

1 **The role of the histone variant H2A.Z/Htz1p on TBP recruitment, chromatin**
2 **dynamics and regulated expression at oleate-responsive genes**

3
4 Yakun Wan,¹ Ramsey Saleem,¹ Alexander Ratushny,¹ Oriol Roda,¹
5 Jennifer Smith,¹ Chan-Hsien Lin,^{1,2} Jung-Hsien Chiang,^{1,2} John Aitchison^{1*}

6 1. Institute for Systems Biology, Seattle, WA 98103

7 2. Department of Computer Science and Information Engineering,

8 National Cheng Kung University, Tainan, Taiwan

11 *Corresponding author

12 Institute for Systems Biology

13 1441 N 34th St. Seattle, WA 98103

14 Phone: (206) 732-1344

15 Fax: (206) 299-6574

16 E-mail: jaitchison@systemsbiology.org

18 Word count for Materials and Methods: 874 words

19 Word count for the Introduction, Results and Discussion: 4324 words

21 Running Title: Control of gene induction by Htz1p

1 **Abstract**

2 The histone variant H2A.Z (Htz1p) has been implicated in transcriptional
3 regulation in numerous organisms, including *Saccharomyces cerevisiae*. Genome-wide
4 transcriptome profiling and chromatin immunoprecipitation studies identified a role for
5 Htz1p in the rapid and robust activation of many oleate-responsive genes encoding
6 peroxisomal proteins, and in particular, *POT1*, *POX1*, *FOX2* and *CTA1*. Swr1p, Gcn5p
7 and Chz1p dependent association of Htz1p into these promoters in their repressed states
8 appears to establish an epigenetic marker for rapid and strong expression of these highly
9 inducible promoters. Isw2p also plays a role in establishing the nucleosome state of these
10 promoters, and associates stably in the absence of Htz1p. Analysis of the nucleosome
11 dynamics and Htz1p association with these promoters suggests a complex mechanism in
12 which Htz1p-containing nucleosomes at fatty acid-responsive promoters are
13 disassembled upon initial exposure to oleic acid leading to the loss of Htz1p from the
14 promoter. These nucleosomes reassemble at later stages of gene expression. While these
15 new nucleosomes do not incorporate Htz1p, the initial presence of Htz1p appears to mark
16 the promoter for sustained gene expression and the recruitment of TBP.

1 Introduction

2 The organization of DNA into chromatin provides cells with a key regulatory
3 mechanism for gene expression by limiting access of the genome to the transcriptional
4 machinery. The nucleosome represents a basic structural unit of chromatin and post-
5 translational modifications of histones serve as signals to define active, repressed or inert
6 chromatin states. In addition, chromatin states and gene expression can be influenced by
7 the dynamics of histones and their nonallelic variants. Indeed, exchange of canonical
8 histones for histone variants appears to be a key mechanism by which the transcriptional
9 machinery overcomes the restricted access imposed by nucleosome positioning (1). Of
10 the many classes of histone variants discovered, the Z variant of H2A is perhaps the best
11 characterized. H2A.Z differs from the canonical H2A histone in both the length and
12 sequence of the C-terminus (37), and is conserved from yeast to mammals (15). Early
13 studies with H2A.Z in *Tetrahymena* showed that H2A.Z incorporation is linked with
14 transcriptionally active chromatin (35). The *Saccharomyces cerevisiae* orthologue of
15 H2A.Z is called Htz1p and is encoded by *HTZ1*. Although *HTZ1* is not essential gene
16 under standard laboratory growth conditions, Htz1p is implicated in transcriptional
17 regulation. Global chromatin studies have revealed that Htz1p preferentially associates
18 with the two nucleosomes flanking the nucleosome free region of promoters (12, 18, 26,
19 41), and this association is inversely proportional to transcription rates (3, 18).

20 Studies of the role of Htz1p in transcriptional regulation at specific promoters,
21 such as those of *GAL1* and *PHO5* (1, 30) indicate that the presence of Htz1p at promoters
22 is dynamic; Htz1p is bound in their repressed states, but dissociates during the activation
23 process. Accordingly, it is proposed that nucleosomes containing Htz1p are poised to
24 undergo nucleosome displacement allowing for rapid transcriptional responses (41).

25 The yeast *S. cerevisiae* is an excellent model for understanding the mechanisms of
26 cellular responses to induced perturbations. Upon exposure of yeast to fatty acids, such as
27 oleate, cells respond by dramatically altering their gene expression patterns, inducing
28 genes required for peroxisomal β -oxidation and peroxisome biogenesis (32). Genetic
29 screens to identify proteins specifically required for efficient fatty acid metabolism in *S.*
30 *cerevisiae* (34) identified metabolic enzymes, proteins required for biogenesis of the
31 organelle, signaling proteins and transcriptional regulators, and chromatin modifiers.

1 Among this latter class of proteins, this approach identified genes encoding Htz1p, RNA
 2 Polymerase II, Mediator subunits and components of chromatin remodeling complexes.
 3 We thus seek to understand the nature of how chromatin is regulated and remodeled in
 4 response to exposure to fatty acids and the specific role Htz1p plays in these
 5 regulation/remodeling processes.

6 In this report, transcriptomes of WT and *htz1Δ* strains were compared during
 7 exposure to oleic acid. While loss of Htz1p reduced the expression of many genes, genes
 8 involved in the fatty acid response were particularly sensitive. A model is proposed, in
 9 which Htz1p-containing nucleosomes at fatty acid-responsive promoters are
 10 disassembled upon initial exposure to oleic acid leading to the loss of Htz1p from the
 11 promoter. These nucleosomes reassemble, at later stages of gene expression. While these
 12 nucleosomes do not incorporate Htz1p, the initial presence of Htz1p appears to mark the
 13 promoter for sustained gene expression and the recruitment of TBP.

1 **Materials and Methods**

2 **Strains and growth conditions.**

3 All yeast strains used in this study are indicated in Table 1. Haploid strains with
 4 myc-tagged genes were made by genomically tagging target genes with the sequence
 5 encoding 13 copies of the c-myc epitope from pFA6a-13MYC (20) by homologous
 6 recombination into BY4742 (wild type) using a previously described PCR-based
 7 procedure (2). Strains were verified by PCR analysis of the tagged gene loci and Western
 8 blot analysis of the fusion proteins. Examination of growth characteristics of each strain
 9 suggests that the chimeras did not alter protein function. For all experiments, control
 10 strains were otherwise isogenic to test strains. Strains were cultured at 30°C in the
 11 following media: YPD (1% yeast extract, 2% peptone, 2% glucose), SCIM (0.17% yeast
 12 nitrogen base without amino acids and ammonium sulfate (YNB-aa-as), 0.5% yeast
 13 extract, 0.5% peptone, 0.079% complete supplement mixture, 0.5% ammonium sulfate)
 14 containing 0.5% Tween 40 (w/v) and 0.2% (w/v) oleate.

15

16 **RNA preparation and microarray analysis.**

17 Yeast cultures were grown at 30°C to a density of $\sim 1 \times 10^7$ cells/ml. Cells were
 18 collected and immediately frozen in liquid nitrogen. Total RNA was isolated by hot acid
 19 phenol extraction. Total RNA was treated with RNase-free DNase I and purified with a
 20 Qiagen RNeasy kit. Microarray labeling and hybridization reactions were performed as
 21 previously described (7). Two color microarrays, comparing RNA from experimental
 22 conditions (wild-type (WT) and *htz1*Δ cells grown in oleate (SCIM) for 6 h) to RNA
 23 from control WT cells grown in glucose-containing medium (YPD), were performed
 24 using Agilent whole-genome *S. cerevisiae* arrays. All experiments were performed with
 25 duplicate experimental and duplicate technical replicates of each condition and the Log₁₀
 26 of the average mRNA abundance ratios are reported. Differentially expressed genes were
 27 identified by maximum-likelihood analysis ($\lambda \geq 100$) (14, 32) and significantly affected
 28 genes in the mutants were identified by a change in expression of two-fold or more
 29 compared to the expression in the relevant WT strains.

30

1 For quantitative reverse transcription-PCR, total RNA was directly reverse
2 transcribed using the First Strand cDNA Synthesis Kit from Fermentas (Catalog: K1611).
3 cDNAs were treated by RNase H and diluted 1:100 for quantitative PCR. RT-PCR was
4 done using a 7900 HT Fast Real-time PCR systems and DyNAmoTM Flash SYBR Green
5 qPCR Kit (NEB, F-415L) with gene-specific oligonucleotides. mRNA levels were
6 normalized relative to *ACT1* mRNA levels from three independent RT-PCR analyses.
7 Primers for RT-qPCRs are available on request.

8 9 **ChIP and Real Time PCR.**

10 For each chromatin immunoprecipitation (ChIP) experiment, yeast strains were
11 first grown in glucose medium (YPD) to a density of $\sim 1 \times 10^7$ cells/ml, and then
12 transferred to oleate medium (SCIM) for indicated times. ChIP experiments were
13 performed as described by (33) with the following modifications: For HA-Htz1p ChIP,
14 cells were cross-linked with 1% formaldehyde for 45 min at room temperature. 2 μ g of
15 anti-HA antibody (12CA5) was prebound with to 50 μ l of pan-mouse IgG Dynabeads
16 (DynaL Biotech) and then incubated with 1mg (protein) of supernatant from the sheared
17 chromatin overnight at 4°C. TBP (Spt15p-Myc) ChIP was performed as described by
18 (33). Cells were cross-linked with 1% formaldehyde for 2 hours at room temperature. 2 μ l
19 of anti-Myc antibody (9E11; Abcam) was pre-bound to 50 μ l of pan-mouse IgG
20 Dynabeads and then incubated with 1mg (protein) of supernatant from sheared chromatin
21 overnight at 4°C.

22 All ChIP experiments were performed in triplicate. The purified ChIP samples
23 were used in quantitative PCR (qPCR) analysis. Real-Time qPCR was performed by
24 using an iCycler instrument (ABI 7900) and DyNAmoTM Flash SYBR Green qPCR Kit.
25 The average of three independent replicates is reported as relative amplification of each
26 target of interest compared to a normalization control amplicon, within the non-promoter
27 IGRi *YMR325W*. Primer sequences are available on request. Occupancy level was
28 determined by dividing the relative abundance of an experimental target by the relative
29 abundance of a control target. This ratio represents the enrichment of ChIP DNA over the
30 input DNA for a specific target versus the control target.

31

1 **FACS analysis.**

2 Procedures were performed as previously described (27). Fluorescence intensities
3 of individual cells were measured using a FACS Calibur flow cytometer (BD
4 Biosciences). Data analysis was performed using WinMDI 2.8 (available from
5 <http://FACS.scripps.edu/>).
6

7 **Nucleosome scanning assay (*NuSA*).**

8 Two hundred ml of cells at OD₆₀₀ of 1.0 in either glucose or after transfer to
9 oleate-containing media for the indicated time were treated with 1% formaldehyde for 20
10 min, followed by 5 min incubation in 125 mM glycine. Cell permeabilization,
11 micrococcal nuclease digestion, protein degradation and DNA purification steps were
12 performed as described in (38). DNA samples were then treated with RNase A and
13 analyzed in a 2% agarose gel to quantify nucleosomal content. The bands corresponding
14 to mononucleosomal DNA were extracted using a Qiagen gel extraction kit. Q-PCR
15 analysis on digested DNA was performed. Q-PCR primers are available on request and
16 cover the promoter regions of *POT1*, *CTAI*, *POXI* and *FOX2* with overlapping
17 amplicons averaging 100 bp in size. To define nucleosome occupancy, the protection
18 value of each amplicon was normalized to *CEN3* values as described (6). The N+1
19 nucleosome refers to the first nucleosome downstream of the transcription start site which
20 is located at open reading frame regions. The N-1 nucleosome refers to the first
21 nucleosome upstream of the transcription start site which is located at promoter regions.

1 Results

2 **Htz1p is required for transcriptional activation of a subset of oleic acid-responsive** 3 **genes.**

4 Transcriptome profiling was used to obtain a global understanding of how Htz1p
5 contributes to gene expression in response to external stimuli. To do so, we focused on
6 gene induction upon shift from glucose to oleic acid growth conditions. This condition
7 was chosen because we and others have previously shown that this transition leads to
8 dramatic alterations in gene expression patterns (16, 32, 33), genes involved in fatty acid
9 metabolism are significantly induced under these conditions, and because it has been
10 shown that *S. cerevisiae* *htz1Δ* strains have a specific growth defect when grown on fatty
11 acids (19, 34). In accordance with previous genome-wide analyses of oleate responses
12 (16, 32, 33), a large portion of the genome responds to the transition (Fig 1A; column 1).
13 Reflecting the non-fermentative metabolism of oleate by the coordinated activities of
14 peroxisomes and mitochondria, the most significantly enriched classes of induced genes
15 include genes linked to mitochondrial respiration and peroxisomal lipid metabolism (GO
16 terms oxidative phosphorylation, electron transport chain and aerobic respiration,
17 hypergeometric p values $<10^{-10}$; components of the mitochondrial respiratory chain -
18 $p\sim10^{-13}$; fatty acid oxidation and peroxisome organization and biogenesis related - $p\sim10^{-6}$,
19 and the peroxisomal compartment - $p\sim10^{-12}$). By comparison, there were many genes that
20 were relatively unresponsive in *htz1Δ* cells (Fig 1A, column 2; Fig 1B). This included
21 genes that were both poorly induced and genes that were poorly repressed in *htz1Δ* cells
22 compared to WT (Fig 1A). Among the genes induced upon oleate exposure, 292 were
23 expressed at least two-fold less in *htz1Δ* cells than in WT cells (Fig 1B). Interestingly,
24 these poorly induced genes were most enriched for those annotated with peroxisomal
25 functions and components; but were not enriched for annotations of mitochondrial
26 components or aspects of mitochondrial respiration (fatty acid and lipid oxidation -
27 $p\sim4.0\times10^{-12}$; and peroxisomes - $p\sim9\times10^{-20}$) (Fig. 1C). Indeed, 26 genes (of 57 total)
28 encoding peroxisomal proteins showed significantly reduced transcription in an *htz1Δ*
29 background (Fig. 1C). These data suggest that Htz1p is required for the regulated
30 expression of a large number of genes upon transition from one state to another. In the

1 case of transition to oleate, genes linked to peroxisomal fatty acid oxidation are normally
2 highly induced and their expression is the most significantly affected in the absence
3 Htz1p.
4

5 ***HTZ1* is required for normal peroxisomal beta-oxidation.**

6 The finding that normally highly induced genes linked to fatty-acid oxidation are
7 poorly expressed in *htz1Δ* cells is consistent with the finding that cells lacking *HTZ1*
8 show a specific impairment of fatty acid metabolism (19, 34). Like mutants defective in
9 peroxisomal function (e.g. *pex3Δ*), *htz1Δ* cells exhibit a growth defect on fatty acid-
10 containing medium (YPBO), but not on glucose (YPD) containing media (Fig 2A), nor
11 on other non-fermentable carbon sources such as glycerol (YPG) or acetate (YPA)
12 requiring mitochondrial function (34). As expected, the WT cells grew normally on
13 different carbon sources.

14 To examine the effect of Htz1p on the organelle itself, we examined peroxisomes
15 by fluorescence microscopy. WT and *htz1Δ* cells expressing peroxisomal thiolase Pot1p,
16 tagged by genomic integration with GFP, were incubated in oleate medium and observed
17 over a time course of induction by direct fluorescence confocal microscopy (Fig. 2B). In
18 glucose-containing medium peroxisomes were barely detectable. However, upon shift to
19 oleic acid, WT cells induced the expression and import of Pot1p-GFP as indicated by the
20 accumulation of punctate fluorescent structures (29). However, there was a dramatic
21 delay in the appearance of punctate GFP fluorescence in *htz1Δ* cells compared with WT
22 cells induced over the same time period. Together these data suggest that peroxisome
23 biogenesis *per se* is not defective in *htz1Δ* cells. Rather the defect in the ability to
24 metabolize oleate effectively is a result of relatively poor expression of genes required for
25 (peroxisomal) fatty acid metabolism.
26

27 **Transcriptional response of *POT1*, *POX1*, *FOX2* and *CTA1*.**

28 To further examine the molecular defects associated with the loss of Htz1p, we
29 focused on 4 strongly induced peroxisomal matrix enzymes, encoded by *POT1*, *FOX2*,
30 *POX1* and *CTA1* (Fig. 3A, red). These genes are normally repressed on glucose, and
31 strongly induced on oleic acid (32). Quantitative RT-PCR of these mRNAs demonstrated

1 that in the absence of Htz1p, each of these genes was repressed as in WT cells, but their
2 induction was impaired upon transition to oleate medium (Fig.3A). Interestingly, the
3 expression of each of these genes appeared to be most significantly affected at the later
4 time points after transition to oleate (compare 4 and 6 hours of induction to 0.5 and 1
5 hour of induction). These data suggest that loss of Htz1p did not dramatically alter the
6 initial response, but was important for the sustained expression of these four genes.

7 Having demonstrated a role for Htz1p in the normal regulation of *POT1*, *FOX2*,
8 *POX1* and *CTA1* expression in the presence of oleic acid, we next sought to determine if
9 Htz1p binds the cognate promoters of these genes using chromatin immunoprecipitation
10 (ChIP) of a strain expressing an HA-tagged version of Htz1p. Cells were grown in either
11 repressed (glucose) or activated (oleate) conditions. Htz1p-HA was immunoprecipitated
12 with anti-HA antibody and isolated DNA was analyzed by PCR. This analysis revealed
13 that Htz1p was bound to each of the four promoters (*POT1*, *FOX2*, *POX1* and *CTA1*) in
14 their repressed states (Fig. 3B). These data are consistent with genome wide
15 characterization of levels of Htz1p association with these promoters (41). The association
16 of Htz1p with these promoters was dynamic; when cells were shifted to oleic acid
17 activating conditions, Htz1p levels on the *POT1*, *POX1*, and *FOX2* promoters were
18 dramatically reduced. Dissociation from the *CTA1* promoter was not observed. These
19 data suggest that loss of Htz1p from promoters is coincident with gene activation, but that
20 dissociation is not required for the induction of all genes.

21 **Swr1p, Chz1p and Gcn5p - dependent association of Htz1p to promoters.**

22 Swr1p, Chz1p and Gcn5p have been implicated in modulating Htz1p association
23 at promoter regions. Swr1p is part of the SWR1-C multisubunit protein complex,
24 necessary for Htz1p deposition at repressed promoters (24). Chz1p, was recently
25 identified as a histone chaperone that preferentially interacts with Htz1p (21) and Gcn5p
26 is the histone acetyltransferase subunit of the SAGA complex (36). To investigate
27 whether these factors affect Htz1p binding to the oleate-responsive promoters and
28 subsequent expression, the association of Htz1p with *POT1*, *POX1*, *FOX2* and *CTA1*
29 promoters was investigated in cells lacking these proteins under conditions of repression
30 (2% glucose) and expression of these genes was monitored upon oleate induction (Fig.
31

4). Similar to its role at the well-studied *GALI* promoter, Swr1p is required for Htz1p binding to oleate responsive promoters suggesting a common role for Swr1p at disparate, highly inducible promoters. Likewise, Gcn5p was required for efficient Htz1p binding. This suggests that Gcn5p, which plays a role as a coactivator of transcription through histone acetylation (11), controls the binding or stability of Htz1p at repressed promoters. This may also be via histone acetylation. In the absence of Chz1p, Htz1p occupancy at each of the four promoters was decreased. As expected the amount of Htz1p on each of these promoters in mutant strains remained low upon switch to oleate (data not shown).

Microarray analyses in *gcn5Δ*, *swr1Δ*, and *chz1Δ* mutants support a model in which initial Htz1p association with the promoter is required for subsequent full induction. The expression levels of *POT1*, *FOX2*, *POX1* and *CTA1* were significantly reduced in mutant strains compared to WT upon oleate induction. Moreover, all 26 genes encoding peroxisomal proteins that showed transcriptional defects in *htz1Δ* cells (Fig. 1C) were similarly reduced in their expression at least two-fold in *gcn5Δ*, *swr1Δ*, and *chz1Δ* mutants compared to WT (Fig. 4B). Together, these data suggest that factors functionally associated with Htz1p, such as the chromatin remodeling complex component Swr1p, histone acetyltransferase Gcn5p and chaperone Chz1p, regulate the deposition or maintenance of Htz1p at repressed promoters, which in turn, facilitates rapid activation of transcription.

Acetylation of Htz1p is required for efficient transcriptional induction.

Acetylation of Htz1p is known to occur at sites of active transcription (23). The finding that Gcn5p is required for expression of oleate responsive genes suggests that acetylation on Htz1p is required for the oleate response. To address this question, plasmids expressing either one of two acetylation mutants of Htz1p (pCM314 (Htz1p-K14A) or pCM330 (Htz1p-K14R)) were introduced into *htz1Δ* cells and expression was monitored by FACS, confocal microscopy and quantitative RT-PCR. FACS and confocal microscopy demonstrated that Pot1p-GFP fluorescence in cells carrying pCM305 (WT *HTZI*) was stronger than that in those cells carrying empty plasmid (pRS416), or acetylation mutants (pCM330, or pCM314) during a time course of oleate incubation but

1 the peroxisomes were morphologically normal (data not shown). mRNA levels of *POT1*,
 2 *CTAI*, *FOX2* and *POXI*, determined by quantitative RT-PCR were consistent with the
 3 GFP reporter analysis (Fig 5A). The K14A acetylation mutant of Htz1p showed a defect
 4 in the normal induction of *POT1*, *CTAI*, *FOX2* and *POXI*. In addition, cells expressing
 5 Htz1p K14A also exhibited a growth defect on fatty-acid containing media, but not on
 6 glucose containing media. This growth defect was less pronounced than the null mutant
 7 of *HTZ1* (data not shown). The association of Htz1p-K14R with these oleate responsive
 8 promoters at two time points (0 h and 6 h), also revealed that Htz1p-K14R association
 9 was diminished under glucose conditions (Fig. 5B). Significant differences in association
 10 of Htz1p-K14R on these promoters were not observed during 6 h of oleate induction.
 11 These data indicate that acetylation of Htz1p is required for association with oleate
 12 responsive promoters during repressed conditions and the acetylation of Htz1p is not
 13 required for the dissociation of Htz1p from oleate responsive promoters during oleate
 14 induction (Fig. 5B). These data collectively indicate that acetylation of Htz1p is
 15 important for expression of fatty acid responsive genes and normal peroxisomal matrix
 16 protein assembly.

17
 18 **TBP is not efficiently recruited to oleate inducible promoters in the absence of**
 19 **Htz1p.**

20 We next directly analyzed *in vivo* binding of the transcriptional machinery to
 21 repressed and activated promoters in both WT and *htz1Δ* strains (Fig. 6A). As expected,
 22 the binding of TBP to the *POT1*, *POXI*, and *CTAI* promoters increased with gene
 23 expression in oleate in WT cells. The abundance of TBP did not significantly increase on
 24 the *FOX2* promoter following oleic acid induction but was present at higher initial levels
 25 than the other three other promoters studied. Nonetheless at all four promoters in *htz1Δ*
 26 cells, TBP binding was significantly reduced compared to WT cells. The reduced levels
 27 of TBP binding were not attributable to decreased cellular levels of TBP. Western blot
 28 analysis of both WT and *htz1Δ* cells demonstrated that TBP levels were equivalent
 29 between the strains and did not significantly change during oleate induction (Fig 5B).
 30 These results suggest a positive function for the Htz1p-containing nucleosomes in the

1 recruitment of TBP to the repressed promoters during the process of transcriptional
2 activation.

3 **Htz1 regulates nucleosome-promoter association during activation.**

4 A nucleosome scanning assay (NuSA) was used to investigate the role of Htz1p in
5 modulating chromatin structure by measuring nucleosome occupancy and location within
6 oleate responsive promoters during activation (*POT1*, *POX1*, *FOX2* and *CTA1*) (Fig. 7).
7 Mononucleosome-associated DNA was isolated from yeast cells before and after oleate
8 induction and quantitative real time PCR (qPCR) was used to measure dynamic
9 nucleosome occupancy during activation in WT and *htz1Δ* cells. The precise positions of
10 the nucleosomes were determined by qPCR corresponding to their known positions (17).
11 Overall the gross nucleosome position pattern at each of the four promoters under
12 repressed conditions was the same in WT and *htz1Δ* cells. The major nucleosome
13 changes were observed at position N-1 in each promoter. These nucleosomes appeared to
14 begin disassembly from each promoter at the earliest time point measured (5 min) and
15 continued through to the 30 min time point. After this initial disassembly, nucleosomes
16 were detected to have begun reassembly after 1 h of induction (Fig. 7). These
17 reassembled nucleosomes likely do not contain Htz1p. As shown in Fig. 3B, Htz1p is
18 progressively lost from these promoters during the 6 h period of induction. Notably, the
19 nucleosomes of each promoter appeared to be more protected at the later time points (6 h)
20 in *htz1Δ* cells compared to WT. This was most evident at the N-1 position of the
21 promoters of *POX1* and *CTA1* (and at the N-2 position of *POX1*). These data suggest that
22 upon oleate treatment the nucleosome proximal to initiation site in each promoter
23 disassembles leading to Htz1p loss and initial transcriptional activation. During
24 prolonged expression, nucleosomes reassemble, but these reassembled nucleosomes do
25 not contain Htz1p.

26

27 **Interplay between Htz1p and chromatin remodeling factor Isw2p.**

28 While Htz1p is proposed to contribute to nucleosome disassembly during
29 induction, the results presented above indicate that the overall chromatin structure at the
30 promoters was not extensively perturbed in *htz1Δ* cells. To gain insight into the potential

1 additional mechanisms at play during the transcriptional induction, we considered
 2 additional chromatin bound proteins. One such protein is Isw2p. Isw2p is an ATP-
 3 dependent chromatin remodeling factor that has previously been shown to be required for
 4 maintenance of chromatin structure at the *POT1* promoter (8, 9, 39) We therefore
 5 investigated Isw2p function at the *POT1*, *POX1*, *FOX2* and *CTA1* promoters in WT and
 6 *htz1Δ* cells.

7 Nucleosome protection assays in *isw2Δ* cells led to significant changes in the
 8 nucleosome structure in all four promoters (Fig 8A). These data indicate that Isw2p plays
 9 a role in chromatin structure of these four promoters and are consistent with previous
 10 work on the *POT1* promoter (8, 9, 39).

11 Next we used ChIP to assay the ability of Isw2p to associate with the four oleate
 12 responsive promoters. Previous work has shown that in WT cells Isw2p does not stably
 13 associate with these promoters, which suggests that under normal conditions, Isw2p
 14 contributes to the nucleosome structure at these Htz1p-containing *POT1*, *POX1*, *FOX2*
 15 and *CTA1* promoters through transient interactions (9). Similarly, we found very low
 16 levels of Isw2p association with these promoters in WT cells, in glucose and after oleate
 17 induction. However, substantial amounts of Isw2p were observed in association with
 18 each of these promoters in the *htz1Δ* cells. Isw2p remained associated with these
 19 promoters during their activation, suggesting a role in establishing chromatin dynamics in
 20 the absence of Htz1p (Fig. 8B).

Discussion

Exposure of yeast cells to oleate results in large scale reorganization of gene expression regulatory networks and provides an excellent experimental system for understanding the mechanisms of gene expression at both the molecular and network levels (28, 33). The resulting changes in gene expression are widespread, representing the reorganization of regulatory networks governing numerous categories of gene function. For example genes involved in protein translation and glycolysis are repressed, reflecting the shift in growth rates and metabolism (16, 32, 33). Likewise, genes linked to mitochondrial respiration and peroxisomal fatty acid metabolism is induced, reflecting the cells shift to non-fermentative β -oxidation as an energy source (16, 32, 33). The ability of yeast cells to adapt to this shift is dependent on the *HTZ1* gene encoding the histone variant Htz1p/H2A.Z (19, 32). The data presented here demonstrate that Htz1p plays a critical role in this transition by contributing to the recruitment of TBP to oleate responsive genes leading to rapid and robust expression of highly inducible genes.

Transcriptome profiling studies presented here demonstrate that expression of genes that are normally highly responsive to oleate is impaired in the absence of Htz1p. Because, under these conditions, many of the most strongly induced genes are required for peroxisomal β -oxidation and peroxisome proliferation, lack of Htz1p renders cells unable to respond efficiently to the transition and metabolize the fatty acids.

In order to elucidate the step-wise molecular function of Htz1p in the transcriptional regulation of these genes, we generated and compared various time course datasets to analyze chromatin states before and after the switch to oleic acid. We used chromatin immunoprecipitations to assay the dynamic association of Htz1p at the promoters of four model genes (*POT1*, *POX1*, *FOX2*, and *CTA1*) encoding peroxisomal matrix enzymes, the expression of which was perturbed by deletions of *HTZ1*. Htz1p has been proposed to preferentially bind repressed promoters facilitating the rapid activation of the associated genes (41). Consistent with the current models, we demonstrate that Htz1p tends to be bound to these promoters in their repressed states (glucose) and disassociates from these promoters once the cells are exposed to oleate; however, this association and dissociation pattern occurs at levels that are promoter specific. The

1 methods employed here did not reveal significant dissociation of Htz1p from the *CTA1*
2 promoter. The data suggest that Htz1p levels on the *CTA1* promoter are lower (~2-fold
3 over control regions) than the other promoters examined. Thus, Htz1p does not appear to
4 dissociate from the *CTA1* promoter following oleic acid induction. The mechanisms
5 underlying promoter specific effects of Htz1p and other epigenetic factors remain fertile
6 ground for future study.

7 In addition, data presented here support previous studies in both yeast and
8 mammalian cells that demonstrate that Htz1p is deposited at promoters by the chromatin
9 remodeling protein Swr1p (24, 40). Similarly, as in other transcriptional responses (23),
10 Gcn5p/Esa1p mediated acetylation at Lys14 of Htz1p is required for efficient
11 transcriptional activation. Moreover, Gcn5p/Esa1p mediated acetylation at Lys14 of
12 Htz1p is required for efficient association of Htz1p at some oleate responsive promoters.
13 The significantly decreased association of the Htz1p-K14R mutant was observed under
14 repressive conditions (i.e. glucose), and this decreased binding of Htz1p was also
15 observed in cells lacking the enzyme (Gcn5p) responsible for Htz1p acetylation. Htz1p
16 acetylation mutant cells also displayed defects in peroxisome proliferation and growth on
17 oleic acid, similar to an *HTZ1* null mutant. These data demonstrate that acetylation of
18 Htz1p, mediated by Gcn5p, is required for association with oleate responsive promoters
19 during repressed conditions and for normal transcriptional induction contributed by
20 Htz1p.

21 Among the known effectors of Htz1p, Chz1p is relatively less well characterized.
22 Luk et al (21) identified a role for Chz1p as a nuclear chaperone for Htz1p though the
23 functional relationship between Chz1p and Htz1p with respect to transcriptional
24 regulation remained uncharacterized. Here, we report that Chz1p, like Swr1p and Gcn5p,
25 is also involved in the deposition of Htz1p at repressed promoters.

26 With respect to the role of Htz1p in TBP recruitment, studies of the *GAL*
27 promoters have drawn different conclusions. In a recent study, TBP recruitment to the
28 *GAL1* promoter in *htz1Δ* strains was indistinguishable from that of WT cells (10).
29 However, earlier studies showed Htz1p-dependent enrichment of TBP to *GAL1* and
30 *GAL10* promoters during a time course of galactose induction (1). In the case of the fatty-
31 acid inducible promoters tested here, absence of Htz1p led to significant reduction in the

1 recruitment of TBP during oleate induction. We did not observe increased enrichment of
2 TBP at *FOX2* promoter during oleate induction. The dynamics of TBP binding appear to
3 be promoter specific. The relative abundance of TBP at the *FOX2* promoter prior to
4 induction by oleic acid (compared to later time points) suggests that activation of *FOX2*
5 does not require additional TBP binding and that the gene exists in a state poised for its
6 activation upon receiving the correct signals (i.e. oleic acid). Comprehensive
7 investigation of the dynamic and quantitative role of Htz1p in the recruitment of factors
8 such as mediator and TBP to different promoters throughout the genome remains for
9 future studies.

10 Our data suggest that activation of repressed genes leads to a dynamic
11 reorganization of chromatin structure. Specifically, upon oleate treatment the nucleosome
12 proximal to initiation site in each promoter disassembles. This coincides with the ejection
13 of Htz1p from the promoter. These data are in agreement with previous studies that
14 indicate nucleosome disassembly from promoters during activation provides access to the
15 transcriptional machinery (25). While Htz1p is proposed to contribute to nucleosome
16 disassembly during induction, surprisingly, the apparent rate of nucleosome disassembly
17 at the oleate responsive promoters was not dramatically different in *htz1Δ* cells. After
18 initial disassembly, the nucleosomes reassemble (~1 h after induction). Interestingly,
19 these new nucleosomes do not appear to contain Htz1p, but levels of transcription are
20 nonetheless higher in WT cells than in cells lacking Htz1p. These data suggest the initial
21 presence of Htz1p ensures a normal transcriptional response and provides an epigenetic
22 mark that persists after its loss, ensuring high levels of expression. Close examination of
23 the data from nucleosome protection assays suggest that, in the absence of Htz1p,
24 nucleosomes in the promoter regions of oleate responsive genes are relatively more
25 assembled, which may cause reduced expression levels at these later time points. The
26 reassembly of nucleosomes during the coincident high levels of gene expression, suggests
27 that transcriptional activity is not simply related to an overall openness of chromatin at
28 activated promoters and obstruction at repressed promoters. Rather, the precise dynamic
29 placement and specific constituents of individual nucleosomes at promoters
30 mechanistically regulates transcription by modulating access of transacting factors to
31 specific sites. Further, characterization of the dynamics of the epigenetic marks, protein

1 components of the nucleosomes and chromatin remodeling complexes at these promoters
2 is required to delineate the mechanistic basis of the links between chromatin state and
3 transcriptional activation.

4 The observed lower expression levels in cells lacking Htz1p may also be
5 contributed by Isw2p. Isw2p is an energy-dependent chromatin remodeling factor and
6 negative regulator of gene expression (8, 9). When assayed for genome binding by ChIP-
7 chip Isw2p association with the *POT1*, *POX1*, *FOX2* and *CTA1* promoters was not
8 detected (9). Similarly, we found no enrichment of Isw2p at these promoters in WT cells.
9 However, Isw2p bound to each promoter in the absence of Htz1p, and this association
10 persisted through the six hours of induction. Therefore, the increased association of
11 Isw2p to these four oleate responsive promoters may account for the reduced expression
12 levels in *htz1Δ* cells. It is also possible that in WT cells Isw2p provides a complementary
13 mechanism for chromatin structural changes independent of Htz1p. Loss of Htz1p
14 provides an opportunity for Isw2p binding that is not normally functional in Htz1p-
15 containing regions of chromatin. Further studies are required to understand the global
16 relationship between Isw2p and Htz1p.

17 In mammalian cells histone variant H2A.Z can serve as a novel epigenetic marker
18 of breast cancer progression as it is associated with lymph node metastasis and decreased
19 breast cancer survival (13). In the plant *Arabidopsis thaliana*, histone H2A.Z is required
20 for immune resistance to the phytopathogenic bacteria *Pseudomonas syringae* pv. tomato
21 (22). In zebrafish, histone variant 2a z (H2afza) is essential for larval development
22 through the generation of a lethal locus with a truncation of conserved carboxy-terminal
23 residues in the protein (31). Taken together these studies implicate histone H2A.Z in a
24 number of diverse functions in different organisms. Because peroxisomes are highly
25 dynamic and responsive eukaryotic organelles whose dysfunction are linked to a host of
26 human conditions (4, 5), it is important to understand the roles of proteins like Htz1p, that
27 control aspects of chromatin structure and transcriptional responses preceding the
28 proliferation of peroxisomes and fatty acid metabolism in *S. cerevisiae*.

1 **Acknowledgements**

2 We thank Bradley R. Cairns, Haiying Zhang and Michael Grunstein for providing
 3 plasmids and strains; Jeff Ranish and members of the Aitchison laboratory for helpful
 4 comments and discussion during the course of this project. This work was supported by
 5 grants GM067228, GMO76547 and RR022220 from the U.S. National Institutes of
 6 Health.

7
 8
 9
 10
 11
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25
 26
 27

References

1. **Adam, M., F. Robert, M. Larochelle, and L. Gaudreau.** 2001. H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Mol Cell Biol* **21**:6270-9.
2. **Aitchison, J. D., M. P. Rout, M. Marelli, G. Blobel, and R. W. Wozniak.** 1995. Two novel related yeast nucleoporins Nup170p and Nup157p: complementation with the vertebrate homologue Nup155p and functional interactions with the yeast nuclear pore-membrane protein Pom152p. *J Cell Biol* **131**:1133-48.
3. **Albert, I., T. N. Mavrich, L. P. Tomsho, J. Qi, S. J. Zanton, S. C. Schuster, and B. F. Pugh.** 2007. Translational and rotational settings of H2A.Z nucleosomes across the *Saccharomyces cerevisiae* genome. *Nature* **446**:572-6.
4. **Bensinger, S. J., and P. Tontonoz.** 2008. Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature* **454**:470-7.
5. **Berger, J., and D. E. Moller.** 2002. The mechanisms of action of PPARs. *Annu Rev Med* **53**:409-35.
6. **Biddick, R. K., G. L. Law, and E. T. Young.** 2008. Adr1 and Cat8 mediate coactivator recruitment and chromatin remodeling at glucose-regulated genes. *PLoS ONE* **3**:e1436.
7. **Dudley, A. M., J. Aach, M. A. Steffen, and G. M. Church.** 2002. Measuring absolute expression with microarrays with a calibrated reference sample and an extended signal intensity range. *Proc Natl Acad Sci U S A* **99**:7554-9.

- 1 8. **Fazzio, T. G., M. E. Gelbart, and T. Tsukiyama. 2005.** Two distinct
2 mechanisms of chromatin interaction by the Isw2 chromatin remodeling complex
3 in vivo. *Mol Cell Biol* **25**:9165-74.
- 4 9. **Gelbart, M. E., N. Bachman, J. Delrow, J. D. Boeke, and T. Tsukiyama. 2005.**
5 Genome-wide identification of Isw2 chromatin-remodeling targets by localization
6 of a catalytically inactive mutant. *Genes Dev* **19**:942-54.
- 7 10. **Gligoris, T., G. Thireos, and D. Tzamarias. 2007.** The Tup1 corepressor directs
8 Htz1 deposition at a specific promoter nucleosome marking the GAL1 gene for
9 rapid activation. *Mol Cell Biol* **27**:4198-205.
- 10 11. **Govind, C. K., F. Zhang, H. Qiu, K. Hofmeyer, and A. G. Hinnebusch. 2007.**
11 Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in
12 transcribed coding regions. *Mol Cell* **25**:31-42.
- 13 12. **Guillemette, B., A. R. Bataille, N. Gevry, M. Adam, M. Blanchette, F. Robert,
14 and L. Gaudreau. 2005.** Variant histone H2A.Z is globally localized to the
15 promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS*
16 *Biol* **3**:e384.
- 17 13. **Hua, S., C. B. Kallen, R. Dhar, M. T. Baquero, C. E. Mason, B. A. Russell, P.
18 K. Shah, J. Liu, A. Khramtsov, M. S. Tretiakova, T. N. Krausz, O. I.
19 Olopade, D. L. Rimm, and K. P. White. 2008.** Genomic analysis of estrogen
20 cascade reveals histone variant H2A.Z associated with breast cancer progression.
21 *Mol Syst Biol* **4**:188.

- 1 14. **Ideker, T., V. Thorsson, A. F. Siegel, and L. E. Hood.** 2000. Testing for
2 differentially-expressed genes by maximum-likelihood analysis of microarray
3 data. *J Comput Biol* **7**:805-17.
- 4 15. **Jackson, J. D., V. T. Falciano, and M. A. Gorovsky.** 1996. A likely histone
5 H2A.F/Z variant in *Saccharomyces cerevisiae*. *Trends Biochem Sci* **21**:466-7.
- 6 16. **Koerkamp, M. G., M. Rep, H. J. Bussemaker, G. P. Hardy, A. Mul, K.**
7 **Piekarska, C. A. Szigarto, J. M. De Mattos, and H. F. Tabak.** 2002.
8 Dissection of transient oxidative stress response in *Saccharomyces cerevisiae* by
9 using DNA microarrays. *Mol Biol Cell* **13**:2783-94.
- 10 17. **Lee, W., D. Tillo, N. Bray, R. H. Morse, R. W. Davis, T. R. Hughes, and C.**
11 **Nislow.** 2007. A high-resolution atlas of nucleosome occupancy in yeast. *Nat*
12 *Genet* **39**:1235-44.
- 13 18. **Li, B., S. G. Pattenden, D. Lee, J. Gutierrez, J. Chen, C. Seidel, J. Gerton,**
14 **and J. L. Workman.** 2005. Preferential occupancy of histone variant H2AZ at
15 inactive promoters influences local histone modifications and chromatin
16 remodeling. *Proc Natl Acad Sci U S A* **102**:18385-90.
- 17 19. **Lockshon, D., L. E. Surface, E. O. Kerr, M. Kaeberlein, and B. K. Kennedy.**
18 2007. The sensitivity of yeast mutants to oleic acid implicates the peroxisome and
19 other processes in membrane function. *Genetics* **175**:77-91.
- 20 20. **Longtine, M. S., A. McKenzie, 3rd, D. J. Demarini, N. G. Shah, A. Wach, A.**
21 **Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for
22 versatile and economical PCR-based gene deletion and modification in
23 *Saccharomyces cerevisiae*. *Yeast* **14**:953-61.

- 1 21. **Luk, E., N. D. Vu, K. Patteson, G. Mizuguchi, W. H. Wu, A. Ranjan, J.**
2 **Backus, S. Sen, M. Lewis, Y. Bai, and C. Wu.** 2007. Chz1, a nuclear chaperone
3 for histone H2AZ. *Mol Cell* **25**:357-68.
- 4 22. **March-Diaz, R., M. Garcia-Dominguez, J. Lozano-Juste, J. Leon, F. J.**
5 **Florencio, and J. C. Reyes.** 2008. Histone H2A.Z and homologues of
6 components of the SWR1 complex are required to control immunity in
7 *Arabidopsis*. *Plant J* **53**:475-87.
- 8 23. **Millar, C. B., F. Xu, K. Zhang, and M. Grunstein.** 2006. Acetylation of H2AZ
9 Lys 14 is associated with genome-wide gene activity in yeast. *Genes Dev* **20**:711-
10 22.
- 11 24. **Mizuguchi, G., X. Shen, J. Landry, W. H. Wu, S. Sen, and C. Wu.** 2004. ATP-
12 driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin
13 remodeling complex. *Science* **303**:343-8.
- 14 25. **Petes, S. J., and J. T. Lis.** 2008. Rapid, transcription-independent loss of
15 nucleosomes over a large chromatin domain at Hsp70 loci. *Cell* **134**:74-84.
- 16 26. **Raisner, R. M., P. D. Hartley, M. D. Meneghini, M. Z. Bao, C. L. Liu, S. L.**
17 **Schreiber, O. J. Rando, and H. D. Madhani.** 2005. Histone variant H2A.Z
18 marks the 5' ends of both active and inactive genes in euchromatin. *Cell* **123**:233-
19 48.
- 20 27. **Ramsey, S. A., J. J. Smith, D. Orrell, M. Marelli, T. W. Petersen, P. de**
21 **Atauri, H. Bolouri, and J. D. Aitchison.** 2006. Dual feedback loops in the GAL
22 regulon suppress cellular heterogeneity in yeast. *Nat Genet* **38**:1082-7.

- 1 28. **Ratushny, A. V., S. A. Ramsey, O. Roda, Y. Wan, J. J. Smith, and J. D.**
2 **Aitchison.** 2008. Control of Transcriptional Variability by Overlapping Feed-
3 Forward Regulatory Motifs. *Biophys J* **8**:3715-23
- 4 29. **Saleem, R. A., B. Knoblach, F. D. Mast, J. J. Smith, J. Boyle, C. M. Dobson,**
5 **R. Long-O'Donnell, R. A. Rachubinski, and J. D. Aitchison.** 2008. Genome-
6 wide analysis of signaling networks regulating fatty acid-induced gene expression
7 and organelle biogenesis. *J Cell Biol* **181**:281-92.
- 8 30. **Santisteban, M. S., T. Kalashnikova, and M. M. Smith.** 2000. Histone H2A.Z
9 regulates transcription and is partially redundant with nucleosome remodeling
10 complexes. *Cell* **103**:411-22.
- 11 31. **Sivasubbu, S., D. Balciunas, A. E. Davidson, M. A. Pickart, S. B. Hermanson,**
12 **K. J. Wangensteen, D. C. Wolbrink, and S. C. Ekker.** 2006. Gene-breaking
13 transposon mutagenesis reveals an essential role for histone H2afza in zebrafish
14 larval development. *Mech Dev* **123**:513-29.
- 15 32. **Smith, J. J., M. Marelli, R. H. Christmas, F. J. Vizeacoumar, D. J. Dilworth,**
16 **T. Ideker, T. Galitski, K. Dimitrov, R. A. Rachubinski, and J. D. Aitchison.**
17 2002. Transcriptome profiling to identify genes involved in peroxisome assembly
18 and function. *J Cell Biol* **158**:259-71.
- 19 33. **Smith, J. J., S. A. Ramsey, M. Marelli, B. Marzolf, D. Hwang, R. A. Saleem,**
20 **R. A. Rachubinski, and J. D. Aitchison.** 2007. Transcriptional responses to fatty
21 acid are coordinated by combinatorial control. *Mol Syst Biol* **3**:115.
- 22 34. **Smith, J. J., Y. Sydorsky, M. Marelli, D. Hwang, H. Bolouri, R. A.**
23 **Rachubinski, and J. D. Aitchison.** 2006. Expression and functional profiling

- 1 reveal distinct gene classes involved in fatty acid metabolism. *Mol Syst Biol*
- 2 **2**:2006 0009.
- 3 35. **Stargell, L. A., J. Bowen, C. A. Dadd, P. C. Dedon, M. Davis, R. G. Cook, C.**
- 4 **D. Allis, and M. A. Gorovsky.** 1993. Temporal and spatial association of histone
- 5 H2A variant hv1 with transcriptionally competent chromatin during nuclear
- 6 development in *Tetrahymena thermophila*. *Genes Dev* **7**:2641-51.
- 7 36. **Sterner, D. E., and S. L. Berger.** 2000. Acetylation of histones and transcription-
- 8 related factors. *Microbiol Mol Biol Rev* **64**:435-59.
- 9 37. **Suto, R. K., M. J. Clarkson, D. J. Tremethick, and K. Luger.** 2000. Crystal
- 10 structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat*
- 11 *Struct Biol* **7**:1121-4.
- 12 38. **Whitehouse, I., O. J. Rando, J. Delrow, and T. Tsukiyama.** 2007. Chromatin
- 13 remodelling at promoters suppresses antisense transcription. *Nature* **450**:1031-5.
- 14 39. **Whitehouse, I., and T. Tsukiyama.** 2006. Antagonistic forces that position
- 15 nucleosomes in vivo. *Nat Struct Mol Biol* **13**:633-40.
- 16 40. **Wong, M. M., L. K. Cox, and J. C. Chrivia.** 2007. The chromatin remodeling
- 17 protein, SRCAP, is critical for deposition of the histone variant H2A.Z at
- 18 promoters. *J Biol Chem* **282**:26132-9.
- 19 41. **Zhang, H., D. N. Roberts, and B. R. Cairns.** 2005. Genome-wide dynamics of
- 20 Htz1, a histone H2A variant that poises repressed/basal promoters for activation
- 21 through histone loss. *Cell* **123**:219-31.
- 22
- 23
- 24
- 25

Figure Legends

2

Figure 1. Robust expression of oleate-responsive genes expression is dependent on

HTZ1. (A) Comparison of changes in mRNA levels of all yeast genes in WT (left column) and *htz1Δ* cells (middle column) after induction in oleate medium for 6 h. Shown are the relative expression levels (Log_{10}) of genes that were determined to be significantly ($\lambda \geq 100$) altered in cells on oleate (compared to WT cells on glucose). Relative expression levels are shown using the scale of the yellow-blue heat map (top). Genes are ordered top-bottom based on relative expression in WT cells on oleate. Approximately 1000 genes were significantly induced and 1000 genes were repressed and changed in expression at least 2-fold. Genes that were reduced in expression were significantly enriched for functions related to ribosomal biogenesis (hypergeometric distribution analysis of GO terms – $p \sim 10^{-50}$). Induced genes were enriched for oxidative phosphorylation, electron transport chain and aerobic respiration ($p \sim 10^{-10}$; components of the mitochondrial respiratory chain ($p \sim 10^{-13}$), fatty acid oxidation and peroxisome organization and biogenesis related ($\sim p \sim 10^{-6}$), and the peroxisomal compartment ($p \sim 10^{-12}$). For comparison the relative expression of each gene in *htz1Δ* cells in oleate (middle column) and glucose (right column) are shown. (B) As in Panel A, but shown are the relative expression levels of 292 genes significantly ($\lambda \geq 100$) altered in WT cells on oleate and expressed at least two-fold less than their expression levels in WT cells. This list is enriched for genes linked to fatty acid and lipid oxidation ($p \sim 4.0 \times 10^{-12}$) and peroxisomes ($p \sim 9 \times 10^{-20}$). (C) As in B, but shown are genes encoding peroxisomal proteins significantly ($\lambda \geq 100$) altered in WT cells on oleate and expressed at least 2-fold less in *htz1Δ* cells.

25

Figure 2. Deletion of HTZ1 leads to delayed peroxisome biogenesis. (A) Deletion of *HTZ1* impairs cell growth on oleate-containing media. Strains were grown to mid-logarithm phase in liquid YPD medium, and equal amounts of cells were serially diluted ten-fold onto YPD and incubated at 30 °C for 3 days and onto oleate-containing YPBO and incubated at 30 °C for 5 days. (B) Fluorescent images of WT and *htz1Δ* cells shown are expressing the peroxisomal matrix protein Pot1p fused with GFP (Pot1p-GFP) at

31

1 different time points of oleate incubation were captured on a TCS SP2 Laser Scanning
2 Spectral Confocal Microscope.

3
4 **Figure 3. Htz1p dynamically dissociates from oleate responsive promoters upon**
5 **induction.** (A) *POT1*, *POX1*, *FOX2* and *CTA1* mRNA levels were determined by RT-
6 PCR in WT and *htz1Δ* strains over a time course of oleate induction. The signal obtained
7 from *ACT1* mRNA was used as a loading control for normalization. Error bars represent
8 standard deviation from the mean of three independent experimental values. (B) Htz1p
9 enrichment at four promoters was determined by qPCR during oleate induction. Relative
10 enrichment values (Y axes) are the average of three independent ChIPs with qPCR
11 determination performed twice per each biological replicate. Non-promoter IGRI
12 *YMR325W* was used as an internal control to normalize signals of promoter enrichment.
13 In response to oleate induction, Htz1p was lost from the *POT1*, *POX1*, *FOX2* and *CTA1*
14 promoters.

15
16 **Figure 4. Swr1p, Chz1p and Gcn5p - dependent association of Htz1p to promoters.**
17 (A) *In vivo* association of Htz1p with *POT1*, *POX1*, *FOX2* and *CTA1* promoters was
18 measured by ChIP in the WT, *chz1Δ*, *gcn5Δ* and *swr1Δ* strains in 2% glucose medium.
19 ChIP was performed in glucose-containing medium. Error bars represent standard
20 deviation from the mean of three independent experimental values and two technical
21 replicates of each. (B) Comparison of changes in mRNA levels of all yeast genes in WT,
22 *chz1Δ*, *gcn5Δ* and *swr1Δ* strains after induction in oleate medium for 6 h. As in Figure 1
23 C, genes are encoding peroxisomal proteins significantly ($\lambda \geq 100$) altered in WT cells on
24 oleate and expressed at least 2-fold less in *htz1Δ* cells.

25
26 **Figure 5. Acetylation of Htz1p is required for efficient transcriptional induction.** (A)
27 *POT1*, *POX1*, *FOX2* and *CTA1* mRNA levels were determined by RT-PCR in WT and
28 *htz1Δ* and Htz1p K14A mutant strains over a time course of oleate induction. The signal
29 obtained from *ACT1* mRNA was used as a loading control for normalization. Error bars
30 represent standard deviation from the mean of three independent experimental values. (B)
31 Enrichment of WT Htz1p and Htz1p K14A mutant at four promoters was determined by

1 qPCR during glucose and oleate induction for 6 hours. Relative enrichment values (Y
2 axes) are the average of three independent ChIP experiments with two technical
3 replicates of each. Non-promoter IGRi *YMR325W* was used as an internal control to
4 normalize signals of promoter enrichment.

5
6 **Figure 6. Recruitment of TBP during oleate induction requires Htz1p.** (A) The
7 association of TBP with *POT1*, *POX1*, *FOX2* and *CTA1* promoters was determined by
8 chromatin immunoprecipitation (ChIP) using anti-Myc antibodies, followed by gene-
9 specific PCR. The relative enrichment ratio is plotted at 4 time points (0, 1, 4, 6 h) of
10 induction in oleate. *ACT1* was used as an internal control to normalize signals of
11 promoter enrichment. Error bars show the standard deviation from three independent
12 experimental values with two technical replicates of each. (B) Deletion of Htz1p did not
13 affect TBP expression during oleate induction. The WT strain and *HTZ1* deletion strains
14 expressing genomically integrated TBP were grown in 2% glucose overnight and then
15 transferred to oleate-containing SCIM medium at the indicated time points. Samples
16 containing equal protein were analyzed by Western blotting with anti-Myc antibody to
17 visualize TBP expression. A polyclonal antibody directed against Gsp1p was used as
18 loading control.

19
20 **Figure 7. Htz1p regulates the occupancy of specific nucleosomes on *POT1*, *POX1*,**
21 ***FOX2* and *CTA1* promoters.** The NuSA assay was used to determine the nucleosome
22 positioning and density at *POT1*, *POX1*, *FOX2* and *CTA1* promoters during oleate
23 induction (time of induction is indicated at left) in WT and *HTZ1* deletion strains. Each
24 point represents the relative protection of each PCR amplicon, quantified by real-time
25 PCR and normalized to a centromeric control. The position of each amplicon (referenced
26 to the middle of each amplicon) within the promoter is shown on the x-axis. The
27 approximate location of nucleosome is represented by grey circle with the nucleosome
28 number referred to in the text shown on the circle.

29
30 **Figure 8. Isw2p can associate with oleate responsive promoters in the absence of**
31 **Htz1p.** (A) The NuSA assay was used to determine the nucleosome positioning and

1 density at *POT1*, *POX1*, *FOX2* and *CTA1* promoters during repression (2% glucose) in
 2 WT, *htz1Δ* and *isw2Δ* strains. Each point represents the relative protection of each PCR
 3 amplicon, quantified by real-time PCR and normalized to a centromeric control. (B) The
 4 association of Isw2p (as a C-terminal myc fusion) with *POT1*, *POX1*, *FOX2* and *CTA1*
 5 promoters was determined by chromatin immunoprecipitation (ChIP) using anti-Myc
 6 antibodies, followed by gene-specific PCR. The relative enrichment ratio is plotted at 4
 7 time points (0, 1, 4, 6 h) of induction in oleate. *ACT1* was used as an internal control to
 8 normalize signals of promoter enrichment. Error bars show the standard deviation from
 9 three independent experimental values with two technical replicates of each.

1 **Table 1: Strains and plasmids used in this study**

Strain	Genotype	Reference
BY4741	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	Open
BY4742	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	Open
YWY004	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, htz1::kanMX4</i>	Open
YWY042	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, POT1-GFP::natMX</i>	This study
YWY007	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, POT1-GFP::natMX, pRS416</i>	This study
YWY005	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, htz1::kanMX4, POT1-GFP::natMX</i>	This study
YWY009	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, htz1::kanMX4, POT1-GFP::natMX, pRS416</i>	This study
YWY010	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, htz1::kanMX4, POT1-GFP::natMX, pCM305</i>	This study
YWY011	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, htz1::kanMX4, POT1-GFP::natMX, pCM330</i>	This study
YWY012	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, htz1::kanMX4, POT1-GFP::natMX, pCM314</i>	This study
YWY013	<i>MATa, leu2Δ0, ura3Δ0, HA-HTZ1</i>	Zhang haiying, 2005
YWY0165	<i>MATa, leu2Δ0, ura3Δ0, HA-HTZ, chz1::kanMX4</i>	This study
YWY0166	<i>MATa, leu2Δ0, ura3Δ0, HA-HTZ, gcn5::kanMX4</i>	This study
YWY0177	<i>MATa, leu2Δ0, ura3Δ0, HA-HTZ, swr1::kanMX4</i>	This study
YWY206	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, SPT15-13MYC::kanMX4</i>	This study
YWY207	<i>MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, SPT15-13MYC::kanMX4, htz1::hphMX</i>	This study
Plasmid	Description	Reference
pRS416	CEN6-ARS4 URA3	Open
pCM314	CEN6-ARS4 URA3 HA-htz1K14A	Millar CB et al, 2006
pCM330	CEN6-ARS4 URA3 HA-htz1K14R	Millar CB et al, 2006
pCM305	CEN6-ARS4 URA3 HA-HTZ1	Millar CB et al, 2006

Fig 1

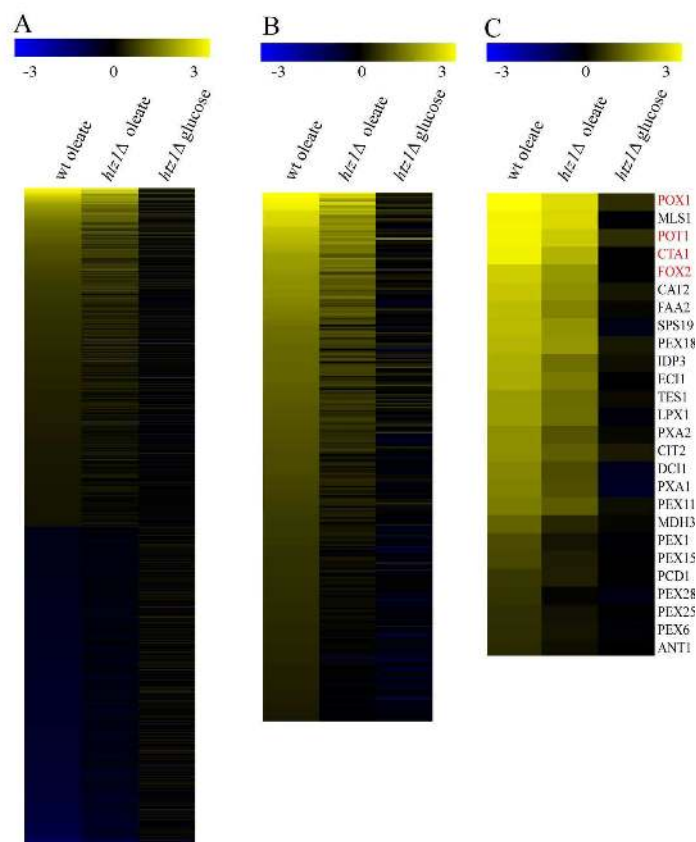
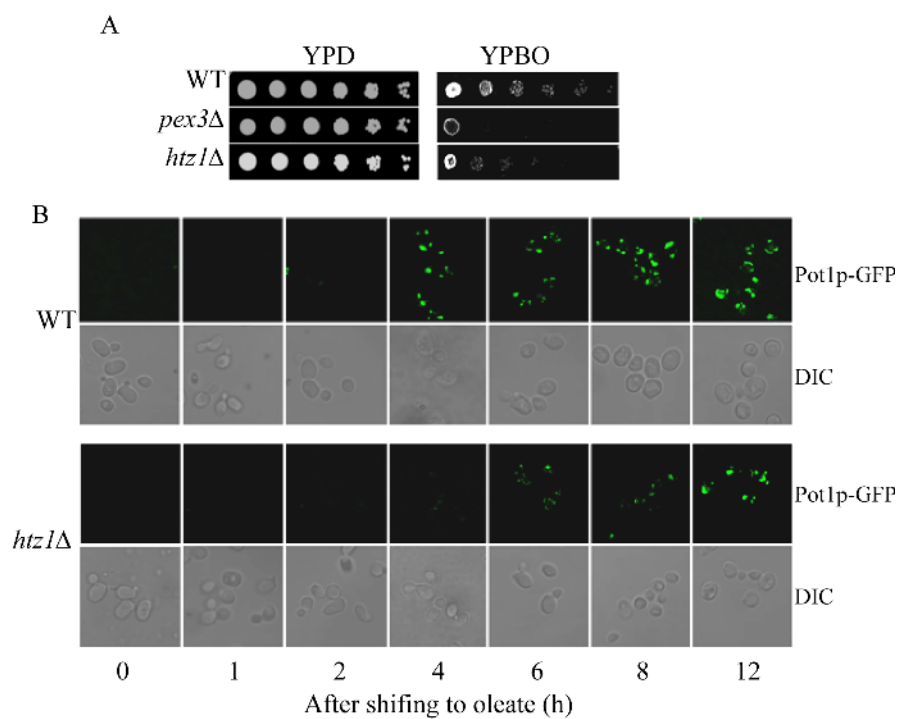


Fig 2



1
2
3
4
5
6
7
8
9
10
11
12
13

Fig 3

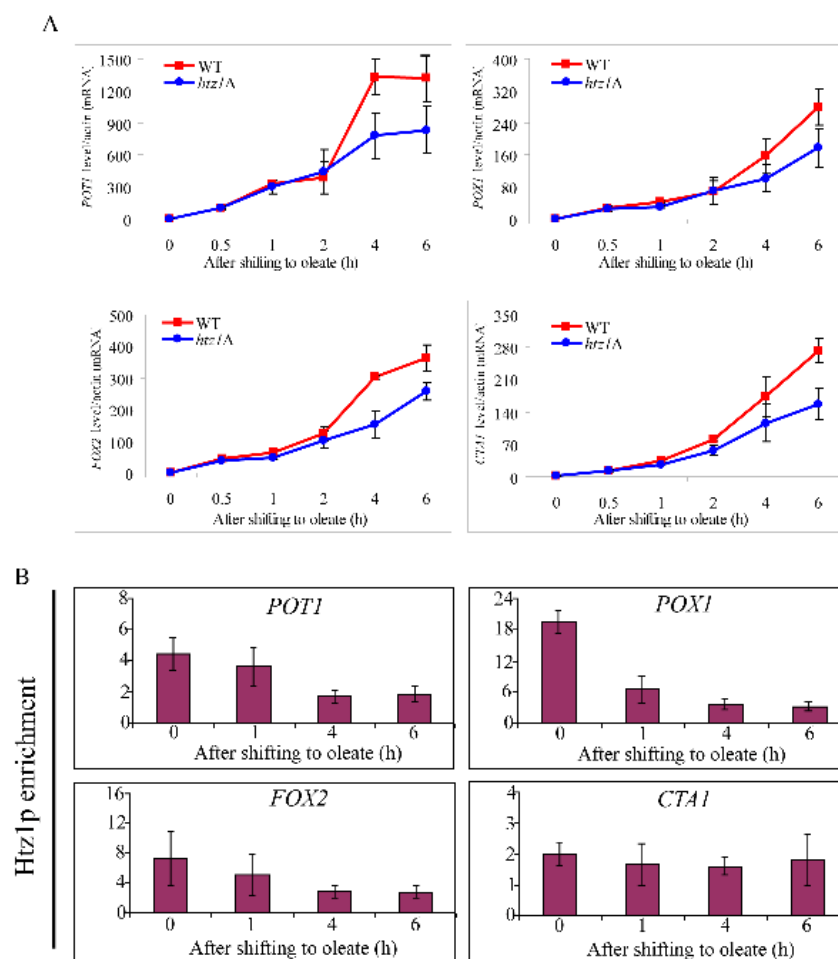
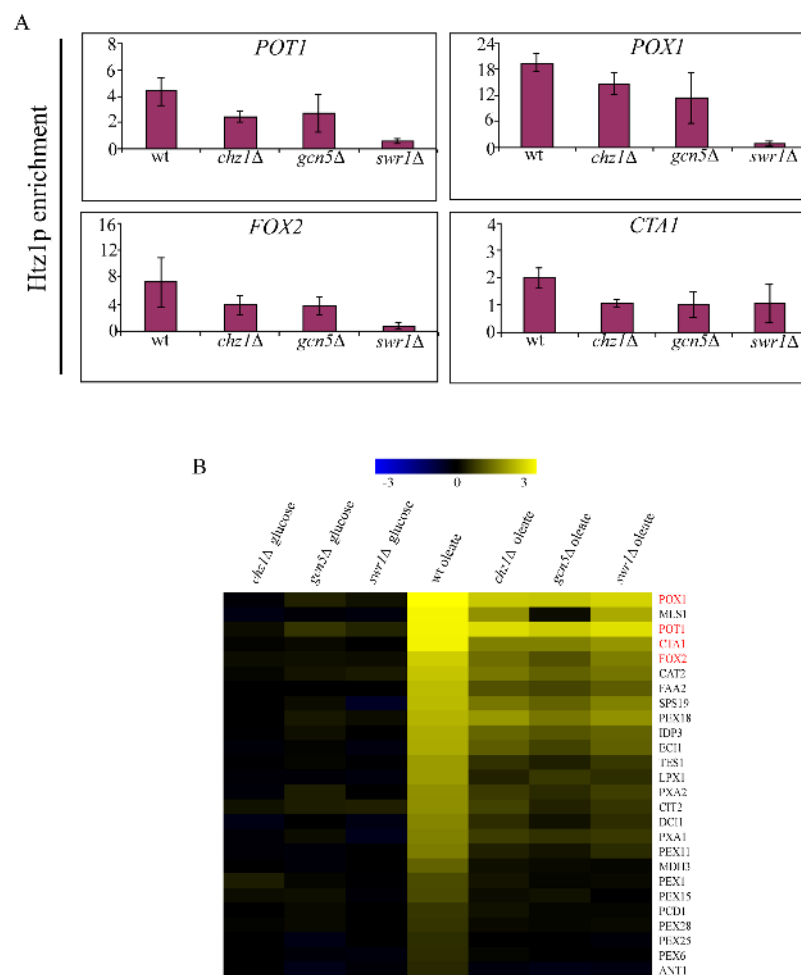
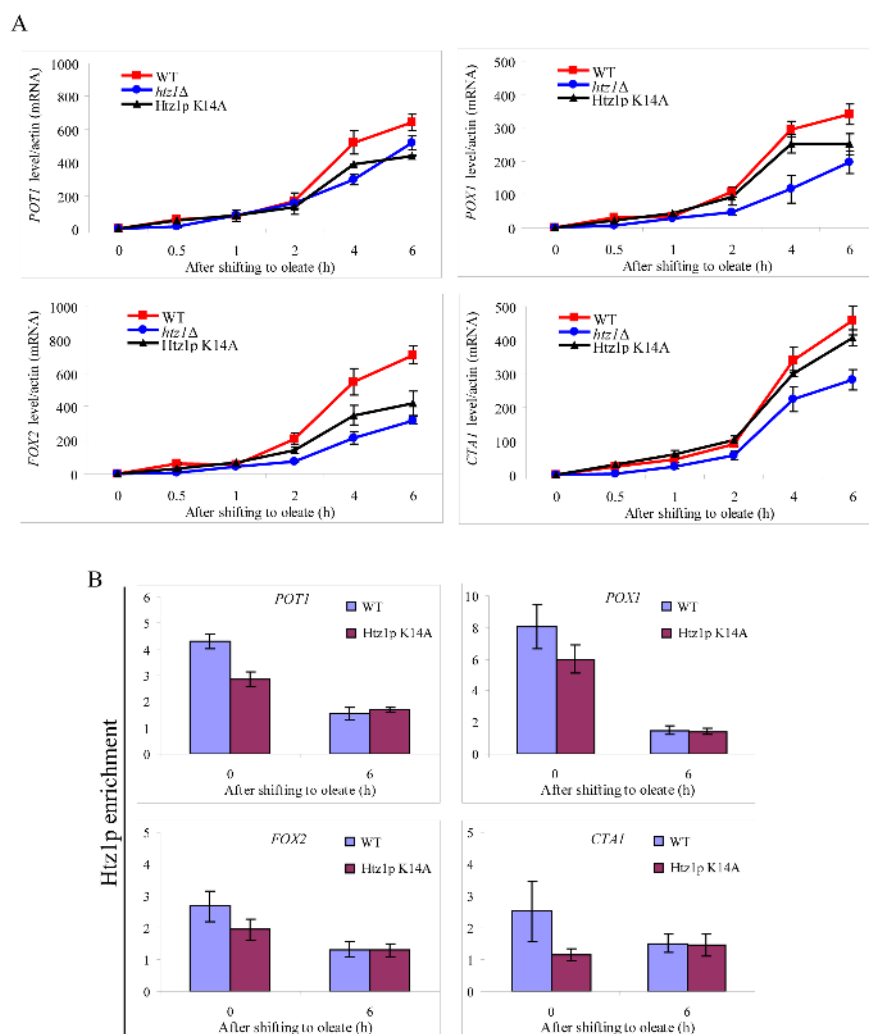


Fig 4



1
2
3
4
5
6
7

Fig 5



1
2
3
4
5
6

Fig 6

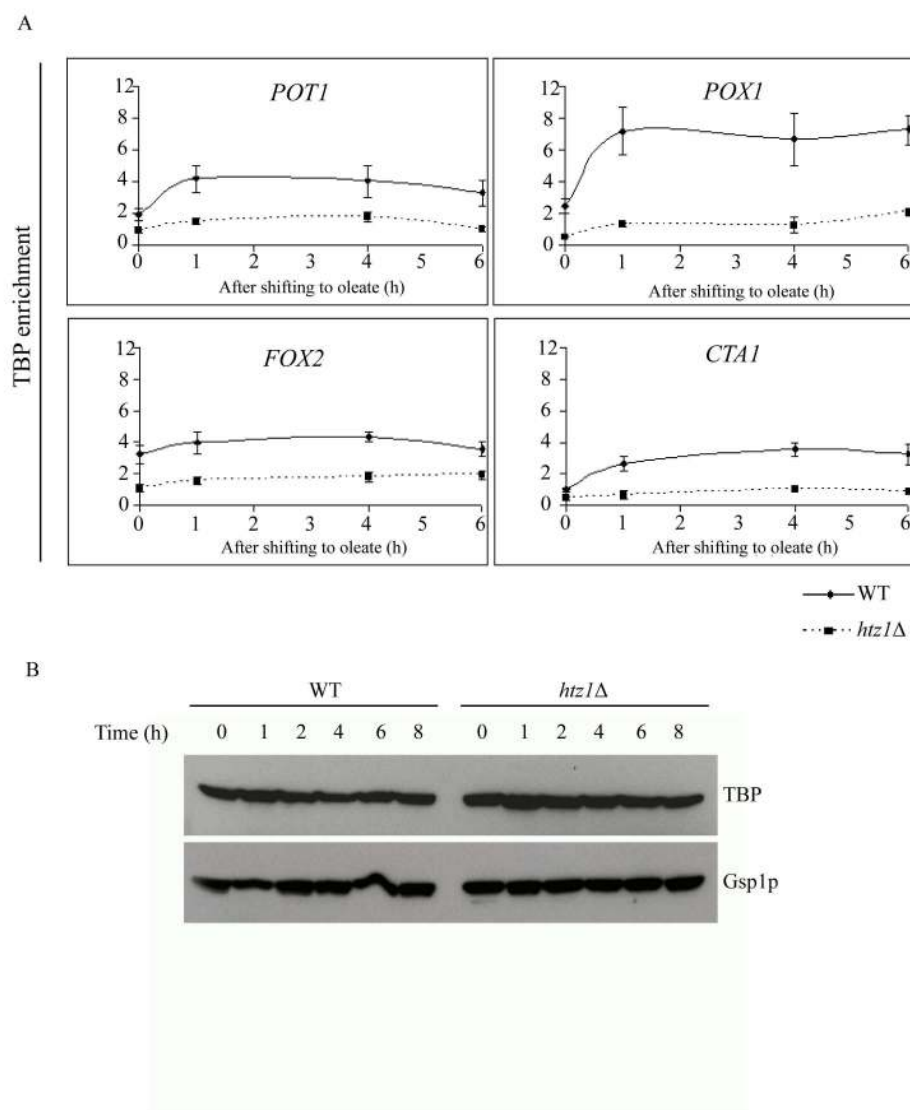
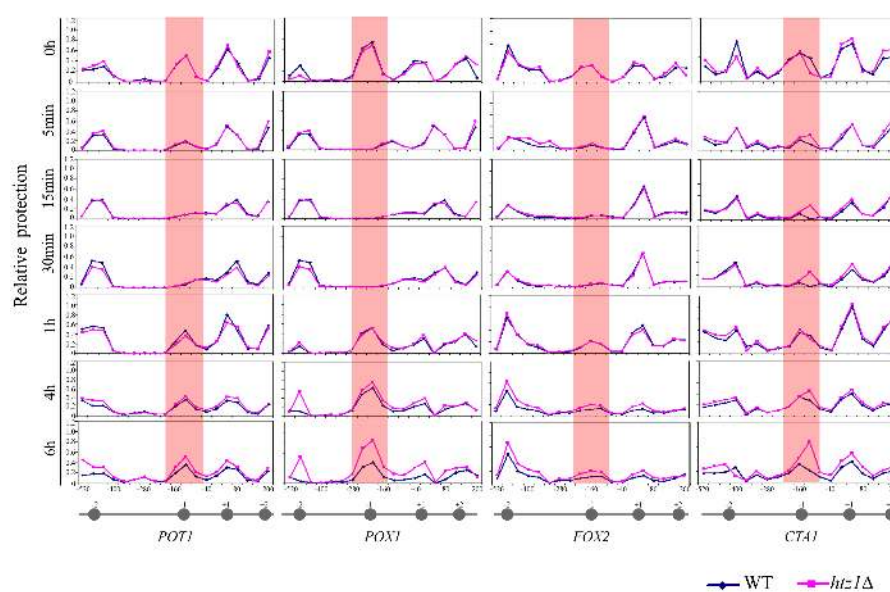
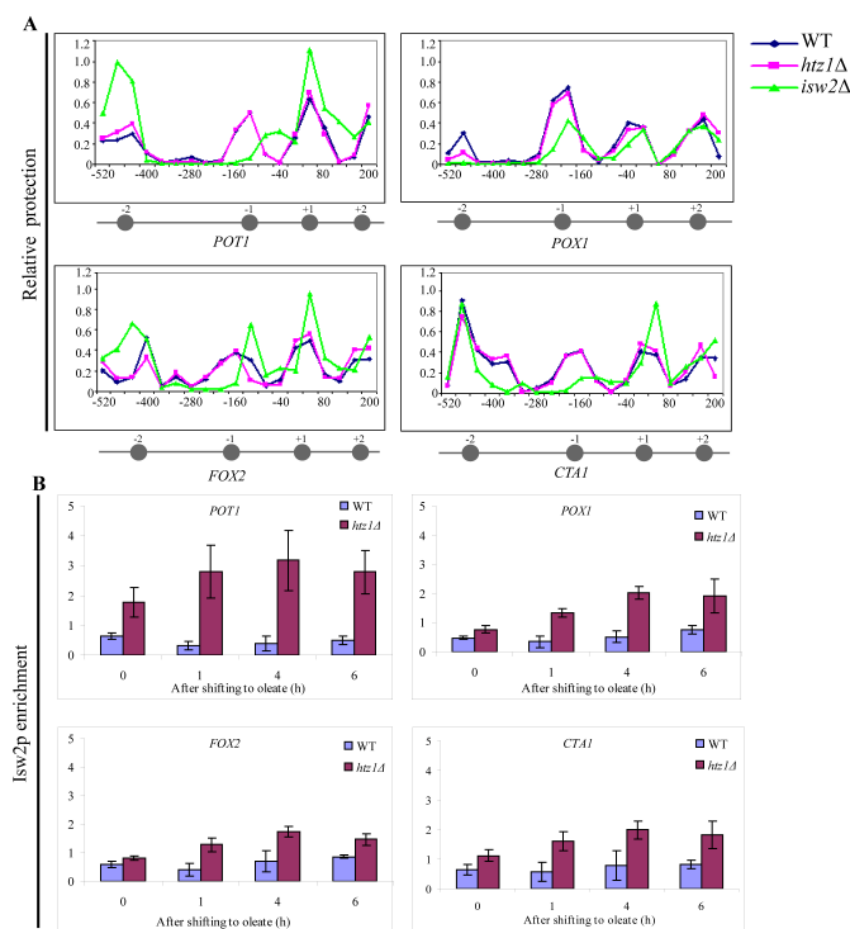


Fig. 7



1
2
3
4
5
6
7
8
9
10
11
12
13
14

Fig. 8



1
2
3
4
5
6
7