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Roles for H2A.Z and Its Acetylation in \textit{GAL1} Transcription and Gene Induction, but Not \textit{GAL1}-Transcriptional Memory

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Abstract

H2A.Z is a histone H2A variant conserved from yeast to humans, and is found at 63\% of promoters in \textit{Saccharomyces cerevisiae}. This pattern of localization suggests that H2A.Z is somehow important for gene expression or regulation. H2A.Z can be acetylated at up to four lysine residues on its amino-terminal tail, and acetylated-H2A.Z is enriched in chromatin containing promoters of active genes. We investigated whether H2A.Z’s role in \textit{GAL1} gene regulation and gene expression depends on H2A.Z acetylation. Our findings suggested that H2A.Z functioned both in gene regulation and in gene expression and that only its role in gene regulation depended upon its acetylation. Our findings provided an alternate explanation for results that were previously interpreted as evidence that H2A.Z plays a role in \textit{GAL1} transcriptional memory. Additionally, our findings provided new insights into the phenotypes of \textit{htz1\Delta} mutants: in the absence of H2A.Z, the SWR1 complex, which deposits H2A.Z into chromatin, was deleterious to the cell, and many of the phenotypes of cells lacking H2A.Z were due to the SWR1 complex’s activity rather than to the absence of H2A.Z per se. These results highlight the need to reevaluate all studies on the phenotypes of cells lacking H2A.Z.

Introduction

In addition to their role in genome packaging, histones also play a role in the functional organization of eukaryotic genomes. Clear causal relationships have been established between some specific modifications of histones at specific loci and the subsequent events that occur at these loci. Histones are modified by enzymes that couple acetyl, methyl, phosphoryl, ubiquitin, or sumo moieties to specific locations either on histone tails, which extend outward from the nucleosome core, or at positions in the core, such as acetylation of H3 lysine 56, near where the DNA helix enters and leaves the nucleosome [1]. Modified histone tails serve in some cases as docking sites for protein complexes. Thus, in principle, a particular collection of modifications on the nucleosomes of a locus can recruit specific complexes to that locus to achieve a particular outcome [2–7].

In addition to histone modifications, nucleosomes can also be specialized by virtue of the presence of histone variants. \textit{Saccharomyces} encodes three histone variants: H2A.Z, which is conserved from yeast to humans; a variant of H2B called H2B2, conserved among yeasts; and Cse4, an H3 variant, which functions at the nucleosomes at centromeres [8].

Like Cse4p, H2A.Z is also localized to specific chromosomal locations with specialized functions. In \textit{S. cerevisiae}, H2A.Z is incorporated into nucleosomes near, but not at, centromeres, at the borders of heterochromatic domains, and near the promoters of 63\% of genes [9–12]. H2A.Z is incorporated into chromatin by the SWR1 complex (SWR1-Com) a multi-subunit enzyme whose catalytic subunit, Swr1, is a member of the Swi2/Snf2 family of chromatin remodeling enzymes [13–15].

H2A.Z’s localization at promoters suggests that it plays an important role in gene expression. Yet genome-wide micro-array analyses indicate that H2A.Z affects the steady-state mRNA levels of only 5\% of \textit{S. cerevisiae} genes [16]. Interestingly, most of the genes downregulated in cells lacking H2A.Z were near the boundaries of SIR-silenced heterochromatin. This observation revealed that H2A.Z functions as part of the boundary separating euchromatin and heterochromatin [16].

H2A.Z is acetylated at up to four positions on its N-terminal tail by the Nua4 and SAGA histone-acetyltransferase complexes [17–19]. Moreover, H2A.Z’s heterochromatin-boundary function depends on its acetylation [17]. Promoter-proximal H2A.Z is also acetylated and, as measured on a cell population, the level of acetylation correlates with the gene’s expression level [19]. Recent work suggests that acetylated-H2A.Z promotes transcription of adjacent genes. Specif-
inducing conditions prior to short-term growth in repressing conditions are able to reinstate GAL1 gene expression upon induction as rapidly as cells grown continuously in nonrepressing conditions [27–29]. This “memory” of recent inducing conditions is reported to be H2A.Z dependent [27], although other explanations have been offered [29].

The role of H2A.Z in galactose induction extends beyond its role in GAL1 transcriptional memory. Cells that are grown in nonrepressing conditions prior to galactose induction require H2A.Z for the rapid induction of GAL1 [21,22]. H2A.Z promotes the rapid induction of GAL1 by recruiting the Mediator complex to the GAL1 promoter [30,31].

The work presented in this paper was aimed at testing the potential role of H2A.Z acetylation in gene induction and transcriptional memory. We found no evidence for a role for H2A.Z in GAL1 transcriptional memory, discovered a role for H2A.Z acetylation in gene induction, and discovered a confounding influence of SWR1-Com on gene regulation in cells lacking H2A.Z.

Results

Acetylated H2A.Z Was Important for Primary Induction of GAL1 Transcription but Did Not Play a Specialized Role in GAL1-transcriptional Memory

Upon galactose induction, cells previously grown long-term in repressing conditions induce GAL1 expression more slowly than cells previously grown in noninducing-nonrepressing conditions. The conclusion that H2A.Z is essential for GAL1 transcriptional memory was based on the following two observations. First, when transferred to inducing conditions from long-term growth in repressing conditions HTZ1 and htz1Δ cells induce GAL1 slowly and at a similar rate [27]. Second, when transferred to inducing conditions from short-term growth in repressing conditions, HTZ1 cells induce GAL1 transcription rapidly, but htz1Δ cells are reported to not induce GAL1 any more rapidly than htz1Δ cells that had been grown long term in repressing conditions prior to galactose induction [27]. We reasoned that if H2A.Z acetylation were required exclusively for transcriptional memory, then cells carrying an unacetylatable allele of HTZ1, htz1-K3,8,10,14R, would exhibit defective GAL1 induction following short-term growth in glucose, but exhibit normal GAL1 induction following long-term growth in glucose.

To determine first whether H2A.Z-acetylation had any role in galactose expression, GAL1 mRNA levels were evaluated by quantitative reverse transcriptase (Q-RT) PCR in HTZ1 [JRY7971], htz1Δ [JRY9001], and htz1-K3,8,10,14R [JRY7983] cultures grown in long-term repressing conditions prior to galactose induction. Cells grown continuously in glucose medium were transferred to galactose medium and GAL1 induction was evaluated at 2-h intervals for 14 h. One characteristic shared between all three strains’ GAL1 induction phenotypes was an approximately 3-h lag period with little to no GAL1 expression. Quantitative analysis suggested that neither htz1Δ nor htz1-K3,8,10,14R cultures exhibited substantially different lag periods prior to the onset of GAL1 expression than those exhibited by HTZ1 cultures (Figure 1A; Table 1, column A). These results suggested that neither H2A.Z nor its acetylation influenced how rapidly the cultures exited glucose repression and began GAL1 transcription.

Other than their lag periods the two mutant cultures exhibited induction phenotypes that were significantly different GAL1 induction phenotypes than those of HTZ1 cultures. Cultures of the two mutant strains had lower steady-state GAL1 expression levels than HTZ1 cultures (Figure 1A;
Acetylated H2A.Z was important for GAL1 induction. Q-RT PCR of GAL1 mRNA performed on HTZ1 (JRY9791), htz1Δ (JRY7754), and htz1-K3,8,10,14R (JRY7983) cultures that were grown long-term in YP-glucose (2%) prior to being transferred into YP-galactose (2%). Open circles represent the average of three biological replicates. Bars represent standard deviations of values from these replicates. Solid lines represent the best-fit curve for the measured data. See text for details. (B) ChIP analysis of H2A.Z-FLAG at the replicates. Solid lines represent the best-fit curve for the measured data. Bars represent standard deviations of values from these replicates. (C) Q-RT PCR of GAL1 promoter in cells grown long-term in YP-glucose (2%) or being transferred into YP-galactose (2%) prior to 12 h of growth in YP-glucose (2%) prior to being transferred into YP-galactose (2%). Open circles represent the average of three biological replicates. Bars represent standard deviations of values from these replicates. Solid lines represent the best-fit curve for the measured data. See text for details.

Table 1, column C). Quantitative analysis suggested that htz1Δ and htz1-K3,8,10,14R cultures required 54.7% and 60.2% more time, respectively, than HTZ1 cultures to reach half-steady-state GAL1 expression levels (Figure 1A; Table 2, column E; note that half-steady-state levels were used instead of half-maximum levels because the level of expression during induction typically overshooted the induced steady-state level). These values, however, underplayed the severity of the htz1Δ and htz1-K3,8,10,14R cultures’ GAL1-transcription rate phenotypes because all three strains spent the majority of time that was required to reach half-steady-state levels in the lag period prior to GAL1 activation (Figure 1A; Table 1, columns A and E; note that half-steady-state levels were used instead of half-maximum levels because the level of expression during induction typically overshooted the induced steady-state level). To accurately compare the GAL1 transcription rates of the three strains it was necessary to determine the amount of time that cultures of these strains required to reach half-steady-state levels of GAL1 expression from the time of GAL1 activation. These values were determined for each culture by subtracting its GAL1 activation time from the time required to reach the half-steady-state level of GAL1 expression. This analysis revealed that once they had begun expressing GAL1, htz1Δ and htz1-K3,8,10,14R cultures required 503% and 625% of the time required for HTZ1 cultures, respectively, to express GAL1 at half-steady-state levels (Table 2, column G). Thus, both H2A.Z and its acetylation contributed to the rate of GAL1 expression in cultures grown under long-term glucose repression prior to galactose induction.

Because the expression of GAL1 in htz1Δ and htz1-K3,8,10,14R strains was similar, the role of H2A.Z in GAL1 expression was presumably dependent upon its acetylation. To determine whether H2A.Z acetylation affected the level of H2A.Z at the GAL1 promoter, chromatin immunoprecipitation experiments were performed with qPCR to quantitate the level of enrichment. Both acetylatable and unacetylatable H2A.Z were present at approximately equal levels at GAL1 (Figure 1B). Therefore, acetylation of H2A.Z was important for GAL1 induction at some point after H2A.Z’s incorporation at the GAL1 promoter.

To determine whether H2A.Z acetylation had a role in transcriptional memory, GAL1 mRNA levels were evaluated in HTZ1 (JRY7971), htz1Δ (JRY9001), and htz1-K3,8,10,14R (JRY7983) cultures that were grown short-term in repressing conditions prior to galactose induction. Cells grown in galactose medium prior to short-term growth in glucose medium (12 h) were transferred to galactose medium and GAL1 induction was evaluated for 14 h in inducing conditions. None of the three strains exhibited a significant lag in GAL1 expression (Figure 1C; Table 1, column B). Quantitative analysis of these data suggested that all three strains, when grown short-term in repressing conditions, expressed GAL1 in half the time, or less, than when the same strains were induced following long-term growth in repressing conditions (Table 3, column D). The combined effect of near-zero onset times and increased GAL1 transcription rates was that all three strains reached half steady-state GAL1 expression levels in 90% less time than was required for the same strains to reach this level when they were grown long-term in repressing conditions prior to galactose induction (Table 3, column C). Thus, all three strains exhibited transcriptional memory with respect to GAL1 transcription. Importantly, relative to the HTZ1 strain, the two mutant strains exhibited less severe phenotypes when they were grown short-term in repressing conditions prior to induction than when they were grown long term in repressing conditions prior to induction (Table 2, compare column G with H).
neither H2A.Z nor its acetylation played an important role in \textit{GAL1}-transcriptional memory.

Because the results described above differed substantially from ostensibly equivalent experiments [27], we obtained the previously published results. Just as described above, both \textit{HTZ1} (CRY 1) and \textit{htz1} (DBY 50) cultures exhibited a similar lag period before \textit{GAL1} mRNA was detectable (Figure 2A; Table 1, column A). As before, when grown under long-term repressing conditions prior to galactose induction, galactose-induced \textit{HTZ1} (CRY 1) cells had both higher steady-state \textit{GAL1} mRNA levels and faster \textit{GAL1} transcription rates than \textit{htz1} (DBY 50) cells (Figure 2A; Table 1, columns D and G). Quantitative analysis suggested that once both cultures had begun expressing \textit{GAL1}, the \textit{htz1} (DBY 50) cultures required about 3.5 x more time than \textit{HTZ1} (CRY 1) cultures to reach half-steady-state \textit{GAL1} expression levels (Table 2, column G). Additionally, as was the case with the other set of strains, both \textit{HTZ1} (CRY 1) and \textit{htz1} (DBY 50) cultures induced \textit{GAL1} expression significantly more rapidly when grown short-term (12 h) in repressing conditions prior to galactose induction than when the same cultures were grown long term in repressing conditions prior to galactose induction (Figure 2B; Table 1, column B). Cultures of both strains also required significantly less time to accumulate half-steady state levels of \textit{GAL1} mRNA when grown short term rather than long term in repressing conditions prior to galactose induction: \textit{HTZ1} (CRY 1) and \textit{htz1} (DBY 50) cultures required 88% and 69% less time, respectively, under these conditions to accumulate half-steady levels of \textit{GAL1} mRNA transcripts (Table 3, column C). Thus, as before, both \textit{HTZ1} and \textit{htz1} cultures exhibited transcriptional memory of prior \textit{GAL1} induction. Therefore, H2A.Z was important for \textit{GAL1} induction regardless of whether cells were induced from short-term or long-term growth in repressing conditions prior to induction.

**Acetylated-H2A.Z Allowed Cells to Activate \textit{GAL1} Expression Efficiently**

Two factors contribute to the \textit{GAL1} expression level in a culture of cells: the proportion of cells that are expressing \textit{GAL1}, and the level of \textit{GAL1} expression in the fraction of cells in which it is expressed. \textit{S. cerevisiae} regulates \textit{GAL1} expression in response to different growth conditions both by increasing the number of \textit{GAL1}-expressing cells and by increasing the level of \textit{GAL1} expression. Both parameters respond independently to different aspects of growth conditions [32].

### Table 1. Quantitative analysis of \textit{GAL1} transcription phenotypes.

| Column | A | B | C | D | E | F | G | H |
|--------|---|---|---|---|---|---|---|---|
| Strain | Primary Induction \textit{GAL1} Transcription Activation Time (min) | Secondary Induction \textit{GAL1} Transcription Activation Time (min) | Primary Induction \textit{GAL1} Steady State Expression Level (\textit{GAL1}/\textit{ACT1}) | Secondary Induction \textit{GAL1} Steady State Expression Level (\textit{GAL1}/\textit{ACT1}) | Primary Induction Time to Half-Steady-\textit{GAL1} Expression Level (min) | Secondary Induction Time to Half-Steady-\textit{GAL1} Expression Level (min) | Primary Induction from Activation to Half-Steady-\textit{GAL1} Expression Level (min) | Secondary Induction Time from Activation to Half-Steady-\textit{GAL1} Expression Level (min) |
| \textit{HTZ1} | 204.8 | 0* | 0.9 | 0.9 | 234.3 | 14.8 | 29.5 | 14.8 |
| \textit{htz1Δ} | 214.1 | 0* | 0.7 | 0.6 | 362.4 | 38.9 | 148.3 | 38.9 |
| \textit{htz1K38,M10.14R} | 191.3 | 0* | 0.8 | 0.7 | 375.4 | 29.7 | 184.2 | 29.7 |
| \textit{HTZ1 (CRY1)} | 204.4 | 0* | 0.8 | 1.0 | 243.7 | 28.4 | 39.3 | 28.4 |
| \textit{htz1Δ (DBY 50)} | 218.9 | 0* | 0.7 | 0.7 | 356.3 | 110.4 | 137.4 | 110.4 |

*The data suggested that the time to first induction of \textit{GAL1} for all strains in the secondary induction experiments was very close to zero, and could not be distinguished from it.*

### Table 2. \textit{GAL1} induction phenotypes relative to \textit{HTZ1} phenotypes (percent of \textit{HTZ1} values).

| Column | A | B | C | D | E | F | G | H |
|--------|---|---|---|---|---|---|---|---|
| Strain | Primary Induction \textit{GAL1} Transcription Activation Time (min) | Secondary Induction \textit{GAL1} Transcription Activation Time (min) | Primary Induction \textit{GAL1} Steady State Expression Level (\textit{GAL1}/\textit{ACT1}) | Secondary Induction \textit{GAL1} Steady State Expression Level (\textit{GAL1}/\textit{ACT1}) | Primary Induction Time to Half-Steady-\textit{GAL1} Expression Level (min) | Secondary Induction Time to Half-Steady-\textit{GAL1} Expression Level (min) | Primary Induction from Activation to Half-Steady-\textit{GAL1} Expression Level (min) | Secondary Induction Time from Activation to Half-Steady-\textit{GAL1} Expression Level (min) |
| \textit{HTZ1} | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| \textit{htz1Δ} | 95.7 | 100.0 | 73.2 | 57.7 | 154.7 | 262.5 | 503.5 | 262.5 |
| \textit{htz1K38,M10.14R} | 107.1 | 100.0 | 84.6 | 78.0 | 160.2 | 200.4 | 625.3 | 200.4 |
| \textit{HTZ1 (CRY1)} | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| \textit{htz1Δ (DBY 50)} | 107.0 | 100.0 | 79.5 | 73.0 | 146.2 | 389.0 | 349.4 | 389.0 |

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Table 3. Percent change in GAL1 induction phenotypes between primary and secondary inductions.

| Column   | Strain  | GAL1 Transcription Activation Time | GAL1 Steady State Expression Level | Time to Half-Max Steady-State GAL1 Expression Level | Time from Activation to Half-Max Steady-State Levels |
|----------|---------|-----------------------------------|-----------------------------------|-------------------------------------------------|-------------------------------------------------|
|          | HTZ1    | −100.0                            | 3.3                               | −93.7                                           | −49.8                                           |
|          | htz1Δ   | −100.0                            | −17.9                             | −89.3                                           | −73.8                                           |
|          | htz1-K3,8,10,14R | −100.0                        | −3.9                               | −92.1                                           | −83.9                                           |
|          | HTZ1 (CRY1) | −100.0                          | 15.5                               | −88.4                                           | −27.7                                           |
|          | htz1Δ (DBY50) | −100.0                         | 6.0                                | −69.0                                           | −19.7                                           |

To determine whether htz1Δ and htz1-K3,8,10,14R cultures’ GAL1 expression defects were attributable to decreased proportions of GAL1-expressing cells, or to decreased GAL1 expression level per cell, flow cytometry was used to monitor galactose induction of a fusion protein containing the entire GAL1 coding sequence, with a C-terminal fusion to green fluorescent protein (GFP), in htz1-K3,8,10,14R, htz1Δ, and HTZ1 cells (Figures 3 and S6, S7, S8).

If H2A.Z were to contribute to the probability that a cell enters the galactose-induced state per unit of time, but not to the expression level in those induced cells, then htz1Δ cultures should have a smaller proportion of GFP-positive cells at each postinduction time point than HTZ1 cultures, but the GFP-positive cells should have similar fluorescence intensities to those in HTZ1 cultures. However, if H2A.Z were important for achieving high expression levels but did not influence the probability of induction per se, then htz1Δ and HTZ1 cultures should have similar proportions of GFP-positive cells, but the GAL1-GFP-expressing cells from htz1Δ mutant cultures would have lower GFP fluorescence than GAL1-GFP-expressing cells from HTZ1 cultures. The same logic would apply to the possible roles of H2A.Z acetylation.

To compare the results of these experiments, a threshold value of GFP-intensity was used to classify cells as either GFP-positive or GFP-negative. This threshold was set so that between 1% and 2% of cells from noninduced HTZ1 cultures were classified as GFP-positive. On average htz1-K3,8,10,14R cultures had 33% fewer GFP-positive cells than HTZ1 cultures at all postinduction time points (Figures 3 and 4A). Additionally, GFP-positive cells from htz1-K3,8,10,14R cells had, on average, 17% lower mean-GFP intensity than HTZ1 cultures (Figures 3 and 4B).

The simplest interpretation of these findings was that H2A.Z-acetylation influenced both the time required to induce GAL1-GFP expression and the rate at which Gal1-GFP accumulated once induced. Another possibility was that the differences between HTZ1 and htz1-K3,8,10,14R cells were due exclusively to differences in either the time required for induction or to the rate of Gal1-GFP accumulation. To distinguish between these two possibilities, the GAL1-induction times and Gal1-GFP accumulation rates were determined for both cultures by fitting a simple mathematical model of gene expression to the data for each culture (the model is described in Materials and Methods; Figures 5 and S1, S2, S3, S4). The model simulated the galactose induction phenotype of a culture by estimating the distribution of activation times and expression rates of the measured cells. The model’s parameters were fitted to the observed data for each strain by optimizing the fit to cell-specific measurements of Gal1-GFP levels. Each culture’s average induction time and average accumulation rate are presented in Tables 4 and 5, respectively.
This analysis revealed that \textit{htz1-K3,8,10,14R} cells induced \textit{GAL1} expression 31\% (+/- 3.3\%) more slowly than did \textit{HTZ1} cells (Table 4), and that induced cells in both \textit{HTZ1} and \textit{htz1-K3,8,10,14R} cultures accumulated Gal1-GFP at similar rates (Table 5). Thus, with respect to \textit{GAL1} induction, H2A.Z-acetylation reduced the amount of time required to induce \textit{GAL1}, but did not influence the rate at which induced cells accumulated Gal1-GFP.

Interestingly, \textit{htz1\Delta} cells had a more severe defect in \textit{GAL1}-GFP expression phenotypes than did cells with unacetylatable H2A.Z (Figures 3, 4A, and 4B; Tables 4 and 5). On average, \textit{htz1\Delta} cultures had 28\% fewer GFP-positive cells than \textit{htz1-K3,8,10,14R} cultures at the 4-h and 6-h time points. At these same time points, the average GFP-intensity of GFP-positive cells in \textit{htz1\Delta} cultures was 46\% lower than that of GFP-positive cells in \textit{htz1-K3,8,10,14R} cultures. Moreover, \textit{htz1\Delta} cells induced \textit{GAL1}-GFP 18.2\% (+/- 3.8\%) later and accumulated Gal1-GFP 38.1\% (+/- 5.6\%) more slowly than \textit{htz1-K3,8,10,14R} cells (Tables 4 and 5). These results were surprising because \textit{htz1\Delta} and \textit{htz1-K3,8,10,14R} cultures had similar \textit{GAL1} mRNA induction phenotypes.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Acetylated H2A.Z was important for \textit{GAL1} gene induction. Flow cytometry analysis was performed using Gal1-GFP on \textit{HTZ1} (JRY9002), \textit{htz1\Delta} (JRY9004), and \textit{htz1-K3,8,10,14R} (JRY9003) cells grown long-term in \textit{YP-glucose (2\%)} prior to being transferred into \textit{YP-galactose (2\%)}. The histograms in this figure represent the distribution of cells within each culture as a function of their GFP intensity. doi:10.1371/journal.pbio.1000401.g003}
\end{figure}
mRNA analysis revealed that the \textit{htz1}D cultures used in these experiments accumulated \textit{GAL1-GFP} transcripts, in contrast to the \textit{GAL1} transcripts in Figure 1A, more slowly than either \textit{HTZ1}, \textit{htz1}A, or \textit{htz1-K3,8,10,14R} culture (Figure 4C). These results suggested that \textit{htz1}A cells accumulated \textit{Gall}-\textit{GFP} mRNA more slowly than \textit{htz1-K3,8,10,14R} cells because they produced \textit{GAL1-GFP} mRNA more slowly than \textit{htz1-K3,8,10,14R} cells.

All of the mRNA measurements performed in this study were performed on bulk cultures, whereas the flow cytometry measurements were made on single cells within cultures. To determine whether the flow cytometry measurements of \textit{Gall}-\textit{GFP} accumulation in \textit{HTZ1}, \textit{htz1}A, and \textit{htz1-K3,8,10,14R} strains corresponded well with each strain’s \textit{GAL1-GFP} mRNA accumulation phenotype, the average \textit{GFP} intensity of each culture was determined (Figure 4D). The galactose-induction phenotypes of all three strains, as measured by average \textit{GFP} accumulation, were qualitatively similar to their galactose induction phenotypes as measured by \textit{GAL1-GFP} mRNA accumulation. Thus, the flow-cytometry data in these studies reflected \textit{GAL1-GFP} mRNA accumulation.

At face value, the more severe galactose-induction phenotypes of \textit{htz1}Δ than of \textit{htz1-K3,8,10,14R} cells suggested that H2A.Z’s role in \textit{GAL1} induction was only partially dependent on its acetylation. However, as described below, the more severe \textit{GAL1}-expression defects in \textit{htz1}Δ cells resulted from secondary complications that arose from the action of SWR1-Com in cells lacking H2A.Z.

**Figure 4. \textit{htz1}Δ cells’ galactose induction phenotypes are more severe than those of \textit{htz1-K3,8,10,14R} cells.** A threshold level of \textit{GFP}-intensity was set so that between 1% and 2% of glucose-grown \textit{HTZ1} cultures were classified as \textit{GFP}-positive cells. (A) The frequency of \textit{GFP}-positive cells within \textit{HTZ1}, \textit{htz1}A, and \textit{htz1-K3,8,10,14R} cultures. (B) The average \textit{GFP} intensity of the \textit{GFP}-positive populations of \textit{HTZ1}, \textit{htz1}A, and \textit{htz1-K3,8,10,14R} cultures. (C) Q-RT PCR of \textit{GAL1-GFP} mRNA performed on \textit{HTZ1} (JRY9002), \textit{htz1}Δ (JRY9004), and \textit{htz1-K3,8,10,14R} (JRY9003) cultures that were grown long-term in YP-glucose (2%) prior to being transferred into YP-galactose (2%). (D) The average \textit{GFP} intensity of the entire population of cells, both \textit{GFP} positive and negative, within \textit{HTZ1}, \textit{htz1}A, and \textit{htz1-K3,8,10,14R} cultures. Bars in all panels represent the standard deviations of values from three biological replicates.

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**Overlap of H2A.Z Acetylation Sites to \textit{GAL1} Induction**

Acetylation of lys14 on H2A.Z is important for its role in \textit{FOX2} and \textit{POT1} induction [20]. To determine whether the acetylation of lys14 or other lysine residues of H2A.Z contributed to \textit{GAL1} induction, the \textit{GAL1} induction phenotypes of diploid cultures each with one null allele and individual lys-to-arg mutations as the other allele (\textit{htz1-K3R}/\textit{htz1}Δ, \textit{htz1-K3R}/\textit{htz1}A, \textit{htz1-K10R}/\textit{htz1}Δ, and \textit{htz1-K14R}/\textit{htz1}Δ) were determined using flow-cytometry. Surprisingly, none of the single acetylation-site mutants exhibited \textit{GAL1-GFP} expression defects (Figure 6; example FACS profiles are in Figures. S6, S7, S8). Thus, H2A.Z’s role in \textit{GAL1} induction depended on its acetylation, but did not depend exclusively on the
H2A.Z-Acetylation and Transcriptional Memory

A. Distribution of GAL1-Activation Times

B. Distribution of GAL1-Expression Rates

C. 0 Hours of Induction

D. 2 Hours of Induction

E. 4 Hours of Induction

F. 6 Hours of Induction

GFP Fluorescence Intensity

# Cells

Hours

GFP Fluorescence / Hour

HTZ1-Replicate 1
HTZ1-Replicate 2
HTZ1-Replicate 3
Best-Fit Model
acetylation of any single tail-lysine residue. These results were surprising given the focus on the acetylation of H2A.Z lys14 in previous studies in *S. cerevisiae* [18,19], but they are consistent with discoveries made in *Tetrahymena*. In *Tetrahymena*, acetylation of H2A.Z’s tail lysines contributes to H2A.Z’s function simply by decreasing the positive charge of H2A.Z’s tail and thus all sites of acetylation function equally well in this respect [33].

**SWR1-Com Enhanced Many *htz1Δ* Mutant Phenotypes**

SWR1-Com deposits H2A.Z into chromatin in a two-step process, removing H2A from nucleosomes and subsequently replacing it with H2A.Z [13]. We hypothesized that if H2A.Z were not available, then SWR1-Com might still perform the first step of this mechanism, disrupting the structure of nucleosomes at those positions at which H2A.Z would normally reside, and that this disruption could affect normal promoter function. Thus, the phenotype of cells lacking H2A.Z might be a composite of two different defects: the lack of H2A.Z’s function per se, and SWR1-Com’s nucleosome-disrupting activity in the absence of H2A.Z. If this hypothesis were correct, then a subset of *htz1Δ*’s phenotypes should be suppressed in cells lacking SWR1-Com function. Indeed as predicted by this model, strains with the *htz1Δ* mutation in combination with a null mutation in any gene encoding an important component of the SWR1 complex (*SWR1, SWC2, SWC3, SWC5, and SWC6*) exhibited less severe mutant phenotypes than *htz1Δ* single-mutant strains on medium containing compounds that each cause a different type of stress (Figure 7).

To determine if the *htz1Δ* mutant’s galactose-induction was more defective than that of the unacetylatabale H2A.Z mutant for a similar reason, the *GAL1* expression phenotypes of both *swr1Δ HTZ1* (JRY9005) and *swr1Δ htz1Δ* (JRY9006) double-mutant cultures were determined using flow cytometry. Prior to induction, *htz1*-K3,8,10,14R, *swr1Δ HTZ1*, and *swr1Δ htz1Δ* cultures had similar proportions of GFP-positive cells, and fewer GFP-negative cells than *htz1Δ* cultures (Figures 8 and 9A). Thus, the *swr1Δ* mutation completely suppressed the *htz1Δ* mutant’s apparent glucose-repression defect. At every postinduction time point, *swr1Δ HTZ1* and *swr1Δ htz1Δ* cultures had similar proportions of GFP-positive cells to *htz1*-K3,8,10,14R cultures and significantly higher proportions of GFP-positive cells than *htz1Δ* cultures (Figures 8 and 9A). The *swr1Δ HTZ1* and *swr1Δ htz1Δ* cells induced *GAL1* expression as rapidly as *htz1*-K3,8,10,14R cells and significantly earlier than *htz1Δ* cells (Table 4). Thus, the severity of the *htz1Δ* mutant’s delayed *GAL1*-induction phenotype was suppressible by the *swr1Δ* mutation and therefore likely resulted from the SWR1 complex’s activity in the absence of H2A.Z. Furthermore, because *htz1*-K3,8,10,14R cells and *htz1Δ swr1Δ* cells needed approximately the same amount of time to induce *GAL1*, H2A.Z’s role in promoting rapid *GAL1* activation completely depended on its acetylation.

Interestingly, *GAL1*-expressing cells from *swr1Δ HTZ1* and *swr1Δ htz1Δ* cultures had significantly higher average GFP intensities than those from *htz1Δ* cultures but they had significantly lower average GFP intensities than those in *htz1*-K3,8,10,14R cultures (Figures 8 and 9B). Quantitative analysis revealed that *GAL1*-expressing cells from both *swr1Δ HTZ1* and *swr1Δ htz1Δ* cultures accumulated *GAL1*-GFP 18.8% (+/−5.3%) more rapidly than *htz1Δ* cells and 23.8% (+/−6.7%) more slowly than *htz1*-K3,8,10,14R cells (Table 5). Thus the severity of the *htz1Δ* mutant’s *GAL1*-acquisition-rate phenotype was suppressible by the *swr1Δ* mutation and therefore likely resulted from the activity of SWR1-Com in H2A.Z’s absence. Moreover, our finding that *swr1Δ HTZ1* and *swr1Δ htz1Δ* cells accumulated *GAL1*-GFP more slowly than *htz1*-K3,8,10,14R cells suggested that H2A.Z has an acetylation-independent role in increasing *GAL1*-expression rate.

**Discussion**

H2A.Z Was Important for *GAL1* Induction, but Not for Transcriptional Memory

In this work, we showed that H2A.Z, through its acetylation, contributed to induction of the *GAL1* gene, a paradigmatic example of a highly inducible gene of *Saccharomyces*. Acetylated H2A.Z contributed to *GAL1* induction both by increasing the fraction of cells that induced at each time point, and by increasing the level of expression per induced cell. Earlier work established that *GAL1* induction has a property termed transcriptional memory, reflecting the ability of cells that were recently induced to be more easily reinduced following short incubations in repressing conditions than after extended incubations in repressing conditions [27–29]. Moreover, H2A.Z has been thought to be a key participant in this transcriptional memory [27]. The conclusion that H2A.Z is important for *GAL1* transcriptional memory is based on experiments involving the induction of *GAL1* as a function of its expression history: when induced from

**Table 4. *GAL1*-activation times.**

| Strain          | Mean Time of Activation (h) | Percent Difference From *HTZ1* |
|-----------------|-----------------------------|--------------------------------|
| *HTZ1*          | 4.2                         | 0.0                            |
| *htz1Δ*         | 6.5                         | 54.6                           |
| *htz1*-K3,8,10,14R | 5.5                        | 31.2                           |
| *swr1Δ HTZ1*    | 5.2                         | 25.2                           |
| *swr1Δ htz1Δ*   | 5.3                         | 27.6                           |

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**Table 5. *GAL1*-expression rates.**

| Strain          | Mean Expression Rate (GFP Counts/h) | Percent Difference From *HTZ1* |
|-----------------|-------------------------------------|--------------------------------|
| *HTZ1*          | 2.2                                 | 0.0                            |
| *htz1Δ*         | 1.3                                 | −38.8                          |
| *htz1*-K3,8,10,14R | 2.1                             | −2.4                           |
| *swr1Δ HTZ1*    | 1.6                                 | −24.0                          |
| *swr1Δ htz1Δ*   | 1.6                                 | −26.1                          |

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Figure 5. The distribution of *GAL1*-induction times and Gala-GFPp accumulation rates among cells as modeled as a Gamma distribution of values. See text for details. (A) shows the Gamma distribution of *GAL1*-induction times that were used in the best-fit simulation of *HTZ1*’s (JRY9002) *GAL1*-acetylation phenotype. (B) shows the Gamma distribution of *GAL1*-GFPp accumulation rates that were used in the best-fit simulation of *HTZ1*’s (JRY9002) *GAL1*-expression phenotype. (C) compares the *GAL1*-GFPp induction phenotypes observed for *HTZ1* cultures with the phenotype predicted for *HTZ1* based on its best-fit simulation.

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long-term growth in repressing conditions, both $htz1\Delta$ and $HTZ1$ cultures were reported to have induced $GAL1$ at similar rates. $htz1\Delta$ cultures were reported to have induced $GAL1$ at a similar rate regardless of whether they had been grown under repressing conditions for either short or long periods of time. However, $HTZ1$ cultures that were grown in repressing conditions for short periods of time were reported to induce $GAL1$ expression much more rapidly than those grown in repressing conditions for long periods of time [27].

Our work was originally directed at understanding the importance of $H2A.Z$ acetylation to the role of $H2A.Z$ in $GAL1$-transcriptional memory. To this end, we determined the $GAL1$-induction phenotypes of $htz1-K3,8,10,14R$ cultures, which carry an unacetylable allele of $H2A.Z$. Surprisingly, both $htz1\Delta$ and $htz1-K3,8,10,14R$ cultures grown in inducing conditions prior to short-term growth in repressing conditions induced $GAL1$ expression more rapidly than those grown long-term in repressing conditions prior to galactose induction. Thus, both $htz1\Delta$ and $htz1-K3,8,10,14R$ cultures exhibited $GAL1$-transcriptional memory. Moreover, regardless of whether they were grown long-term or short-term in repressing conditions prior to induction, $htz1\Delta$ and $htz1-K3,8,10,14R$ cultures induced $GAL1$ more slowly than $HTZ1$. 

The discrepancies between the previously published data [27] and those presented here, concerning the role of $H2A.Z$ in primary inductions of $GAL1$, have a straightforward explanation. The conclusion that $H2A.Z$ was not important for primary galactose inductions was based upon $htz1\Delta$ cells having induced $GAL1$ expression less well than $HTZ1$ cultures after a 2-h induction following short-term growth in repressing conditions, whereas
HTZ1 and htz1Δ cells induced GAL1 equally well following long-term growth in repressing conditions. Our observations were quantitatively similar. However, the critical point is that the magnitude of induction at this early time point was negligible in both htz1Δ and HTZ1 cultures. At all longer periods of galactose induction, htz1Δ cells induced GAL1 expression significantly less well than HTZ1 cells. We believe the earlier conclusions were based upon inadequate induction periods in some experiments.

The original work implicating H2A.Z in transcriptional memory of GAL1 also reached the same conclusion for INO1. However, the data offered in support of these conclusions are weaker than those offered in support of H2A.Z’s role in GAL1 induction memory. First, these studies fail to establish that S. cerevisiae exhibits transcriptional memory of INO1 in the same way that it exhibits transcriptional memory of GAL1. Unlike GAL1, cells that are grown short term in repressing conditions prior to induction induce INO1 more slowly and at lower levels than cells that had been grown long term in repressing conditions prior to induction [27]. Thus, transcriptional memory of INO1 functions in the opposite way of how it functions in GAL1 transcription—decreasing rather than increasing a cell’s response to inducing conditions. Second, since INO1-transcriptional memory results in slower INO1 reinductions, cells lacking INO1-transcriptional memory should induce INO1 more rapidly than cells that have INO1-transcriptional memory. These studies show that htz1Δ cells both induce and reinduce INO1 more slowly than HTZ1 cells [27]. Therefore, htz1Δ cells do not seem to lack transcriptional memory of INO1, rather they seem to exhibit defective INO1 transcription regardless of whether they had recently induced INO1 expression.

SWR1-Com Was Deleterious in Cells Lacking H2A.Z

Because SWR1-Com catalyzes a two-step reaction removing H2A from nucleosomes and replacing it with H2A.Z, we considered the possibility that SWR1-Com’s function, in the absence of H2A.Z, might leave those nucleosomes normally destined to receive H2A.Z compromised in some way. Thus the overall phenotype of htz1Δ would be a composite of those consequences due to the lack of H2A.Z, and those due to uncoupled H2A removal from nucleosomes. Two lines of evidence supported this hypothesis. First, the severity of htz1Δ cells’ sensitivities to various agents with different mechanisms and targets were substantially suppressible by mutations in genes encoding subunits of SWR1-Com. Second, the difference between GAL1 induction in htz1Δ cells and in cells with unacytlatable H2A.Z was largely suppressed by the swc5Δ mutation, creating the less severe phenotype of the unacytlatable H2A.Z mutant. This model is further supported by the observation that htz1Δ cells have chromatin that is in the partially open configuration at the PHO5 promoter under noninducing conditions [21]. We predict that this partially open configuration is a physical manifestation of the mischief wrought by the Swr1-Complex in the absence of H2A.Z.

The benomyl-sensitivity phenotype of the swc5Δ htz1Δ double mutant suggests another possible explanation for why SWR1-Com is dangerous for cells that lack H2A.Z. Unlike the swc5Δ, swc2Δ, swc3Δ, and swc6Δ mutations that strongly suppressed the htz1Δ mutant’s benomyl sensitivity phenotype, the swc5Δ mutation only weakly suppressed this phenotype. In vitro studies have shown that SWR1-Com complexes lacking Swc2p, Swc3p, Swc4p, Yaf9, or Arp6 bind nucleosomes less well than complete SWR1-Com complexes. In contrast, SWR1-Com complexes that lack Swc5p bind nucleosomes better than complete SWR1-Com complexes [35]. Since Swc5p is required for SWR1-Com’s function, the simplest model for why the swc5Δ mutation does not strongly suppress the htz1Δ mutant’s benomyl sensitivity is that mutant SWR1-Com complexes lacking Swc5 may persist in chromatin, perhaps removing H2A, but be unable to replace it with H2A.Z.
H2A.Z Had Two Distinct Roles in GAL1 Expression

Our observation that swr1Δhtz1Δ cells required more time to induce GAL1 expression, and expressed GAL1 more slowly once induced, suggested that H2A.Z had two distinct roles in GAL1 expression—one allowing efficient induction of GAL1, and another to increase the rate of GAL1 expression. That H2A.Z had a role in GAL1 induction was not surprising given H2A.Z's enrichment at the GAL1-promoter. However, that H2A.Z had a role in increasing GAL1's expression rate, as inferred from our model, was unexpected.

There are two lines of evidence that H2A.Z may be important for the expression, per se, of actively transcribed genes. First, even though H2A.Z predominantly localizes to promoters, it is not completely absent from open reading frames (ORFs). The ACT1 and PRP8
The average GFP intensity of the GFP-positive populations of HTZ1 biological replicates. All panels represent the standard deviations of values from three intensity was as above. (A) The frequency of GFP positive cells within induction phenotypes resemble those of htz1-K3,8,10,14R cultures. A threshold level of GFP-expression in different populations of HTZ1, htz1-Δ, htz1-K3,8,10,14R, swr1Δ HTZ1, and swr1Δ htz1Δ cultures. Bars in all panels represent the standard deviations of values from three biological replicates. DOI: 10.1371/journal.pbio.1000401.g009

Figure 9. swr1Δ mutants’ GAL1 induction phenotypes resembled those of htz1-K3,8,10,14R cultures. A threshold level of GFP-intensity was as above. (A) The frequency of GFP positive cells within HTZ1, htz1Δ, htz1-K3,8,10,14R, swr1Δ HTZ1, and swr1Δ htz1Δ cultures. (B) The average GFP intensity of the GFP-positive populations of HTZ1, htz1Δ, htz1-K3,8,10,14R, swr1Δ HTZ1, and swr1Δ htz1Δ cultures. Bars in all panels represent the standard deviations of values from three biological replicates. DOI: 10.1371/journal.pbio.1000401.g009

Materials and Methods

Strain Construction

All of the strains used in this study are presented in Table 6. All of these strains were from the W303 background. One-step integration of knockout cassettes has been previously described [38]. JRY9002 was constructed by transforming the KanMX cassette into JRY7752. To generate KJVY2512, the DNA sequence encoding GFP was inserted before the stop codon of the GAL1 open reading by transforming a HIS3-marked construct encoding the GFP protein. JRY9002, JRY9003, and JRY9004 were segregants from crosses of JRY7972, JRY7985, and JRY9001 to KJVY2512, respectively. JRY9005 and JRY9006 were segregants from crosses of JRY7752 to JRY9002 and JRY9004, respectively. JRY9011, JRY9012, JRY9013, JRY9014, JRY9015, and JRY9016 were segregants from crosses of JRY9000 to JRY7972, JRY7985, JRY9007, JRY9008, JRY9009, and JRY9010, respectively. MKY1029/MKY1029, MKY1030/MKY1031, MKY1032/MKY1033, MKY1034/MKY1035, and MKY1036/MKY1037 were created by disrupting SWR1, SWC2, SWC5, and SWC6 respectively in MKY1030 using a SpHISMX knock-out cassette that was amplified from pFA6a-His3MX6 [38].

Culturing of Yeast

Yeast media were as described [39]. Seed culture density affected GAL1 induction phenotypes, so precautions were taken to ensure that seed cultures of all strains had similar growth histories. Specifically, seed cultures for all experiments were grown in YP-Dextrose (D-glucose, 2%) except DBY50 and CRY1, which were grown in CSM-Dextrose (D-glucose, 2%). 50 ml seed cultures were inoculated with cells from a single colony and grown overnight with shaking at 30 °C to OD 0.2, and were then fed by centrifugation at 2,000 g for 1 min. The cells were then washed with 25 ml of prewarmed 30 °C YP-galactose and resuspended in 50 ml of 30 °C YP-galactose, except in experiments performed with DBY50 and CRY1, in which CSM-Galactose was used instead of YP-galactose for both washing and resuspending in order to follow precisely the procedures of others [27]. The volume of culture removed for each time point was replaced with the same volume of 30 °C YP-galactose.

RNA Analysis and ChIP

Both determination of mRNA levels by quantitative reverse-transcriptase (Q-RT) PCR and ChIP were performed as described [17] except that SYBR GreenER (Invitrogen) PCR reagents were used. H2A.Z-3Flag, and H2A.Z-K3,8,10,14R-3Flag were immunoprecipitated using the αFlag M2 resin (Sigma).

Flow Cytometry

Cells were harvested by centrifugation, fixed in a 4% paraformaldehyde/3.4% sucrose solution for 10 min at room temperature and then stored overnight at 4 °C in a 1.2 M sorbitol solution with KPO4 buffer at pH 7.5. GFP expression data were collected for each sample using the FC-500 (Beckman-Coulter) flow cytometer and analyzed using the Flow-Jo software package. The GAL1-GFP expression status of individual cells within cultures on a cell-by-cell basis in each culture was determined by plotting flow-cytometry measurements as a histogram of GFP fluorescence (y-axis number of cells; x-axis Log GFP intensity relative to GFP-negative values). The threshold of GFP intensity was set so that between 1% and 2% of glucose-grown HTZ1 cultures would be classified as GFP-positive. Cells that had GFP-intensity greater than this threshold value were counted as GFP-positive (GAL1-GFP expressing). The level of GAL1 expression in different populations was calculated by determining the geometric mean GFP intensity.

Quantitative Analysis of mRNA Expression Levels

We developed a simple mathematical model to analyze the dynamics of GAL1 mRNA expression levels. This model allowed us to robustly quantify the onset time of GAL1 induction, steady state GAL1 mRNA level, and the time needed to reach half of the steady-state level. The model is based on three parameters, which we optimized to

ORFs, two loci that have been historically considered nonenriched for H2A.Z, are slightly enriched for H2A.Z relative to no-tag controls (Figure 5). Second, the htz1Δ mutant is sensitive to 6-azauracil, a toxic compound that slows the growth rate of cells that are defective (Figure S5). One of the best-studied examples of eukaryotic gene regulation. In contrast, the htz1Δ mutant is sensitive to 6-azauracil, a toxic compound that slows the growth rate of cells that are defective in mRNA transcript elongation [36]. Thus, it is possible that H2A.Z plays a direct role in transcript elongation. Recent reports raise that possibility further, showing that H2A.Z may aid expression by suppressing antisense transcripts [37].

In summary, our results established that H2A.Z plays no significant role in GAL1 transcriptional memory. In contrast H2A.Z, and its acetylation contributed to both the induction of the gene and to its expression per se, adding valuable new insights into one of the best-studied examples of eukaryotic gene regulation. In addition, we showed that SWR1-Com caused defects in gene expression and induction in the absence of H2A.Z, presumably due to nucleosome disruption, that force a reevaluation of all previously described phenotypes of cells lacking H2A.Z.
maximize the fit of the model to the measured GAL1 mRNA levels. These include: (1) the time \( x \) when induction of GAL1 mRNA begins; (2) the rate \( \alpha \) at which GAL1 mRNA is produced; and (3) the rate \( \delta \) at which GAL1 mRNA molecules are being degraded.

According to the model, the relative amount of GAL1 mRNA at time \( t \), \( M(t) \), follows the ordinary differential equation (ODE):

\[
\frac{d}{dt} M(t) = \begin{cases} 
0 & \text{if } t \leq x \\
\alpha - \delta \cdot M(t) & \text{if } t > x
\end{cases}
\]

Namely, GAL1 is not being expressed at all until time point \( x \), from which point it is produced at a fixed rate \( \alpha \), and being degraded at a fixed ratio \( \delta \), until it reaches the steady state equilibrium:

\[
M(t) = \frac{\alpha}{\delta}
\]

Given the model parameters, and starting from zero \( M(0) = 0 \), we can solve the ordinary differential equation using the Runge-Kutta method (as implemented in MATLAB 7.6), and estimate the mRNA level of GAL1 at every time point \( t \).
We optimized the three parameters \(x, \alpha, \text{ and } \delta\) for every culture to minimize the root-mean-square deviation (RMSD) between the experimental measurements and the modeled values. The values that were used in each of the best-fit models are presented in Table S1. We constrained the parameters \(x, \alpha, \text{ and } \delta\) to non-negative values, and used the active-set optimization algorithm (FMINSEARCH function in MATLAB 7.6). For the memory experiments, the optimized values of the \(GAL1\) expression onset times, for all cultures, were very close to zero, and practically below the time resolution of the model and data. We therefore simplified the model, and explicitly set \(x\) to zero.

Finally, to estimate the half steady-state time point, we used the optimized parameters for each culture to find the steady state level \(E_{\delta}\), and to solve the ordinary differential equation and identify when \(GAL1\) levels reach half of the steady state level.

### Quantitative Modeling of Flow Cytometry Data

To analyze the flow cytometry data, the time-course measurements of single-cell \(GAL1\)-GFPp intensities were transformed into \(GAL1\)-GFP induction times and \(GAL1\)-GFP accumulation rates. To do this, a simplified model of \(GAL1\)-induction was developed, and its six parameters fitted to the measured data for each culture. For every cell, this model assumes that \(GAL1\) is completely repressed until its induction time \(t_i\), when cellular \(GAL1\)-GFPp begins to accumulate at a fixed rate \(x_i\). We therefore model \(E_i(t)\), the \(GAL1\)-GFPp content of the \(i\)th cell at time \(t\) as:

\[
E_i(t) = \begin{cases} 
\varepsilon_i & \text{if } t \leq t_i \\
(t - t_i)x_i + \varepsilon_i & \text{if } t > t_i
\end{cases}
\]

where:

- The induction time of the \(i\)th cell, \(t_i\), is sampled from a Gamma distribution with parameters \((k_i, \theta_i)\).
- The \(GAL1\)-GFPp accumulation rate of the \(i\)th cell, \(x_i\), is independently sampled from a Gamma distribution with parameters \((k_i, \theta_i)\).

The estimated expression is added to a stochastic noise term \(\varepsilon_i\), drawn from a Normal distribution with parameters \((\mu, \sigma^2)\), to simulate a basal level of \(GAL1\) expression.

The model was used to simulate a population of 100,000 cells, whose \(GAL1\)-GFP induction times \(x_i\)'s and accumulation rates \(x_i\)'s were sampled independently from two Gamma distributions: \(x_i \sim \Gamma(k_i, \theta_i)\), and \(x_i \sim \Gamma(k_i, \theta_i)\), and their stochastic noise terms sampled from a Normal distribution: \(\varepsilon_i \sim N(\mu, \sigma^2)\). Given a set of six parameters \((k_i, \theta_i, k_i, \theta_i, \mu, \sigma^2)\) this model sampled activation times, accumulation rates, and noise terms for each of the 100,000 cells in the simulation, and computed the cellular \(GAL1\)-GFPp levels \(E_i(t)\) for each of the four time points that were measured \((0, 2, 4, \text{ and } 6 \text{ h} \text{ following induction})\), which allowed for the simulation of flow-cytometry outputs. Activation times and accumulation rates were sampled from a stochastic distribution rather than being fixed at specific values to account for the natural variability among cells because of biological variables like cell size, position in the cell cycle, cell age, and other factors that were not treated as variables in the model. Gamma distributions were used due to their non-negativity property.

The parameters of the model were optimized by minimizing the root-mean-squared deviation between the measured data (average of triplicates) and the model predictions, summed over the four measured time points \((0, 2, 4, \text{ and } 6 \text{ h})\). To optimize these parameters, genetic algorithms were used (as implemented in the GA function in MATLAB 7.6) followed by a derivative-free optimization using the simplex algorithm (FMINSEARCH function in MATLAB 7.6). These optimization steps were repeated with 200 random starting points for each strain, and the optimal set of parameters were then selected (Tables S2 and S3). The error in our estimation of each strain’s induction time and accumulation rate was calculated by determining the range of values for each parameter that were used in the top 50 best-fit simulations for each strain.

The models that were determined for each strain’s \(GAL1\)-GFPp expression phenotype were used as a proxy to quantitatively compare the \(GAL1\)-activation times and \(GAL1\)-GFPp accumulation rates of \(htz1\), \(htz1\)-K3,8,10,14R, \(htz1\), \(swr1\) \(HTZ1\) and \(swr1\) \(HTZ1\) cells.

### Supporting Information

#### Figure S1

**The distribution of \(GAL1\)-induction times and \(GAL1\)-GFPp accumulation rates among cells from \(htz1\) cultures as modeled as a gamma distribution of values.** See text for details. (A) shows the Gamma distribution of \(GAL1\)-induction times that were used in the best-fit simulations of \(htz1\) \(GAL1\)-GFP expression phenotype. (B) shows the Gamma distribution of \(GAL1\)-GFP accumulation rates that were used in the best-fit simulations of \(htz1\) \(GAL1\)-GFP expression phenotype. (C) compares the \(GAL1\)-GFP induction phenotypes that were observed for \(htz1\) cultures with the phenotype that was predicted for each culture based on its best-fit simulation.

Found at: doi:10.1371/journal.pbio.1000401.s001 (0.81 MB TIF)

#### Figure S2

**The distribution of \(GAL1\)-induction times and \(GAL1\)-GFPp accumulation rates among cells from \(htz1\)-K3,8,10,14R cultures as modeled as a gamma distribution of values.** See text for details. (A) shows the Gamma distribution of \(GAL1\)-induction times that were used in the best-fit simulations of \(htz1\)-K3,8,10,14R \(GAL1\)-GFP expression phenotype. (B) shows the Gamma distribution of \(GAL1\)-GFP accumulation rates that were used in the best-fit simulations of \(htz1\)-K3,8,10,14R \(GAL1\)-GFP expression phenotype. (C) compares the \(GAL1\)-GFP induction phenotypes that were observed for \(htz1\)-K3,8,10,14R cultures with the phenotype that was predicted for each culture based on its best-fit simulation.

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#### Figure S3

**The distribution of \(GAL1\)-induction times and \(GAL1\)-GFPp accumulation rates among cells from \(swr1\) \(HTZ1\) cultures as modeled as a gamma distribution of values.** See text for details. (A) shows the Gamma distribution of \(GAL1\)-induction times that were used in the best-fit simulations of \(swr1\) \(HTZ1\) \(GAL1\)-GFP expression phenotype. (B) shows the Gamma distribution of \(GAL1\)-GFP accumulation rates that were used in the best-fit simulations of \(swr1\) \(HTZ1\) \(GAL1\)-GFP expression phenotype. (C) compares the \(GAL1\)-GFP induction phenotypes that were observed for \(swr1\) \(HTZ1\) cultures with the phenotype that was predicted for each culture based on its best-fit simulation.

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#### Figure S4

**The distribution of \(GAL1\)-induction times and \(GAL1\)-GFPp accumulation rates among cells from \(swr1\) \(htz1\) cultures as modeled as a gamma distribution of values.** See text for details. (A) shows the Gamma distribution of \(GAL1\)-induction times that were used in the best-fit simulations of \(swr1\) \(htz1\) \(GAL1\)-GFP expression phenotype. (B) shows the Gamma distribution of \(GAL1\)-GFP accumulation rates that were used in the best-fit simulations of \(swr1\) \(htz1\) \(GAL1\)-GFP expression phenotype. (C) compares the \(GAL1\)-GFP induction phenotypes that were observed for \(swr1\) \(htz1\) cultures with the phenotype that was predicted for each culture based on its best-fit simulation.

Found at: doi:10.1371/journal.pbio.1000401.s004 (0.81 MB TIF)
phenotypes that were observed for awr1Δ htz1Δ cultures with the phenotype that was predicted for each culture based on its best-fit simulation.

Found at: doi:10.1371/journal.pbio.1000401.s004 (0.80 MB TIF)

Figure S5  H2A.Z localizes to the ORFs of ACT1 and PRP8. ChIP analysis of H2A.Z-FLAG enrichment at the ACT1 and PRP8 ORFs in HTZ1-Flag (JRY9792) cultures that were grown long-term in YP-glucose (2%). Bars represent the standard deviation of three biological replicates.

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Figure S6  Measurements of Gal1-GFP accumulation by flow cytometry were reproducible. Flow cytometry analysis was performed using Gal1-GFP on HTZ1 [JRY9002] cells grown long-term in YP-glucose (2%) prior to being transferred into YP-galactose (2%). The histograms in this figure represent the distribution of cells within each culture as a function of their GFP intensity. The individual FACS plots of three biological replicates are shown for HTZ1.

Found at: doi:10.1371/journal.pbio.1000401.s006 (0.62 MB TIF)

Figure S7  Measurements of Gal1-GFP accumulation by flow cytometry were reproducible. Flow cytometry analysis was performed using Gal1-GFP on htz1Δ [JRY9004] cells grown long-term in YP-glucose (2%) prior to being transferred into YP-galactose (2%). The histograms in this figure represent the distribution of cells within each culture as a function of their GFP intensity. The individual FACS plots of three biological replicates are shown for htz1Δ.

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Figure S8  Measurements of Gal1-GFP accumulation by flow cytometry were reproducible. Flow cytometry analysis was performed using Gal1-GFP on 31-3.8,10,14R [JRY9003] cells grown long-term in YP-glucose (2%) prior to being transferred into YP-galactose (2%). The histograms in this figure represent the distribution of cells within each culture as a function of their GFP intensity. The individual FACS plots of three biological replicates are shown for 31-3.8,10,14R.

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Table S1  Parameters used in mathematical model of GAL1 mRNA data.

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Table S2  Parameters used in mathematical model of GAL1 activation times.

Found at: doi:10.1371/journal.pbio.1000401.s10 (0.06 MB DOC)

Table S3  Parameters used in mathematical model of GAL1 expression rates.

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Author Contributions

The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: JEH MSK. Performed the experiments: JEH AYW. Analyzed the data: JEH TK AYW MSK. Contributed reagents/materials/analysis tools: JEH TK. Wrote the paper: JEH JR.
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