Transcription-dependent targeting of Hda1C to hyperactive genes mediates H4-specific deacetylation in yeast

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In yeast, Hda1 histone deacetylase complex (Hda1C) preferentially deacetylates histones H3 and H2B, and functionally interacts with Tup1 to repress transcription. However, previous studies identified global increases in histone H4 acetylation in cells lacking Hda1, a component of Hda1C. Here, we find that Hda1C binds to hyperactive genes, likely via the interaction between the Arb2 domain of Hda1 and RNA polymerase II. Additionally, we report that Hda1C specifically deacetylates H4, but not H3, at hyperactive genes to partially inhibit elongation. This role is contrast to that of the Set2–Rpd3S pathway deacetylating histones at infrequently transcribed genes. We also find that Hda1C deacetylates H3 at inactive genes to delay the kinetics of gene induction. Therefore, in addition to fine-tuning of transcriptional response via H3-specific deacetylation, Hda1C may modulate elongation by specifically deacetylating H4 at highly transcribed regions.
Histone acetylation is regulated by covalent modifications of histones, including acetylation, methylation, phosphorylation, and ubiquitination. Histone acetylation directly activates RNA polymerase II (RNA Pol II) transcription by disrupting the interaction between histones and DNA, thereby opening the chromatin structure, and/or recruiting factors that affect local chromatin structure and transcription. By contrast, histone deacetylation directly represses transcription.

The opposing activities of histone acetyltransferases (HATs) and histone deacytases (HDACs) dynamically regulate histone acetylation and transcription. In yeast, multiple HATs, including SAGA, NuA3, and NuA4, are responsible for acetylation at promoter regions, whereas histone deacetylation is mainly mediated by the Rpd3 large (Rpd3L) HDAC. Histone acetylation/deacetylation within coding regions affects RNA Pol II elongation, transcription initiation from cryptic promoters, and histone exchange. Furthermore, co-transcriptional histone elongation, transcription initiation from cryptic promoters, and deacetylation within coding regions affects RNA Pol II promoter regions, whereas histone deacetylation is mainly regulated by SAGA, NuA3, and NuA4, responsible for acetylation and transcription. In yeast, multiple HATs, including Set2 and Bur1, suggest that Hda1C may play a negative role in elongation.

To understand the exact function of Hda1C in more detail, the Rpd3 small (Rpd3S) HDAC pathway has overlapping but distinct functions in histone deacetylation, namely, deacetylation of histone H3 at inactive genes and deacetylation of histone H4 within active coding regions. To confirm these hypotheses, acetylation patterns were analyzed at two actively transcribing genes: YEF3 and PMA1. Whereas H4 acetylation within the coding regions of these genes was strongly increased in the hda1Δ strain, deletion of HDA1 did not significantly affect H4 acetylation at the promoter regions or H3 acetylation at the promoter or coding regions (Fig. 1b). The same patterns of histone acetylation were also observed in hda2Δ and hda3Δ strains (Supplementary Fig. 1a–c). These findings provide further evidence that Hda1C specifically deacytlates histone H4 within coding regions of actively transcribed genes.

To explore the roles of Hda1C in histone deacetylation on a genome-wide level, we used chromatin immunoprecipitation sequencing (ChIP-seq) to monitor H3 and H4 acetylation in wild-type and hda1Δ strains. As Hda1C functionally interacts with the general corepressor Tup1, histone acetylation patterns were also determined in tup1Δ cells. Levels of histone acetylation were normalized to total histone H3 levels. In both hda1Δ and tup1Δ cells, H3 acetylation was increased at inactive genes, as sorted by Rpb3 occupancy (Fig. 2a). By contrast, deletion of HDA1 increased H4 acetylation within the coding regions of more active genes (Fig. 2a). We further generated quantitative ChIP-seq data with Schizosaccharomyces pombe spike-in control normalization to ensure the accurate changes in H4 acetylation levels. hda1Δ cells but not tup1Δ cells still exhibited a strong increase of H4 acetylation within more active genes (Fig. 2b). This finding indicates that Hda1C-mediated deacetylation of H4 within coding regions is independent of Tup1.

Next, using the data set from Fig. 2b, we divided the genes into five groups by K-mean clustering to categorize genes based on similarities in acetylation profiles. Approximately 47% of the genes (including groups 3, 4, and 5) showed a considerable increase in H4 acetylation within coding regions upon deletion of HDA1 (Fig. 2c, d). To understand the relationships between the changes in H4 acetylation levels and transcription frequency or gene length, all genes were classified into five groups based on RNA Pol II occupancy in wild-type cells or their length (Fig. 2e, f).

Highly active genes in wild-type cells and longer genes showed more substantial increases in H4 acetylation following
deletion of HDA1 than those with lower RNA Pol II occupancies and shorter genes, respectively (Fig. 2e, f). By contrast, deletion of HDA1 caused a slight increase in H3 acetylation throughout inactive genes (Supplementary Fig. 2a and b). Further analyses of histone acetylation patterns in tup1Δ cells revealed that Tup1 primarily controls histone acetylation at inactive promoters (Supplementary Fig. 2c and d). These results suggest that Hda1C preferentially deacetylates histone H4 within coding regions of hyperactive long genes via a Tup1-independent mechanism.

Hda1 associates with highly transcribed genes. Although previous studies have proposed that Hda1 targets inactive promoters via Tup1, the observed increase in H4 acetylation within the coding regions of highly transcribed genes following deletion of HDA1 suggests that it also binds to these regions. Therefore, we performed ChIP experiments using an anti-myc antibody and cells expressing 18-myc-tagged Hda1 (Hda1-myc). Unlike the untagged control, Hda1-myc was highly enriched at the promoter and coding regions of the active genes YEF3 and PMA1 (Fig. 3a). By contrast, binding of Hda1-myc was relatively depleted at the

Fig. 1 Hda1C preferentially deacetylates histone H4 at actively transcribed genes. a Hda1C specifically deacetylates histone H4 at active GAL3-coding regions. Crosslinked chromatin from the indicated strains grown in YP-Glucose (YPD) or YP-Galactose was precipitated with an anti-H3, anti-acetyl H3, or anti-acetyl H4 antibody as indicated. PCR analysis of the precipitated DNA was carried out on the promoter and coding regions of GAL3. A non-transcribed region located close to the telomere of chromosome VI was used as an internal control. The signals for acetyl H3 and acetyl H4 were quantitated and normalized to the total H3 signal. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. **p < 0.01 and ***p < 0.001 (two-tailed unpaired Student’s t tests). b Hda1C deacetylates histone H4 at coding regions of the actively transcribed genes YEF3 and PMA1. Crosslinked chromatin from the indicated strains grown in YPD was precipitated with an anti-H3 or anti-acetyl H4 antibody as indicated, and a ChIP assay was performed as in a. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. *p < 0.05 and **p < 0.01 (two-tailed unpaired Student’s t tests). Source data are provided as a Source Data file.
inactive gene TKL2, even though Hda1-myc occupancy at this region was approximately threefold higher than that of the untagged control (Fig. 3a).

Next, we examined whether other subunits of Hda1C are required for Hda1 crosslinking. Binding of Hda1 to the YEF3 and PMA1 genes was reduced slightly following deletion of HDA2, HDA3, or both, even though the Hda1 protein level was similar in wild-type and mutants (Fig. 3b and Supplementary Fig. 3a and b). A previous study showed that Hda2 and Hda3 can bind to double- and single-stranded DNA in vitro. Although binding of these subunits to nascent RNA has not been demonstrated, the strong enrichment of Hda1 at highly active genes suggests that it

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Fig. 2 Actively transcribed long genes are preferentially deacetylated by Hda1C. a Hda1, but not Tup1, deacetylates histone H4 within coding regions. Heatmaps of H3 and H4 acetylation levels calculated as the log2 fold change in hda1Δ or tup1Δ cells versus wild-type cells from two independent ChIP-seq experiments. All genes are sorted by descending order of Rpb3 occupancy from Mayer et al.45. The y axis indicates each gene and the x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). b Confirmation of H4-specific deacetylation by Hda1C. Heatmaps of H4 acetylation levels calculated as in a from two independent ChIP-seqs including S. pombe chromatin as spike-in controls. All genes are sorted by descending order of Rpb3 occupancy. The y axis indicates each gene and the x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). c Heatmaps of H4 acetylation patterns for five gene groups identified by K-mean clustering using ChIP-seq data from b. The number of genes and % in genome are indicated on the right. d Average plot of the data shown in c. The x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). The number of genes is indicated in the parenthesis. e Actively transcribed genes exhibit a marked increase in H4 acetylation upon deletion of HDA1. Average plot of histone acetylation patterns based on RNA Pol II occupancy (Rpb3). The "0–20%" group includes genes showing the lowest Rpb3 occupancy and the "80–100%" group includes genes with the highest level of Rpb3 binding. f Longer genes show a larger increase in H4 acetylation than shorter genes upon deletion of HDA1. The plot shows average histone acetylation patterns grouped by gene length.
Fig. 3 The Arb2 domain of Hda1 is required for its binding to chromatin. a Hda1 binds strongly to active genes. Crosslinked chromatin from untagged control (HDA1) or HDA1-myc cells grown in YP-Glucose (YPD) was precipitated with an anti-myc antibody. PCR analysis of the precipitated DNA was carried out on the promoters and coding regions of the indicated genes. A non-transcribed region near the telomere of chromosome VI was used as an internal control. The signals for anti-myc were quantitated and normalized to the input signal. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. *p < 0.05, **p < 0.01, and ***p < 0.001 (two-tailed unpaired Student’s t tests). b Hda2 and Hda3 partially affect Hda1 recruitment. ChIP assays using the indicated strains were performed as in a. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. *p < 0.01 (two-tailed unpaired Student’s t tests). c RNA removal partially reduces Hda1 occupancy. Crosslinked chromatin from cells expressing Hda1-myc was treated with or without RNaseA/T1 and precipitated with an anti-myc antibody. The PCR analysis was performed as in a. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. **p = 0.005 (two-tailed unpaired Student’s t tests). d RNA removal has no effect on Hda1 occupancy in hda2Δhda3Δ mutant. Crosslinked chromatin from the indicated strain was treated with or without RNaseA/T1 and precipitated with an anti-myc antibody. The PCR analysis was performed as in a. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. ***p < 0.001 (two-tailed unpaired Student’s t tests). e Loss of the Arb2 domain increases H4 acetylation. ChIP assay using the indicated strains was performed as in Fig. 1b. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. *p < 0.05 and **p < 0.01 (two-tailed unpaired Student’s t tests). Source data are provided as a Source Data file.

may occur in vivo. To examine whether RNA-binding of Hda1C contributes to its chromatin association, we performed ChIP experiments followed by RNaseA and T1 treatments to remove the RNA. Similar to the effect of hda2Δhda3Δ, removal of the RNA reduced the binding of Hda1 to YEF3 and PMA1 slightly (Fig. 3c). Importantly, this effect was not seen in hda2Δhda3Δ cells (Fig. 3d). These findings suggest that Hda2 and Hda3 contribute partially to Hda1C recruitment, possibly through their RNA-binding activity.

Co-transcriptional histone methylations at H3K4 or H3K36 act as binding sites for other HDACs, including Set3 HDAC and Rpd3S6–8. Loss of Set1 or Set2, which are responsible for methylating H3K4 or H3K36, respectively, had no strong effect on Hda1 occupancy at YEF3 and PMA1 (Supplementary Fig. 3c). In set1Δset2Δ cells, Hda1 binding was reduced only by ~10% suggesting that crosslinking of Hda1 to highly active genes is only partially dependent on co-transcriptional histone methylations at H3K4 and H3K36 (Supplementary Fig. 3c). Ctk1, which phosphorylates serine 2 of the C-terminal domain of Rpb1, was also not required for binding to YEF3 and PMA1 (Supplementary Fig. 3d and e).

Binding of Hda1 to hyperactive genes requires its Arb2 domain. The Hda1 protein has two distinct domains: a HDAC domain at its N-terminal domain and an Arb2 domain at its C terminus (Fig. 3e). The recombinant Arb2 domain is known to interact strongly with nucleosomes in vitro, even though it has no role in histone deacetylation by Hda1 in vitro11,12. Deletion of the Arb2 domain reduced Hda1 protein levels by ~13% in vivo (p = 0.002943) (n = 3) (Fig. 3f). Surprisingly, loss of this domain led to a dramatic decrease in binding of Hda1 to the active genes YEF3 and PMA1 (Fig. 3g). Consistently, the H4 acetylation pattern of the cells expressing Hda1 lacking the Arb2 domain was comparable to that of the hda1Δ cells (Fig. 3h). These results suggest that the Arb2 domain is important for Hda1C binding to highly active genes.

Next, we used quantitative ChIP-seq experiments using normalizing S. pombe spike-in controls to confirm Hda1C crosslinking to highly active genes and the role of the Arb2 domain in this process. Hda1 was highly enriched at actively transcribed genes and this binding was observed throughout all yeast chromosomes (Fig. 4a and Supplementary Fig. 4a). This occupancy was reduced markedly upon deletion of the Arb2 domain (Fig. 4a). The top 25% of genes with the highest level of RNA Pol II also showed the Arb2 domain-dependent association of Hda1 with coding regions (Fig. 4b). In addition, this binding showed a weak, but statistically significant correlation with increases of H4 acetylation in hda1Δ cells (Fig. 4c and Supplementary Fig. 4b). Furthermore, group 4 and 5 in Fig. 2d having the highest level of H4 acetylation upon HDA1 deletion showed relatively high levels of RNA Pol II (Supplementary Fig. 4c). For example, binding of Hda1 to two highly active genes, TEF1 and TEF2 was correlated with Rpb3 occupancy and this binding was greatly reduced upon deletion of the Arb2 domain (Fig. 4d). In addition, H4 acetylation, but not H3 acetylation, at these genes was increased in hda1Δ cells (Fig. 4d). In contrast, only H3 acetylation was increased at an inactive gene, TKL2 (Fig. 4d). Collectively, these data indicate that Hda1 binds directly to hyperactive genes, possibly via its Arb2 domain, to specifically deacetylate histone H4.

An obvious question is whether binding of Hda1 to highly active genes is transcription-dependent. To examine this possibility, the interaction between Hda1 and Rpb3, a subunit of RNA Pol II, was examined by co-immunoprecipitation assay. Paf1, a component of the PAF complex, interacted weakly with Rpb3 (Fig. 4e). However, a strong interaction between Hda1 and Rpb3 was observed (Fig. 4e). Importantly, this interaction was almost absent when the Arb2 domain is deleted (Fig. 4f). These results suggest that Hda1C could be recruited to highly transcribed regions via the interaction between the Arb2 domain of Hda1 and RNA Pol II (Fig. 4f).

To test this possibility, we determined the occupancies of Hda1-myc and RNA Pol II (Rpb3) at various positions of the GAL1 and GAL3 genes before and after glucose shut-off. Rpb3 strongly bound to the GAL genes in medium containing galactose where GAL genes are active. This binding was completely abolished at 4 min post transfer to glucose medium (Fig. 4g). Similarly, Hda1 binding to these genes was also high in galactose medium, but Hda1 rapidly dissociated following transfer to glucose medium, indicating that RNA Pol II transcription is critical for targeting of Hda1C to highly active genes (Fig. 4g). Consistent with this finding, a previous study also showed transcription-dependent binding of Hda1 to ARG1 gene16. Histone acetylation patterns were also examined to determine whether Hda1C binding is directly associated with histone deacetylation. During growth in galactose medium, H4
Acetylation was increased within the coding region but not the promoter of GAL3 in the absence of Hda1 (Fig. 1a and Supplementary Fig. 4d). However, this effect of Hda1 depletion was not seen at 4 min after glucose shut-off. Instead, H3 acetylation was increased at this time-point in hda1Δ cells (Supplementary Fig. 4d), suggesting a dynamic switch of the specificity of Hda1C from histone H4 to H3 depending on the transcription status. Overall, these findings indicate that transcription-dependent targeting of Hda1C via the interaction between the Arb2 domain of Hda1 and RNA Pol II facilitates histone H4-specific deacetylation at highly active genes.

Differential deacetylation of histone H4 by Hda1C and Set2. Co-transcriptional histone methylation at H3K36 by Set2 recruits and/or activates the Rpd3S HDAC to reduce histone acetylation in the 3′ regions of genes7,8. A previous study proposed that the Set2–Rpd3S pathway acts primarily on infrequently transcribed genes.

**Note:** The text includes various figures and graphs that illustrate the acetylation patterns and transcriptional activities of different genes and proteins. The figures are color-coded to differentiate between untreated and treated samples, and they show a clear trend in acetylation changes across different regions and time points. The graphs and plots are used to support the findings discussed in the text.
Fig. 4 Transcription-dependent binding of Hda1C to active coding regions. a Hda1 binds to hyperactive genes. Scatterplot of Hda1 occupancy from two independent ChIP-seqs including S. pombe chromatin as spike-in controls in untagged (gray), Hda1-myc wild-type (red), and Hda1-myc (ΔArb2) (blue) cells, plotted against Rp3β levels (relative Pol II). Pearson’s correlation coefficients are indicated. b Hda1 occupancies for the top 25% of highly transcribed genes (1705 genes) from two independent ChIP-seqs including S. pombe chromatin as spike-in control. The genes were sorted in descending order of Rp3β occupancy in wild-type cells. The y axis indicates each gene and the x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). c Histone acetylation patterns of the genes shown in b. d ChIP-seq tracks at active genes, TKL1, TEF1, and TEF2, and an inactive gene, TKL2 showing the signals for Hda1 occupancy, H4 acetylation levels, H3 acetylation levels, Rp3β occupancy, or histone H3 occupancy. e Hda1 interacts with Rp3β. Total extracts prepared from the indicated strains were incubated with an anti-Rpb3 antibody and protein G beads. The precipitates (IP) were analyzed by immunoblottting for TAP-tagged proteins (TAP) and Rp3β. Two independent experiments showed the same results. f The Arb2 domain is important for the interaction between Hda1 and Rp3β. Co-immunoprecipitation assay using the indicated strains was done as in e. Two independent experiments showed the same results. g Transcription-dependent Hda1C binding to GAL genes. The cells were grown in YP-Galactose (red) and then shifted to YP-Glucose for 4 min (blue). ChIP analyses of GAL3 and GAL1 were performed using anti-Rpb3 or anti-myc antibodies, as in Fig. 3a. Error bars show the standard deviation calculated from three biological replicates, each with three technical replicates. **p < 0.01 and ***p < 0.001 (two-tailed unpaired Student’s t tests). Source data are provided as a Source Data file.

long genes17. This function contrasts with that of Hda1C, which appears to preferentially deacetylate histone H4 within the coding regions of hyperactive genes (Fig. 2a). To confirm the distinct functions of Hda1C and the Set2–Rp3β pathway, we compared the H4 acetylation patterns of hda1Δ and set2Δ cells. As reported previously, deletion of SET2 mainly increased H4 acetylation within less active genes, whereas increased H4 acetylation in hda1Δ cells was correlated with transcription frequency (Fig. 5a, b). As H4 acetylation was increased at many genes in the hda1Δ and set2Δ strains, we examined whether Set2 and Hda1C functionally interact. First, we performed peptide pull-down experiments to monitor the interaction between Hda1C and trimethylated H3K36 (H3K36me3). NuA3 HAT is known to have at least two subunits, Nto1 and Pdp3, that directly recognize H3K36me318,19. Although a strong interaction between Nto1 and H3K36me3 was seen, Hda1C did not recognize H3K36me3 even in low salt conditions (Supplementary Fig. 5a and b). In addition, loss of Hda1 had no effect on H3K36me3 levels in the YEF3- and PMAl-coding regions (Supplementary Fig. 5c). H4 acetylation at the coding regions of two genes, STT4 and HAP1, was significantly increased in mutants for Hda1 and Set2. A double deletion strain lacking Hda1 and Set2 showed higher levels of H4 acetylation than the single deletion mutants (Fig. 5c). Taken together, these findings suggest that the Hda1C and the Set2–Rp3β pathway differentially control histone acetylation within coding regions.

To understand the distinct functions of Hda1C and Set2 further, genes displaying hyperacetylation of H4 within coding regions following HDA1 or SET2 deletion were divided into three groups: Hda1-specific, Set2-specific, and common genes. Approximately 68% of the genes displayed H4 hyperacetylation in both mutants (1863 of 2739 and 2734 genes in hda1Δ and set2Δ cells, respectively, corresponding to 68.0% and 68.1%, respectively; Fig. 5d–g, yellow). The proportions of genes that were Hda1-specific (876 of 2739 genes, 32.0%; Fig. 5d–g, green) and Set2-specific (871 of 2734 genes, 31.9%; Fig. 5d–g, red) were comparable. Surprisingly, the Set2-specific genes showed lower levels of H3K36me3 than the Hda1-specific genes (Fig. 5d, e), suggesting that Set2 may affect histone acetylation independently of H3K36 methylation or Rp3β at these genes. Gene Ontology analyses of the Hda1-specific, Set2-specific, and common genes revealed their distinct biological functions (Supplementary Fig. 5d). Whereas Hda1-specific genes were linked to cytoplasmic translation and biosynthetic processes, Set2-specific genes were enriched for adaptation of signaling pathways and base-excision repair (Supplementary Fig. 5d). Overall, these results indicate that Hda1C functions independently of the Set2–Rp3β pathway to deacetylate histone H4 at highly transcribed genes.

Hda1C may negatively affect elongation. Histone acetylation/deacetylation within transcribed regions affects RNA Pol II elongation3. The Set2–Rp3β pathway negatively regulates elongation. Cells that are defective in this pathway show resistance to 6-azauracil or mycophenolic acid and bypass the requirement for Bur1, a positive elongation factor8. In addition, the Set2–Rp3β pathway inhibits initiation from cryptic promoters and suppresses histone exchange7,20.

Interestingly, loss of Hda1C slightly suppressed the growth defect of bur1Δ (Fig. 5h). A previous study showed that overexpression of the histone demethylases Jhd1 and Rph1, which target H3K36 methylation, partially supports the growth of bur1Δ cells21. A synergistic effect on cell growth was seen when Jhd1 or Rph1 was overexpressed in hda1Δ cells, suggesting that Hda1C may inhibit RNA Pol II elongation via H4-sepcific deacetylation at highly transcribed genes (Supplementary Fig. 5e). Although the Set2–Rp3β pathway represses cryptic promoters within coding regions by deacetylating histones, deletion of HDA1 had no effect on cryptic initiation from the PCA1 and STE11 genes (Supplementary Fig. 5f and g). Unexpectedly, genes with H4 hyperacetylation in hda1Δ cells had relatively high histone occupancy (Supplementary Fig. 5h). Furthermore, longer genes also showed a slightly increased histone density within coding regions (Supplementary Fig. 5i). In contrast, reduced histone density was seen at 3′-transcribed regions in set2Δ cells (Supplementary Fig. 5h and i). Although how Hda1C maintains optimal histone occupancy remains elusive, the absence of cryptic initiation in mutants for Hda1C may be due to increased histone density. Taken together, these results suggest that Hda1C-mediated H4 deacetylation at highly active genes may negatively regulate RNA Pol II elongation.

H3 deacetylation by Hda1C delays gene induction. Although chromatin regulators have been thought to play important roles in global gene regulation, little or no obvious effect on global gene expression was seen in a steady-state condition22. Instead, they seem to function as modulators that regulate the kinetics of transcriptional responses to support cell adaptation and fitness upon environmental changes23,24. Hda1C and Tup1 contribute to gene repression and both proteins were required for H3 deacetylation at inactive genes in our ChIP-seq analyses (Fig. 2a). Previous studies showed that Hda1C had a minimal effect on global transcription under steady-state conditions but affected the kinetics of gene induction upon diamide stress22,23. To further understand the exact function of Hda1C, we monitored the transcriptional responses of wild-type and hda1Δ cells during carbon source shifts24,25. In this experiment, wild-type, hda1Δ, and hda3Δ cells were initially
grown in synthetic complete medium containing raffinose, and subsequently shifted to galactose for 120 min, glucose for 120 min, and then back to galactose for 30 min (Fig. 6a). The basal expression level of HXT5, a gene induced by galactose, was not affected by deletion of HDA1 or HDA3 in raffinose medium; however, a strong and rapid induction was seen in the Hda1C mutants during galactose incubation (Supplementary Fig. 6a).

To explore how Hda1C affects global gene expression dynamics, total RNAs from the wild-type and hda1Δ cells were analyzed by strand-specific RNA-sequencing. Our previous...
Fig. 5 Hda1C and Set2 differentially affect H4 acetylation within coding regions. a Heatmaps of H4 acetylation patterns in hda1Δ (6664 genes) and set2Δ (5648 genes) cells. The plots show the log2 fold changes in H4 acetylation in hda1Δ (from Fig. 2b) or set2Δ cells versus wild-type cells. All genes are sorted by descending order of Rpb3 occupancy. The y axis indicates each gene and the x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). Histone acetylation patterns of set2Δ were analyzed using the data set from Venkatesh et al.29. b H4 deacetylation by Hda1, but not Set2, correlates with RNA Pol II occupancy. Scatterplot showing the relationship between H4 acetylation changes in hda1Δ or set2Δ cells and Rpb3 occupancy. Pearson’s correlation coefficients are indicated. c Dual loss of Hda1 and Set2 increases H4 acetylation further. A ChIP assay using the indicated strains was performed as in Fig. 1b. *p < 0.05 and **p < 0.01 (two-tailed unpaired Student’s t-test). Source data are provided as a Source Data file. d Genes displaying H4 hyperacetylation in the hda1Δ or set2Δ cells were divided into three groups: Hda1-specific (green), Set2-specific (red), and common (yellow) genes. Heatmaps of H4 acetylation patterns are shown as in a. The y axis indicates each gene and the x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). e Heatmap of H3K36me3 patterns in the three groups shown in d. The y axis indicates each gene and the x axis indicates relative position to the transcription start site (TSS) and transcription end site (TES). f Heatmap of H4 acetylation patterns in the three groups shown in d. g Venn diagram of the three groups described in d. The significance of the overlap was calculated using Fisher’s exact test. h Loss of Hda1C bypasses the requirement of Bur1, a positive elongation factor. Bur1 plasmid shuffling strains harboring the indicated deletions were spotted in threefold dilutions onto a synthetic complete (SC)/Glu plate (shown after 2 days) or a SC/5-FOA plate (shown after 8 days). The set2Δ mutant was used as a positive control.

studies identified ~1000 genes that differentially respond to distinct carbon sources24,25. Hda1C-regulated genes were identified as those showing at least a 1.7-fold increase in transcript levels at one or more time-points during carbon source shifts. A total of 331 genes induced during galactose incubation were negatively regulated by Hda1C (Fig. 6b). The transcript levels of these genes were similar in hda1Δ and wild-type cells during incubation in raffinose medium, but deletion of HDA1 resulted in rapid and strong induction during galactose incubation (Fig. 6b). Notably, deletion of HDA1 had no strong effect on the induction of these genes when they were hyperactivated by the second galactose pulse (Fig. 6b).

Next, the histone acetylation patterns of the 331 Hda1C-regulated inducible genes were analyzed. Although Hda1C functionally interacts with Tup1, the histone acetylation patterns differed slightly in hda1Δ and tup1Δ cells. In hda1Δ cells, H3 acetylation, but not H4 acetylation, was increased at the promoter regions (Fig. 6c) and within gene bodies (Fig. 6d). However, deletion of TUP1 increased the acetylation of both H3 and H4 at promoter regions, suggesting that Tup1 might functionally interact with Rpd3 to promote H4-specific deacetylation at inactive promoters26 (Fig. 6c, d). These results indicate that, in addition to H4-specific deacetylation within hyperactive coding regions, Hda1C preferentially deacetylates H3 to delay the kinetics of gene induction upon environmental changes.

Discussion

Histone acetylation and deacetylation within coding regions play important roles in RNA Pol II elongation27. The Set2–Rpd3S HDAC pathway slows elongation and inhibits transcription initiation from cryptic promoters7,8. In addition, H3K36 methylation by Set2 inhibits the incorporation of acetylated histones to suppress histone exchange within coding regions28. The Set3 HDAC also represses transcription from internal promoters in 5’-transcribed regions6. The results presented here indicate that Hda1C specifically deacetylates histone H4, but not H3, within hyperactive genes to partially inhibit elongation. In addition, Hda1C preferentially deacetylates histone H3 at inactive genes to delay the kinetics of gene induction upon environmental changes (Fig. 7).

Although Hda1C binds to highly active genes, it lacks domains recognizing co-transcriptional histone modifications, including H3K36me3 and H3K4me2, enriched in transcribed regions. Instead, the Arb2 domain of Hda1 and the N-terminal regions of Hda2 and Hda3 contribute to targeting of the complex to chromatin. Recruitment of Hda1C to hyperactive genes is likely initiated by RNA Pol II transcription. Hda1C interacts directly with Rpb3 via the Arb2 domain of Hda1 and its crosslinking is highly correlated with transcription frequency (Fig. 4a, b). Furthermore, our ChIP data indicated that Hda1C travels with RNA Pol II at GAL1 genes (Fig. 4g). Therefore, we propose that RNA Pol II initially recruits Hda1C to highly transcribed genes, and then multiple interactions including Arb2-RNA Pol II, Arb2-histones, and DNA/RNA-Hda2 and -Hda3 stabilize Hda1C binding to highly active genes. Notably, some mammalian HDACs also bind active genes29. In particular, HDAC6 is highly enriched at active coding regions via an interaction with the phosphorylated C-terminal domain of RNA Pol II, although its function in histone deacetylation remains unknown.

Although Hda1C and the Set2–Rpd3S pathway play important roles in histone deacetylation, they differentially regulate H4 acetylation within coding regions (Fig. 7). Whereas Hda1C is more active at highly transcribed genes, the Set2–Rpd3S pathway preferentially acts on infrequently transcribed genes (Fig. 5a, b). Furthermore, mutants for the Set2–Rpd3S pathway, but not Hda1C mutant cells, showed reduced H4 acetylation at transcription start sites (Fig. 5d, f). These findings suggest independent roles of these HDACs in histone deacetylation. Genes transcribing at intermediate levels are deacetylated by both Hda1C or the Set2–Rpd3S pathway, but these HDACs may act at different times. It is possible that Hda1C deacetylates histone H4 at currently transcribing genes via an interaction with RNA Pol II, whereas the Set2–Rpd3S pathway deacetylates histone H4 at the same genes when they are not currently transcribing by recognizing H3K36me3. Hda1C may deacetylate histone H4 immediately after RNA Pol II passes, but acetylated histone H4 incorporated during nucleosome reassembly may be deacetylated by the Set2–Rpd3S pathway (Fig. 7).

Loss of the Set2–Rpd3S pathway leads to an accumulation of cryptic and antisense transcripts from internal promoters within coding regions7,17,30. As expected, the STE11 and PCA1 genes produced cryptic transcripts in set2Δ cells (Supplementary Fig. 5f and g). Some Set2-repressed cryptic transcripts, including PCA1, are transcriptionally induced upon environmental changes25. Although deletion of HDA1 slightly increased H4 acetylation at PCA1 but not at STE11, cryptic transcripts were not observed, suggesting that elevated H4 acetylation itself is not sufficient to activate cryptic promoters (Supplementary Fig. 5f and g). Unexpectedly, loss of Hda1 results in a slight increase in histone occupancy. Although how Hda1C reduces nucleosome occupancy remains unclear, a slight increase of histone levels may block initiation from cryptic promoter in HDA1 deleting cells (Supplementary Fig. 5h and i). Cells defective in the Set2–Rpd3S pathway bypassed the requirement for Bur1, a positive elongation factor5 (Fig. 5h). The growth defect of bur1Δ cells was also
suppressed by overexpression of the histone demethylases Jhd1 and Rph1, which demethylate H3K36 methylation\(^2\). Notably, deletion of HDA1 also partially supported the growth of bur1Δ cells and a synergistic effect was seen when Jhd1 or Rph1 was overexpressed (Fig. 5h and Supplementary Fig. 5e). Taken together, these findings suggest that Hda1C-mediated deacetylation of histone H4 within coding regions may inhibit RNA Pol II elongation. Although loss of Hda1 resulted in a significant increase in histone acetylation throughout the yeast genome, there was little effect on global transcript levels under a steady-state condition\(^2\).

Recent studies have suggested that chromatin regulators may fine-tune the kinetics of transcriptional responses upon environmental changes\(^3\). Here, we identified 331 genes that were negatively regulated by Hda1C. Whereas basal transcript levels of these genes were not strongly affected by deletion of HDA1, their induction by switching to galactose medium was stronger and more rapid in hda1Δ cells than in wild-type cells (Fig. 6b). Loss of Hda1 increased H3 acetylation but not H4 acetylation at these genes, suggesting that Hda1C-mediated deacetylation of H3 at inactive genes delays gene induction (Fig. 6c, d).

**Fig. 6** H3 deacetylation by Hda1C fine-tunes the kinetics of gene induction. a Schematic representation of the time-course experiments to monitor gene expression changes in wild-type and hda1Δ cells during carbon source shifts. Ra, raffinose; Gal, galactose; Glu, glucose. RNA samples were collected at the indicated time-points. b Hda1C negatively regulates the kinetics of gene induction. RNA samples from the time-course experiments described in a were analyzed by strand-specific RNA-sequencing. The ratios of transcript levels for 331 genes in hda1Δ versus wild-type cells are shown. Hda1-repressed genes were identified as those showing at least a 1.7-fold increase in transcript levels at one or more time-points. c, d Hda1C preferentially deacetylates histone H3 at inducible genes. Average profiles of H3 and H4 acetylation changes upon deletion of HDA1 or TUP1 around the transcription start sites (TSs; –200 bp to +200 bp) (c) and within gene bodies (d) of the Hda1-repressed genes. Significance levels were computed by permutation tests. **p < 0.01 and ****p < 1.0 × 10\(^{-10}\).
In addition, these two proteins are phosphorylated by unknown kinases. Determining whether these modifications affect the specificity of Hda1C will be important to understand the exact function of Hda1C in histone deacetylation and transcriptional regulation.

Whereas Hda1C mainly reduces H3 acetylation at transcriptionally inactive genes, histone H4 is specifically deacetylated by this complex at highly transcribed genes (Fig. 7). Post-translational modifications of Hda1C might affect its specificity. Hda1 and Hda3 are highly sumoylated upon KCl or sorbitol stress. In addition, these two proteins are phosphorylated by unknown kinases. Determining whether these modifications affect the specificity of Hda1C will be important to understand the exact function of Hda1C in histone deacetylation and transcription regulation.

**Methods**

**Yeast strains.** Yeast strains used in this study are listed in Supplementary Table 1. The time-course experiments were done with matched HDA1 and hda1Δ strains as previously described. To generate the mutant lacking the Arb2 domain in Fig. 3, the delitto perfetto strategy was used. The HDA1-myc strain was generated by inserting the 18-myc tag into C-terminus of HDA1. Except for Figs. 1a, 4g and Supplementary Fig. 4d, all strains were grown in YPD medium at 30°C to mid-log phase. Cells were lysed using lysis buffer (50 mM Tris-HCl (pH 7.5), 0.1% NP-40) containing 150 mM, 200 mM, or 300 mM NaCl and protease inhibitors. In all, 1 mg of total extracts was incubated with 1 μg of biotinylated histone peptides (Anaspec) and 25 μl of streptavidin coupled Dynabeads (Invitrogen) at 4°C overnight. Beads were washed five times with 1.5 ml of binding buffer and precipitates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblot analysis.

**Northern blot analysis.** Total RNA was isolated from cells with hot phenol and 10 μg of total RNA was separated on an agarose gel and then transferred to nylon membrane. Northern blot analysis was done as previously described. The sequences of oligonucleotides used for northern blot analysis are listed in Supplementary Table 2. Strand-specific probes were generated by unidirectional PCR in the presence of [α-32P]dATP with only one primer. Hybridization was done in a buffer containing 1% BSA, 7% SDS, 1 mM EDTA (pH 8.0), and 300 mM sodium phosphate buffer (pH 7.2). The membranes were washed with 2× SSC and 0.1% SDS for 20 min and exposed to PhosphoImager. Uncropped Northern-blots can be found in Supplementary Fig. 9.

**Spot assay.** For spotting analysis, cells were resuspended at 2.5 × 10^8/ml and subjected to threefold serial dilutions in synthetic complete (SC) media lacking any carbon sources, and 3 μl of aliquot of each dilution was spotted on the indicated plates. Growth was assayed at 2 days (SC/Glu as a positive control) or 8 days (SC/5-FOA to select against the BURI/URA3 plasmid).

**Western blot analysis.** Cells expressing TAP-tagged or myc-tagged proteins were grown in YPD at 30°C to mid-log phase. Cells were lysed using lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% NP-40) with protease inhibitors (Pepstatin A 1 μg/ml, Aprotinin 0.3 μg/ml, Leupeptin 1 μg/ml, phenylmethylsulfonyl fluoride 1 μg/ml) and subjected to SDS-PAGE followed by immunoblot analysis.

The sequences of oligonucleotides used in this study are listed in Supplementary Table 2.

**Peptide pull-down assay.** Whole cell extracts were prepared with binding buffer (50 mM Tris-HCl (pH 7.5), 0.1% NP-40) containing 150 mM, 200 mM, or 300 mM NaCl and protease inhibitors. In all, 1 mg of total extracts was incubated with 1 μg of biotinylated histone peptides (Anaspec) and 25 μl of streptavidin coupled Dynabeads (Invitrogen) at 4°C overnight. Beads were washed five times with 1.5 ml of binding buffer and precipitates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblot analysis.

**RT-PCR.** RNA was extracted from cells with hot phenol. Total RNAs was treated with DNase I (Thermo Fisher Scientific) and first-strand cDNA was prepared with 1 μg total RNA, ReverTra Ace qPCR RT kit (TOYOBO), and gene-specific primers. cDNA was analyzed by real-time qPCR using SYBR Green Supermix and CFX96 cycler (Bio-Rad).

**Pfu Turbo DNA polymerase.** High-fidelity DNA polymerase was obtained from Agilent Technologies (1 unit). Reactions were done in a 25 μl volume with 2 μl of diluted cDNA and gene-specific primers.
**Co-immunoprecipitation assay.** Whole cell extracts were prepared with binding buffer (50 mM Tris-HCl [pH 7.5], 0.1% NP-40) containing 150 mM NaCl and protease inhibitors (1 μM aprotinin, 1 μM leupeptin, 1 μM pepstatin, 1 μM pepsin, 1 μM n-p-nitrophenylsulfonyl fluoride). In all, 1.5 mg of total extracts was incubated with 25 μl of protein G sepharose (GE Healthcare) and with or without 4 μl of a-Rpb3 antibody (BioLegend) at 4 °C for 4 h. Beads were washed five times with 1.5 ml of binding buffer and precipitates were resolved by SDS-PAGE followed by immuno blot analysis.

**Chromatin immunoprecipitation and ChIP-sequencing.** Cells fixed with 1% formaldehyde were subjected to ChIP as previously described with oligonucleotides as listed in Supplementary Table 2. The following histone antibodies were used: anti-H3K36me3 (Abcam ab9059), anti-acetyl H4 (Millipore 06–598), anti-acetyl H3 (Millipore 06–599), anti-myc (BioLegend 626802) and anti-H3 (Abcam ab1791). Except for H3 acetylation, all ChIP-seqs included S. pombe “spike-in” control (H3) in each experiment. The blots were visualized on a Bio-Rad ChemiDoc MP system. The ChIP and RNA samples were prepared using Accel-NGS 2S Plus DNA Library Kit (Swift Biosciences) and sequenced on the HiSeq2500 platform (Illumina) following the manufacturer’s instructions.

**RNA preparation and sequencing.** Total RNA was isolated by hot-phenol method. The mRNA libraries were prepared using NEBNext Rapid Directional mRNA-Seq Kit (Bio Scientific) and sequenced on the HiSeq2500 sequencer (Illumina). The sequencing was performed on the S. cerevisiae transcriptome using HISAT2–2.0.41. The sequence reads were counted by StringTie–1.3.0.44 and fragments per kilobase of transcript per million mapped reads (FPKM) was calculated as a normalized expression level. The downstream analysis such as drawing heatmap and violin plot, and permutation test, was done by using R packages (ver. 3.4.1).

**Data availability**

The ChIP-seq and RNA-seq data sets that support the findings of this study have been deposited in the Gene Expression Omnibus with the accession code GSE121763 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121763). All other relevant data supporting the key findings of this study are available within the article and its Supplementary Information files or from the corresponding author upon reasonable request. The data underlying Figs. 1, 3a-d, 3g, 3h, 4g, and 5c, and Supplementary Figs. 1, 3b-d, 4d, 5c, and 6a are provided as a Source Data file. A reporting summary for this Article is available as a Supplementary Information file.

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**Author contributions**

S.D.H., T.-Y. R., and T.K. designed the project; S.D.H. and M.Y.K. performed most of the experiments; S.D.H., I.J., and S.H. analyzed the data; J.H.K., B.R.L., I.J., M.K.L., and J.-T. H. contributed to the data analyses; S.D.H., S.H., T.-Y.R., and T.K. wrote the manuscript. All authors discussed the results and commented on the manuscript.

**Additional information**

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