc was built by swapping MET25 with the GAL1 promoter in pRS406-MET25-Fluc and integrating into strain MMY162-2C (40). Both the plasmid and integrated strains were gifts from A. Mazo-Vargas. For constitutive or induced overexpression of GAL3 under the control of the TDI3 or GAL1 promoter, the entire GAL3 gene was integrated in the Gateway destination vectors pAG416GPD-cbdB and pAG416GAL1-cbdB (39). For constitutive overexpression of ENA1 under the control of the PMA1 promoter, the plasmid pRS699-ENA1 (a gift from J. M. Mulet, Valencia, Spain) was used.

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Comparison of the gradual gene expression, RNAPII occupancy, and histone remodeling at the GAL1 and GRE2 genes. (A) The expression of a GAL1-lucCP+ reporter gene is dynamically modulated at the level of the synthesis rate. A live-cell luciferase assay was used to determine the expression rates in raffinose-containing minimal medium after supplementation with the indicated concentrations of galactose. GAL1-luciferase fusions were expressed from centromeric plasmids (upper panel) or after integration in the genome (lower panel). Data for the upper panel are from reference 38. The mean values for three independent biological replicas are shown for each galactose concentration (standard deviation, ~15%). (B) Gradual association of RNAPII with the GAL1 promoter is modulated by the galactose inducer concentration. ChIP of Rpb3-HA-expressing cells was used to determine the RNAPII density at the GAL1 promoter. 

FIG 2
an Applied Biosystems 7500 sequence detector and Fast EvaGreen Mastermix for qPCR (Biotium). All occupancy data are presented as fold IP over the POL1 coding sequence (+1796/+1996) internal control. Each ChIP was performed in triplicate with three different chromatin samples. All primer sequences used for ChIP are available upon request.

Transcriptional memory experiments. For memory experiments at GAL1, cells containing the GAL1-lucCP+ reporter gene were grown overnight in synthetic raffinose (SRaff) medium lacking histidine to exponential growth phase. A first round of induction was then performed for 2 h with 2% galactose, while naïve cells remained in SRaff medium. Both cell cultures were then precipitated, washed once with water, and then incubated in fresh SD medium for 1 h. Finally, cells were changed to SRaff medium containing 0.3 mM luciferin for 90 min before starting the next induction with the indicated galactose concentrations and continuous measurement of luciferase activity. For the memory experiment under gradual Gal3 expression levels, the duration of the first round of galactose induction was reduced to 30 min.

For memory experiments at GRE2, cells containing the GRE2-lucCP+ reporter gene were grown overnight in synthetic glucose (SD) medium lacking histidine to exponential growth phase. A first salt shock was applied by treatment with 0.7 M NaCl for 1 h, while naïve cells remained in SD medium. We confirmed that those salt stress conditions did not cause any decrease in cell viability. Both cell cultures were then precipitated, washed once with water, and then incubated in fresh SD medium with 0.5 mM luciferin for 90 min. The indicated NaCl doses were then applied to aliquots of both cultures and the luciferase activity continuously measured.

Single-cell time-lapse luminescence microscopy. Cells with an integrated GAL1-FLuc reporter gene were grown overnight in synthetic raffinose (SRaff) medium. We sonicated our yeast in a Diagenode Bioruptor UCD-200 sonicator for 30 s at medium intensity to obtain single-cell suspensions before loading them onto microfluidic plates (CellAsic). Bioluminescence imaging of yeast cells was performed with a DV Elite microscope equipped with UltimateFocus, an Evolve EMCCD camera, and a 60x/1.25-numerical-aperture (NA) phase oil objective lens. Cells were grown for 60 min at 30°C in SRaff medium at pH 3.8 with 200 μM beetle 6-luciferin before switching to the SRaff-plus-galactose version of the same medium. We imaged cells every 4 min and processed raw data using same protocols as before (40). Briefly, cell segmentation was done in Cell-Stat (MATLAB plug-in [42]), and single-cell gene expression was fit to an exponential curve using the induction model described previously (40).

RESULTS

Comparison of transcriptional memory at the nutrient-controlled GAL1 and stress-induced GRE2 genes. Transcriptional memory effects in yeast have been found predominantly at nutrient-regulated genes. We therefore sought to compare how the dose-dependent gene expression was modulated after a previous stimulation of the indicated galactose concentrations. We next tested whether the stress-activated GRE2 gene changed upon repeated activation. Memory experiments were performed with the GRE2-luciferase real-time reporter and multiple NaCl induction. We observed in this case (Fig. 1B) that the induction of GRE2 expression was neither faster nor more efficient nor more sensitive at low stress doses in the second round of stimulation. Irrespective of previous gene induction, GRE2 expression always occurred at the same time and with remarkably similar synthesis rates. The only difference we observed was that cells which responded to salt stress for the second round of stimulation aborted the induced GRE2 expression a few minutes earlier than naïve cells. Thus, it seemed that GRE2 induction is not further enhanced or sensitized by previous stress treatment and that it instead reduced the amplitude of the transcriptional burst at GRE2 in the second round of activation.

Next we wanted to gain insights into the mechanisms which sensitized GAL1 gene expression to dose-independent maximal induction rates after previous induction. It has been previously reported that the transcriptional memory at GAL1 was dominantly regulated by signaling molecules such as the Gal3 inducer (33). Thus, we manipulated the Gal3 levels and tested its impact on the dose-dependent induction profile of GAL1. As depicted in Fig. 1C, constitutive overexpression of GAL3 led to a highly efficient GAL1-luciferase expression independently of the galactose promoter in synthetic raffinose medium before and after induction with the indicated galactose concentrations. The mean values for three independent biological replicates are shown with the corresponding standard deviation. (C) Nucleosome remodeling is gradually stimulated by increasing galactose inducer concentrations. We next tested whether the dose-response behavior of the stress-activated GRE2 gene changed upon repeated activation. Memory experiments were performed with the GRE2-luciferase real-time reporter and multiple NaCl induction. We observed in this case (Fig. 1B) that the induction of GRE2 expression was neither faster nor more efficient nor more sensitive at low stress doses in the second round of stimulation. Irrespective of previous gene induction, GRE2 expression always occurred at the same time and with remarkably similar synthesis rates. The only difference we observed was that cells which responded to salt stress for the second round of stimulation aborted the induced GRE2 expression a few minutes earlier than naïve cells. Thus, it seemed that GRE2 induction is not further enhanced or sensitized by previous stress treatment and that it instead reduced the amplitude of the transcriptional burst at GRE2 in the second round of activation.

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inducer concentration. Therefore, high GAL3 levels can mimic the enhanced sensitivity of GAL1 expression acquired during repeated galactose induction. The Gal3 inducer level is therefore a key element in changing the dose-response behavior at the GAL1 gene during memory.

GRE2 expression was modulated by repeated stimulation in a manner opposite to that for GAL1, as the second wave of transient gene expression was shorter than the initial one in this case. We hypothesized that the accumulation of defense proteins in the first round of stimulation could prepare the cells for the second salt shock and thereby permit an efficient adaptation with a diminished transcriptional response. We considered two physiological adaptations as most relevant for the tolerance to salt stress: the accumulation of the osmolyte glycerol and the enhanced extrusion of Na⁺. Both processes can be blocked by single deletions in key structural genes, such as in the gpd1 and ena1-4 mutants, respectively. We repeated the memory experiments in those specific mutant strains. The gpd1 cells were indistinguishable from the wild type (data not shown), but we detected important differences for the ena1-4 mutant, which lacks all copies of the Ena Na⁺ extrusion ATPase. As shown in Fig. 1D, in this mutant the GRE2-lucCP reporter responded almost equally during the first and second exposures to NaCl stress. Finally, we altered the ENA1 expression levels by the use of an additional copy of the gene under the control of the constitutive PMA1 promoter. The effect of increasing ENA1 expression was a subsequent decrease of the GRE2 expression peak (Fig. 1E), thus providing additional evidence that the gradual GRE2 expression depended on the amount of the Ena1 Na⁺ pump. Taken together, these experiments show that important differences exist in the responses of differentially regulated genes to previous exposure. GAL1 gene expression is highly sensitized to previous exposure, which leads to a dose-independent activation likely driven by the Gal3 inducer. The transient GRE2 expression is not sensitized to previous exposure and seems to be modulated principally by the physiology of the cell, which dictates the amplitude of the transcriptional response at this gene.

Dose-dependent expression of GAL1 in cell populations corresponds with gradual histone remodeling and RNAPII association. The pattern of the gradual response of GAL1 to increasing galactose concentrations was recorded with real-time luciferase reporters, which were expressed from centromeric plasmids or integrated into the yeast genome. The GAL1-luciferase expression was stimulated to a detectable level with a minimal galactose concentration of approximately 0.02% for plasmid expression or 0.01% for genomic expression. Increasing stimulus concentrations provoked a continuous increase of the reporter activity until a threshold concentration of 0.5% was reached (Fig. 2A). Greater galactose concentrations did not further increase the reporter activity; however, they slightly decreased the lag time between stimulation and response. Since GAL1 transcript levels are actively repressed in the absence of galactose, we interpreted the GAL1-luciferase expression data as the actual mRNA synthesis rates which are dynamically modulated in a stimulus dependent manner. Galactose concentrations from 0.02% to 0.5% result in a gradual activation of GAL1 promoter activity. We next addressed whether this dynamic behavior was attributable to a galactose-dependent regulation of RNA polymerase II (RNAPII) association at GAL1. We performed in vivo ChIP experiments to quantify the association of the RNAPII subunit Rpb3 with the GAL1 promoter within the range of galactose concentrations which apparently cause graded transcription outputs. As shown in Fig. 2B, RNAPII recruitment is slow and inefficient at low threshold concentrations (0.03% galactose) and is continuously faster and more efficient until an upper threshold concentration of 0.5% galactose is reached. As a result, we can correlate the dynamic behavior observed with the GAL1p-driven luciferase expression system with the gradual association of the transcription machinery at the GAL1 promoter.

We then wanted to know whether the gradual increase of GAL1 promoter activity was accompanied by graded chromatin remodeling. Therefore, we determined the histone H3 density at GAL1 by ChIP over the same range of galactose concentrations. As depicted in Fig. 2C, we found that the speed and efficiency of nucleosome remodeling at GAL1 are gradually increased in the dynamic range of galactose concentrations. Thus, at the nutrient-regulated GAL1 gene, an ample range of inducer concentrations is transduced to a graded remodeling of its nucleosomal promoter structure and the dynamic entry of the transcription machinery.

Dose-dependent expression of GRE2 relies on the temporal regulation of histone remodeling and RNAPII association at constant synthesis rates. We next extended our analysis of graded gene regulation to the transiently activated GRE2 gene. We first determined the complete dose-response profile in response to NaCl stress in vivo by the use of plasmid-borne or integrated GRE2-luciferase reporters. As shown in Fig. 2D, GRE2 responded with characteristic and transient activation profiles in an NaCl concentration range from 0.1 M to 1 M. Moderate salt concentrations (0.15 to 0.4 M) induced GRE2 always at the same time and with almost identical synthesis rates. However, in the same dynamic range of salt concentrations, a gradual increase of the stimulus (NaCl) provoked gradually increasing maximal reporter activities. This apparently was achieved by continuously prolonging the time during which GRE2-lucCP remained actively expressed at maximal synthesis rates. In summary, in contrast to the gradual regulation of synthesis rates seen in the case of GAL1, the GRE2-luciferase reporter was dynamically regulated temporally while maintaining constant gene expression rates.

We then wanted to determine whether this particular dose-response profile correlated with transcriptional events. Thus, we directly measured the association of RNAPII and histone H3 by ChIP at GRE2 at NaCl concentrations which cause dynamic changes in the maximal expression. We found that RNAPII association with the GRE2 promoter occurred very rapidly at low salt concentrations (0.1 to 0.3 M) (Fig. 2E). Clearly, increasing salt concentrations did not stimulate the absolute RNAPII levels but continuously increased the time during which the RNA polymerase remained associated with GRE2. These data correlated well with the rapid loss of histone H3 from the GRE2 promoter region, which was continuously prolonged, but not more efficient, in response to stimulation by increasing NaCl shocks (Fig. 2F). Taking these observations together, we find that the differential expression of GRE2 caused by increased stress doses is achieved mainly by regulating the time during which the promoter remains actively transcribed with practically constant synthesis rates, RNAPII occupancy, and histone eviction.

We then wanted to determine whether this temporal pattern of dose response was general for stress-responsive genes. Therefore, we quantified the dose-sensitive expression patterns of two more osmotic-stress-inducible natural promoters, HOP2 and ALD6. The respective fusions with destabilized luciferase were suitable...
for determination of the entire dose-response profiles for both genes upon NaCl stress (Fig. 3A). The comparison of the stimulus-dependent modulation of both the maximal expression and the synthesis rates revealed almost identical patterns for GRE2, ALD6, and HOR2 (Fig. 3B). At all three genes the maximal synthesis rate was reached with low stress doses (0.2 M NaCl), while the maximal expression further increased until 0.4 M NaCl due to prolonged activation of the respective fusion genes. Finally we tested the dose-response behavior of two additional genes, SOD2 and CTT1, in response to a different type of environmental cue such as oxidative stress (Fig. 3C). As depicted in Fig. 3D, the two genes showed very similar patterns of maximal gene expression and synthesis rates upon increasing stimulation with menadione compared to the previous patterns obtained for salt stress. Thus, the temporal modulation of gene activity in response to increasing stress doses might be a general feature for stress-responsive genes in yeast.

Increasing galactose stimuli gradually decrease the lag phase and cell-to-cell variability to activate GAL1 gene expression. We investigated the dose-sensitive response of GAL1 at the level of single cells. GAL1 gene expression has been reported to occur in a bimodal fashion, especially at lower galactose concentrations (43–46). Therefore, we wanted to test to what degree bimodality was the source of the gradual GAL1 regulation. We performed time-elapsed induction studies and recorded the traces of GAL1-luciferase-expressing single cells upon stimulation with different galactose concentrations. As shown in Fig. 4, a high galactose stimulus (0.5%) leads to a fast and homogeneous induction throughout the cell population, while lower galactose concentrations increase the lag phase and the heterogeneity of gene induction. However, even very low inducer concentrations (0.02%) activated GAL1 expression in most of the cells over time, and the slope of GAL1 induction was largely unaffected by the inducer concentration (Fig. 4d and e). Thus, the gradual decrease of GAL1 expression in a cell population is mostly the result of a heterogeneous induction delay caused by suboptimal inducer concentrations.

SAGA, SWI/SNF, or mediator mutants cause severely reduced dose responses of GAL1. The expression of the GAL1 gene is finely tuned depending on the galactose availability, and we have shown above that this regulation involves the graded modulation of promoter activity and nucleosome eviction in a cell population. We next wanted to know how impaired nucleosome remodeling affected the dynamic adaptation of GAL1 promoter activity to changing inducer concentrations. Therefore, we determined the induction profile of the GAL1-luciferase reporter gene in response to a wide range of galactose concentrations in mutants with defects in various coactivator complexes. We included in this study the gen5 (SAGA histone acetyltransferase), snf2 (SWI/SNF chromatin-remodeling complex), and gal11 (mediator complex) mutants, with mutations in genes previously identified as important for full GAL1 transcriptional activation via Gal4 (22–27), and additionally the htz1 mutant in the histone variant H2AZ. The comparison of the dose-response profiles obtained for all mutant strains (Fig. 5A) revealed that loss of SAGA or SWI/SNF function significantly reduced the dynamic range of luciferase synthesis rates driven by the GAL1 promoter (Fig. 5B). While wild-type cells continuously increase the expression rate until a galactose concentration of 0.5% is reached, gen5 and snf2 mutants have truncated dose responses. Both mutants reach a maximal synthesis rate at very low inducer concentrations, which cannot be further increased. The gal11 and htz1 mutants revealed an even stronger reduction in the dynamic gene expression at GAL1. To attribute the observed loss of dynamic GAL1 promoter activity in response to gradual increment of inducer to impaired chromatin remodeling, we next compared the changes in histone H3 occupancy among the different mutant strains. As shown in Fig. 5C, loss of SAGA, SWI/SNF, or mediator function impaired the efficient and dose-dependent histone eviction from the GAL1 promoter. Thus, a correlation exists between efficient nucleosome removal and the gradual adaptation of GAL1 promoter activity, which relies on the activity of the coactivator complexes investigated here. In the absence of histone variant H2AZ, we still observed efficient histone H3 remodeling at GAL1 (Fig. 5B). Therefore, the defect of htz1 mutants in the galactose-dependent modulation of GAL1 gene expression is likely caused by effects other than impaired nucleosome eviction.

We next applied the same exhaustive analysis of dose-response profiling at the stress-regulated GRE2 gene. An NaCl gradient was applied to the same set of mutants, and their dose-dependent GRE2 expression profiles were determined (Fig. 6A). As the GRE2 gene expression is dynamically regulated in response to increasing salt stress via modulation of its maximal expression level, we chose this parameter to identify alterations in the dose-dependent behavior of this gene. As shown in Fig. 6B, the loss of SWI/SNF activity did not affect the dose-response profile of GRE2. Mutations in SAGA or H2AZ caused a general reduction in the maximal GRE2-luciferase activities; however, a continuous increase in reporter activity was still observed in the dynamic inducer range (0.1 to 0.5 M NaCl). Loss of mediator function caused a very poor expression of GRE2 at any salt concentration. Importantly, and different from the case for the GAL1 gene, SWI/SNF activity is dispensable for the efficient adaptation of GRE2 activity to increased stimulation by salt. Also, in the absence of SAGA, the absolute expression levels of GRE2 decreased; however, the gradual increase in the maximal expression following the NaCl gradient was maintained. In conclusion, chromatin modifiers such as SWI/SNF or SAGA have distinct roles in the establishment of specific dose responses, exemplified here for the GAL1 and GRE2 genes.

Gradual association of Gal3 and temporally regulated recruitment of Hog1 recapitulate the different dose-response behaviors of GAL1 and GRE2. The regulation of the dose-dependent expression of GAL1 and GRE2 depends on different mechanisms. We next investigated the signaling compounds which were responsible to establish a specific dose-response pattern at the two genes. We first focused at the specific transcription factors Gal4 and Sko1, which bind directly to the GAL1 or GRE2 promoter regions and confer galactose- or salt-induced transcriptional activation. We found that Gal4 binding to GAL1 was generally stimulated by galactose but independently of the concentration tested (Fig. 7A). Sko1 binding to GRE2 was slightly increased by low salt doses (Fig. 7B) but did not correlate with the increasing GRE2 promoter activity observed before in this range of salt stimuli. Therefore, the differential binding of the direct transcriptional activator Gal4 or Sko1 was not a mechanism to establish the dynamic dose responses at the GAL1 or GRE2 gene. We then determined the association of a second class of regulators, the Gal3 inducer and the Hog1 MAP kinase. Both signaling molecules are imported into the nucleus upon stimulation, associate with the promoter regions via Gal4 or Sko1, and are required to trigger the transcriptional switch from repression to activation. As shown in
FIG 3 Dose-sensitive modulation of salt and oxidative stress-regulated yeast genes. (A) Live-cell reporter fusions with destabilized luciferase were used to determine the dose-response profiles of the *HOR2* and *ALD6* genes in response to NaCl stress. The mean values for three independent biological replicas are shown for each salt concentration (standard deviation, 15%). (B) Maximal expression levels ($A_{\text{max}}$) and synthesis rates ($V_{\text{max}}$) of the *ALD6*, *HOR2*, and *GRE2* genes upon NaCl stress. Error bars indicate standard deviations. For each gene, the highest value for $A_{\text{max}}$ or $V_{\text{max}}$ was adjusted to 100. (C) Live-cell reporter fusions with destabilized luciferase were used to determine the dose-response profiles of the *SOD2* and *CTT1* genes in response to menadione stress. The mean values for three independent biological replicas are shown for each menadione concentration (standard deviation, 15%). (D) Maximal expression levels ($A_{\text{max}}$) and synthesis rates ($V_{\text{max}}$) of the *SOD2* and *CTT1* genes upon menadione stress. Error bars indicate standard deviations. For each gene, the highest value for $A_{\text{max}}$ or $V_{\text{max}}$ was adjusted to 100.
Fig. 7A, Gal3 association with the GAL1 promoter increases gradually with increasing galactose concentrations. The Gal3 inducer bound slowly and less efficiently with low galactose concentrations and faster and more efficiently with higher galactose stimulation. These data correlated with the dynamic nucleosome remodeling, entry of RNAPII, and modulation of promoter activity in the same range of galactose concentrations. Of note, Gal3 association with GAL1 in response to high galactose concentrations was transient, although expression of GAL1 occurs for longer times. However, our observation is in agreement with previous findings that report transient Gal3 association with GAL genes only in the early phase of galactose induction.

We finally wanted to determine whether the Gal3 inducer level was the decisive factor for the sensitivity and efficiency of GAL gene expression. We therefore placed the GAL3 gene under the control of the GAL1 promoter and monitored the effect of a gradual activation of GAL3 by limiting galactose concentrations on the second round of GAL1-luciferase expression. As shown in Fig. 7C, the gradual increase of GAL3 preactivation mimicked the transition from slow and insensitive to fast and highly sensitive GAL1 gene activation. These data indicate that the Gal3 inducer level is an important determinant of regulating the dose-sensitive GAL gene expression.

FIG 4 Graded dose response of GAL1 expression results from a heterogeneous induction delay across the population. (a to c) Time-lapse luminescence microscopy was used to measure gene expression in single cells after induction (dashed line) in medium with 0.02, 0.06, and 0.5% galactose. Average gene expression is shown as black curve. (d and e) Distributions of the delay (d) and slope (e) of induction across different numbers of cells (n = 90, 136, and 97, respectively). Statistical analysis confirms that the delay becomes longer whereas the slope is not significantly different across all galactose concentrations (***, P < 0.001 by Student’s t test).

DISCUSSION
Cells execute transcriptional programs in response to many different environmental stimuli and threats. A single cell such as a yeast cell has acquired a multitude of gene expression responses triggered by external stimuli, which is well documented by extensive literature published over the past decades. Traditionally these environmental stress responses were investigated with severe insults, which activate the signaling pathways to a maximal level. However, the adaptation to subtle changes in the cell’s environment might be of more physiological importance, and generally we expect that cells are able to adapt their transcriptional re-
The efficiency of dose-sensitive regulation at GAL1 depends on coactivator complexes and histone remodeling. (A) The GAL1-lucCP+ reporter gene was used in live-cell luciferase assays in the indicated yeast strains to determine the expression rates in raffinose-containing minimal medium after supplementation with the indicated concentrations of galactose. The mean values for three independent biological replicas are shown for each galactose concentration (standard deviation, <15%). (B) Comparison of galactose-dependent modulation of GAL1 synthesis rates. Data shown represent the mean values for the maximal synthesis rate for each galactose concentration determined in three independent biological replicates for the indicated yeast strains. Error bars indicate standard deviations. (C) Comparison of nucleosome remodeling at GAL1 in response to increasing galactose inducer concentrations. ChIP of histone H3 was used to determine the nucleosome occupancy at the GAL1 promoter in YP-raffinose medium before and after induction with the indicated galactose concentrations. The mean values for two independent biological replicas are shown with the corresponding standard deviation.
responses gradually in response to the severity of the stress. It is largely unknown how gene regulatory systems adapt to these “suboptimal” signals, mostly because it is experimentally challenging to quantify the dynamic gene expression upon gradually changing stimulation. Here, the recent application of destabilized luciferase reporters for continuous live-cell measurements in yeast turned out to be especially useful (15, 38). This real-time survey of gene expression activity reveals the dynamic range of differentially regulated groups of genes. In the case of the two genes studied in detail in cell populations here, we find a graded...
Dose response in concentration ranges well below the stimuli normally used for these types of genes. GAL1 expression gradually adapts from 0.01 to 0.5% galactose, while GRE2 expression continuously increases from 100 to 400 mM NaCl. These specific stimulus concentrations that provoke gradual outputs might reflect evolutionary adaptation to the naturally occurring environmental changes. It is important to note that the sensitivity of gene expression to a common signal, such as a nutrient or a stress, can be different for specific responsive genes. Here, the chromatin structure and the combination of different cis-regulatory elements in promoters have been implicated in creating characteristic dose sensitivities of yeast genes (14, 15). In our present study, we reveal different strategies that ensure an appropriate transcriptional activation corresponding to subtle environmental changes.

Galactose induction at the GAL1 gene is slow and inefficient at threshold concentrations. This might be due to the repressive chromatin structure at the GAL1 promoter region, which has to be overcome by the activated Gal3 inducer. The initial repressed levels of Gal3 in combination with low galactose concentrations delay the transition of GAL1 to the on state. Consequently, the time point of active gene expression becomes much more variable for individual cells at low galactose doses; however, it is important to note that even at the lowest inducer concentrations, all cells finally actively express GAL1 with comparable induction kinetics. Therefore, the key determinants which explain a gradual galactose response are the signaling events that permit the first round of transcription (Fig. 7D). It is likely that with few Gal3 molecules present in a cell that has not metabolized galactose over a longer time, the rate-limiting step for efficient GAL gene transcription is a threshold concentration of active Gal3 bound at the Gal4 transcriptional activator. In this model, the grade of active Gal3 counteracting the Gal80-mediated repression and additional recruitment of SAGA and Swi/Snf coactivators would increasingly favor GAL1 transcriptional initiation along with growing galactose concentrations. On the other hand, the fact that the expression of GAL3 itself is activated by galactose makes the GAL system especially modulatable. An inducible sensor such as Gal3 allows adaptation of the sensitivity of the GAL gene activation to environmental needs in a way that yeast cells, for example, which frequently encounter galactose as an energy source, would respond more readily in the following round of stimulation (33, 34). Of note, transcriptional memory in yeast has been identified predominantly at genes responsive to nutritional stimuli (31, 48). Thus, gradually regulated promoter activity over a range of metabolite concentrations with the ability to modulate the sensitivity by specific inducible signal transducers might be a general scheme of nutrient-stimulated gene expression in yeast.

The adaptation of gene expression to different grades of cytotoxic stress seems to follow a different principle. Intuitively one might think that a gene product that functions in the detoxification of an acute stress has to be produced as soon as possible and, at least in the beginning of the stress defense, regardless of the strength of the insult. Such an “emergency” response is identified here in the case of the prototypical stress defense gene GRE2. At this gene, maximal levels of nucleosome eviction and preinitiation complex formation are observed almost immediately (experimentally at 2 min) after salt stress exposure. Importantly, and in contrast to the case for GAL1, low stress doses provoke maximal induction at GRE2. Our data suggest that activation of the Hog signaling pathway in the range of mild salt stress always triggers the same signal to its target promoters, which in all cases leads to full transcriptional activation in the first instances of adaptation. Only at salt concentrations above 0.4 M NaCl can a progressive delay in gene expression be observed, which can be explained by general inhibition of the transcription process and a slowdown of signal transduction at high osmolality (49–51). Our results also indicate that the switch-like behavior is a general feature for stress-responsive promoters and not restricted to GRE2 activation by salt stress. We therefore speculate that genes of acute stress responses might generally switch to active transcription easily and independently of the stress dose and that mainly the duration of the on state would be dictated by the strength of the stress. This regulatory mode can provide the cell the most efficient protection, as the absolute production of defense gene mRNAs continuously increases from very low stress levels to stress levels that actually start to inhibit gene expression in general (Fig. 3B). This also implies that the dynamic adaptation of gene expression to stress results not from gradual activation but from the timely shutdown of transcription, a process whose molecular basis is substantially unknown and therefore of special interest for future studies on stress regulation. In general, the osmotic and oxidative stress responses might be optimized to execute very rapid transcriptional activation, which cannot be further enhanced during transcriptional memory. In line with this assumption, the most notable effect of memory on the GRE2 expression is a reduction in the amplitude during repeated salt stress. This reduction is produced by the accumulation of defense proteins such as the cation exporter Ena1 in the case of NaCl stress. Therefore, stress-induced genes might be...
modulated predominantly by the cellular defense capacity, which determines the time needed to maintain maximal gene expression. It is worth noting that a positive memory effect has been reported recently for the yeast response to oxidative stress when the cells were previously treated with a mild dose of salt (52). Future work might therefore reveal the importance of acquired resistance versus transcriptional memory for different stress types and doses.

The nuclear expression of both GAL1 and GRE2 is modulated by signals which originate in the cytoplasm and are then sent to the chromosomal genes via signaling proteins Gal3 and Hog1, respectively. Galactose-bound Gal3 and phosphorylated Hog1 physically interact with their target genes through DNA-bound transcription factors. Here we show that the dynamics of Gal3 or Hog1 association during gradual stimulation faithfully reflect the grade of chromatin remodeling, RNAPII density, and transcriptional output of the regulated genes. Therefore, Gal3 and Hog1 are very likely to be responsible for the specific dose responses observed at their target genes. Importantly, Gal3 protein levels in the uninduced state are very low, which explains the need for high inducer concentrations to efficiently switch on transcription of Gal1 genes. In contrast, Hog1 protein levels are constitutively high (approximately 10-fold more abundant than uninduced Gal3 [36]) independently from the stress condition, thereby ensuring maximal transcriptional responses at low stress doses. Of note, the increasingly longer, but not more efficient, association of Hog1 with its GRE2 target promoter reported here is in agreement with gradually longer phosphorylation of the MAP kinase upon increasing salt stimulation (53). An additional layer of regulation might affect the chromatin structure at stress- versus nutrient-regulated genes. In the case of GAL1, nucleosome remodeling seems to be more important to achieve efficient transcription, and accordingly, we find that the Swi/Snf and SAGA chromatin modifiers are crucial for the dynamic increase of GAL1 activity. In the case of GRE2, nucleosome remodeling either might occur in a much easier fashion or might be less important for activated transcription. This notion is supported by our finding that Swi/Snf is completely dispensable for the dose-dependent GRE2 regulation, and even in the absence of SAGA, the transcriptional output is gradually stimulated by increasing salt stress. Both coactivator complexes, however, have been shown to be recruited to the GRE2 promoter upon salt shock (30). Taking our findings together, gradual stimulation of inducible yeast genes can be conferred by different principles, i.e., modulation of the time in the "on" state in the case of stress genes or gradual modulation of the transition to the "on" state in the case of nutrient-regulated genes. The efficiency of signal transduction is a key determinant for the type of response, and its reinforcement during memory provides a way to switch from one mode to another.

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