

Opposing functions of the Hda1 complex and histone H2B mono-ubiquitylation in regulating cryptic transcription in *Saccharomyces cerevisiae*

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Abstract

Maintenance of chromatin structure under the disruptive force of transcription requires cooperation among numerous regulatory factors. Histone post-translational modifications can regulate nucleosome stability and influence the disassembly and reassembly of nucleosomes during transcription elongation. The Paf1 transcription elongation complex, Paf1C, is required for several transcription-coupled histone modifications, including the mono-ubiquitylation of H2B. In *Saccharomyces cerevisiae*, amino acid substitutions in the Rtf1 subunit of Paf1C greatly diminish H2B ubiquitylation and cause transcription to initiate at a cryptic promoter within the coding region of the *FLO8* gene, an indicator of chromatin disruption. In a genetic screen to identify factors that functionally interact with Paf1C, we identified mutations in *HDA3*, a gene encoding a subunit of the Hda1C histone deacetylase (HDAC), as suppressors of an *rtf1* mutation. Absence of Hda1C also suppresses the cryptic initiation phenotype of other mutants defective in H2B ubiquitylation. The genetic interactions between Hda1C and the H2B ubiquitylation pathway appear specific: loss of Hda1C does not suppress the cryptic initiation phenotypes of other chromatin mutants and absence of other HDACs does not suppress the absence of H2B ubiquitylation. Providing further support for an appropriate balance of histone acetylation in regulating cryptic initiation, absence of the Sas3 histone acetyltransferase elevates cryptic initiation in *rtf1* mutants. Our data suggest that the H2B ubiquitylation pathway and Hda1C coordinately regulate chromatin structure during transcription elongation and point to a potential role for a HDAC in supporting chromatin accessibility.

Keywords: Hda1 histone deacetylase complex; H2B K123 ubiquitylation; Rtf1; cryptic transcription; Paf1 complex

Introduction

Transcription of eukaryotic genes requires passage of RNA polymerase through a generally repressive chromatin template. Nucleosomes, which consist of approximately 147 base pairs of DNA wrapped around an octamer of histones H2A, H2B, H3 and H4, present a barrier to RNA polymerase II (Pol II) progression (Farnung *et al.* 2018; Kujirai *et al.* 2018; Chen *et al.* 2019). Organisms have evolved numerous mechanisms to overcome this barrier. Variants of histones can replace their canonical counterparts at certain locations in the genome, such as the presence of H2A.Z in exchange for H2A in the nucleosome downstream of the transcription start site (TSS) (Bagchi *et al.* 2020). Chromatin remodeling factors, such as those in the SWI/SNF, ISWI, INO80 and CHD families, alter the positioning of nucleosomes to regulate DNA accessibility (Tyagi *et al.* 2016), and the histone chaperones FACT and Spt6 facilitate the reassembly of nucleosomes following Pol II passage (Gurard-Levin *et al.* 2014; Hammond *et al.* 2017; Formosa and Winston 2021). The post-translational modification of histones, though, may represent the most diversified means by which nucleosomes can regulate Pol II transcription.

With high selectivity, histone modifying enzymes carry out the covalent modification (*e.g.* phosphorylation, acetylation, methylation or ubiquitylation) of amino acids within the N- and C-terminal tails or core domains of the histones (Bannister and Kouzarides 2011; Lawrence *et al.* 2016). At the genomic level, these modifications are deposited in specific patterns and, depending on their functions, are enriched in transcribed or non-transcribed regions. Enzymes that modify histones are hypothesized to generate a “histone code” that is interpreted by effector proteins, which bind with high specificity to appropriately modified histones (Jenuwein and Allis 2001). For example, acetylation of lysines by histone acetyltransferases (HATs) (Lee and Workman 2007) loosens the interactions of histone tails with the DNA backbone (Hong *et al.* 1993), providing increased DNA accessibility to the transcription machinery and modulating transcription. In addition, acetylated histones can serve as binding sites for other regulatory factors, such as chromatin remodeling factors (Musselman *et al.* 2012; Marmorstein and Zhou 2014). Histone deacetylases (HDACs) remove the acetyl groups, thus reversing these effects (Seto and Yoshida 2014; Porter and Christianson 2019; Park and Kim 2020).

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Crosstalk among histone modifications allows the integration of multiple signals to control transcription. A well-studied example is the dependency of H3 K4 and H3 K79 di- and trimethylation (me2 and me3) on the mono-ubiquitylation of a conserved lysine in H2B (K123 in *Saccharomyces cerevisiae*; K120 in *H. sapiens*) (Worden and Wolberger 2019). H2B K123 mono-ubiquitylation (H2Bub) is catalyzed by the ubiquitin conjugating enzyme Rad6 and the ubiquitin protein-ligase Bre1 (Robzyk et al. 2000; Hwang et al. 2003; Wood et al. 2003a; Fuchs and Oren 2014) and is enriched on the bodies of actively transcribed genes (Minsky et al. 2008; Batta et al. 2011; Van Oss et al. 2016). The Set1 and Dot1 histone methyltransferases that modify H3 K4 and H3 K79, respectively, require the ubiquitin moiety on H2B for activity (Briggs et al. 2002; Dover et al. 2002; Ng et al. 2002; Sun and Allis 2002; Hsu et al. 2019; Worden and Wolberger 2019; Worden et al. 2020). In turn, H3 K4 methylation as well as H3 K36me3 recruit the NuA3 HAT to the genome through its Yng1 and Pdp3 subunits, respectively, thereby controlling the location of histone acetylation by the Sas3 subunit (Taverna et al. 2006; Martin et al. 2017). In other cases, one histone modification may regulate the removal of another modification. For example, H3 K36me2/3 by Set2 activates the Rpd3S deacetylase complex to remove acetyl groups from histones and restore a repressive chromatin structure that prevents initiation of Pol II transcription within protein coding regions (Carrozza et al. 2005; Joshi and Struhl 2005; Keogh et al. 2005; Drouin et al. 2010; Govind et al. 2010; Ruan et al. 2015; Venkatesh and Workman 2015). Thus, the enzymes that modify histones coordinately control chromatin structure and collectively impose or remove barriers to transcription.

In addition to the histone modifiers themselves, transcription factors that associate with Pol II can significantly influence the epigenetic state of chromatin. Previous work on the *S. cerevisiae* Polymerase-Associated Factor 1 Complex (Paf1C), which is composed of Paf1, Ctr9, Cdc73, Rtf1 and Leo1, has demonstrated a key role for this transcription elongation complex in regulating conserved transcription-coupled histone modifications. Paf1C has not been shown to possess enzymatic activity; however, mutations in genes encoding Paf1C subunits greatly diminish the levels of H2Bub, H3 K4me2/3, H3 K79me2/3 and H3 K36me3 (Krogan et al. 2003; Ng et al. 2003a,b; Wood et al. 2003b; Larabee et al. 2005; Chu et al. 2007; Piro et al. 2012; Van Oss et al. 2016, 2017). We previously identified a small region within the Rtf1 subunit of Paf1C, termed the histone modification domain (HMD), which appears to function as a critical cofactor in the deposition of H2Bub. The HMD is necessary and sufficient to promote H2Bub *in vivo*, stimulates H2Bub *in vitro*, and binds directly to Rad6 (Piro et al. 2012; Van Oss et al. 2016). Mutations that disrupt the Rad6-HMD interaction severely reduce levels of H2Bub, H3 K4me2/3 and H3 K79me2/3 and cause phenotypes indicative of disrupted chromatin structure (Warner et al. 2007; Tomson et al. 2011; Van Oss et al. 2016). For example, certain amino acid substitutions within the HMD lead to transcription initiation at a cryptic TSS within the coding region of a Pol II transcribed gene (Tomson et al. 2011; Van Oss et al. 2016), a mutant phenotype that arises when the repressive chromatin state of transcribed regions is disrupted by the loss of histone chaperones, histone modifiers and chromatin remodeling factors (Kaplan et al. 2003; Cheung et al. 2008; Silva et al. 2012). In this study, we exploited the cryptic transcription initiation phenotype of HMD mutants and performed a genetic screen to investigate the functional interactions involving this domain and the histone modification it facilitates, H2Bub. Our results uncovered an interplay between the factors that establish H2Bub during transcription elongation and the Hda1 deacetylase

complex (Hda1C) and suggest that Hda1C, directly or indirectly, impacts chromatin accessibility within gene bodies.

Materials and methods

Yeast strains and growth conditions

Saccharomyces cerevisiae strains were grown at 30°C in YPD or SC media (Rose et al. 1990). The SC-His+Gal medium used to monitor the cryptic initiation phenotype at the GAL1p-FLO8-HIS3 reporter contained 2% galactose. To assess defects in telomeric silencing, SC media contained 0.1% 5-fluoroorotic acid (5-FOA; USBiological, F5050). *Saccharomyces cerevisiae* strains used in this study are listed in Supplementary Table S1. KY strains are isogenic with FY2, a GAL2⁺ derivative of S288C (Winston et al. 1995). KA strains were derived from crosses originating with strains from a library of histone H3 mutants (Dai et al. 2008). Strains were made by standard methods for genetic crosses or gene replacements (Rose et al. 1990; Vidal et al. 1991). Strains containing a mutation in RTF1 were identified during tetrad analysis by the presence of an HA-tag sequence, using PCR amplification. Strains containing the *htb1-K123R* allele were identified by restriction enzyme digestion as previously described (Tomson et al. 2011). Strains containing the *hda1-H206A* replacement were created by *delitto perfetto* (Storici and Resnick 2006). A portion of the HDA1 gene (+1538 to +1868) was replaced with pCORE using homologous recombination in KY3880 and selecting for Ura⁺, G418^R colonies. The pCORE segment was replaced with a PCR-amplified DNA fragment containing *hda1-H206A* by selecting for 5FOA^R colonies and screening for G418^S. The *hda1-H206A* mutation was created by PCR mutagenesis of a plasmid containing HDA1. Strains containing the phenotypic reporters were derived from genetic crosses.

For phenotypes assessed by replica plating, strains were first purified on YPD. For serial dilution analyses in Figures 2A and 3, strains were grown overnight in liquid YPD, diluted 1:10 and five-fold serial dilutions were pinned (Sigma-Aldrich R2383) to SC Complete, 5FOA, SC-His+Gal or SC-His media. For serial dilution analysis in Figure 7, strains were grown overnight in liquid YPD, diluted to OD₆₀₀ = 0.8, and 3 μl of five-fold serial dilutions were spotted onto SC-His+Gal or SC Complete media.

Identification of suppressors of an *rtf1-108-110A* mutation

With the original goal of identifying high-copy-number suppressors of the *rtf1-108-110A* mutation, KY1232 was transformed with a LEU2-marked high-copy-number plasmid library and replica-plated to SC-His-Leu+Gal and SC-Leu + 5FOA media to monitor suppression of the cryptic initiation and telomeric silencing phenotypes, respectively. Using a strategy previously described (Thompson et al. 1993), the pRS425-based (Christianson et al. 1992) library was made in our laboratory from genomic DNA prepared from an *rtf1Δ* strain (KY957) to avoid recovering RTF1 plasmids that complemented the *rtf1-108-110A* mutation. Two 5FOA resistant (5FOA^R), His⁻ strains were isolated and, surprisingly, retained the suppression phenotypes even after plasmid loss through growth in nonselective conditions, indicating that they harbored chromosomal suppressors of *rtf1-108-110A*. The two strains, following plasmid loss, were saved as KY1410 and KY1411 and studied further. Diploid yeast from genetic matings with *rtf1-108-110A* strains and KY1410 and KY1411 showed that the two suppressor mutations were recessive, as the diploids grew on SC-His+Gal media. Additional crosses between KY1410 and KY1411 and with a strain containing RTF1, KY1228, showed

that the suppression phenotype of each strain was due to a mutation in a single gene and that the suppressor mutation in each strain was unlinked to the *rtf1-108-110A* mutation. Haploid derivatives from these crosses were used in genetic matings, which showed that the two suppressor mutations defined a single complementation group. The suppressor mutations in KY1410 and KY1411, named *sup2-22* and *sup2-23*, respectively, behaved similarly in all phenotypic tests.

Several attempts to identify the *sup2-22* suppressor mutation by complementation of the cryptic initiation phenotype using plasmid-based yeast genomic DNA libraries were unsuccessful. Two genes that reversed the cryptic initiation phenotype of the *rtf1-108-110A sup2-22* strain were *HSF1* and *ASF1*. However, further genetic analysis showed that the suppressor mutation was not in these genes, but rather expression of these genes from a plasmid was likely affecting expression from the cryptic initiation reporter. Specifically, in the *FLO8* gene, upstream of where the *HIS3* reporter is inserted, we noticed a potential Hsf1 binding site 80 bp upstream of the predicted TATA box for the cryptic transcript, suggesting that overexpression of Hsf1 may drive higher expression of the *HIS3* reporter. Further, *RTF1* strains that were transformed with an *Asf1*-expressing plasmid grew on the SC-His+Gal media, suggesting that *Asf1* was bypassing the effect of the suppressor. This is consistent with the role of *Asf1* in promoting histone exchange and increasing histone acetylation on gene bodies (Hammond et al. 2017; Zhang et al. 2018). Therefore, we performed bulk segregant analysis (Birkeland et al. 2010) followed by whole-genome sequencing to identify the *sup2-22* suppressor.

Six tetrads were selected from a cross between KY1232 and KY1498, and genomic DNA was prepared (Gopalakrishnan and Winston 2019) from the two pools of twelve haploid progeny either exhibiting a *sup2-22* phenotype or not exhibiting a *sup2-22* phenotype. Sequences were obtained from libraries prepared using an NEB Ultra II Library Prep Kit (New England Biolabs # E7103), multiplexed and run on an Illumina MiSeq with a 150-cycle v3 Reagent Kit yielding approximately 7.6 million reads per sample. Raw reads were mapped to the *S. cerevisiae* genome (S288C version = R64-2-1) (Cherry et al. 2012; Engel et al. 2014) using Bowtie2 (Langmead and Salzberg 2012). SAMtools were used to calculate genotype likelihoods before calling sequence variants using BCFtools (Li et al. 2009; Li 2011). Using VCFtools (Danecek et al. 2011), VCF files for mutant and wild-type segregants were compared and only the mutations found in the suppressor pool were selected from the output text (Ellison et al. 2020). Variants with a quality score > 50 and found within coding regions were retained, leaving three variants. Two of the variants were synonymous (*Mtl1* S257S and *Flo5* V354V), and a third variant, which created a frameshift (GT to C at +1118), mapped within the *HDA3* gene. Sequence variants of interest were further inspected by visualizing the data in the Integrative Genomics Viewer (IGV) from the Broad Institute (Thorvaldsdottir et al. 2013). The mutation at codon 373 of *HDA3* is predicted to change seven amino acids in the protein product before a stop codon is encountered. Subsequent direct sequencing of the *HDA3* gene in a *sup2-23* strain revealed a C to T substitution at nucleotide 592 of the open reading frame, which replaces codon 198 with a stop codon.

Western blot analysis

Western blots to visualize Hda1 and H2Bub were performed using whole-cell extracts prepared using SUTEB buffer as described (Van Oss et al. 2016). H3 methylation was examined using whole-cell extracts prepared using a TCA extraction method (Van Oss et al. 2016). Proteins were run on 15% SDS-polyacrylamide gels.

The following antibodies were used: α -Hda1 (Santa Cruz sc-393814; 1:200), α -H2B (Active Motif #39237; 1:3000), α -H2Bub (Cell Signaling #5546; 1:1000), α -G6PDH (Sigma #A9521; 1:20000), α -H3K4Me2 (Millipore #07-030; 1:2000), α -H3K4Me3 (Active Motif #39159; 1:2000), α -H3K79Me2/3 (Abcam #ab2621; 1:1000; note that this antibody recognizes both di- and trimethylated H3K79) and α -H3 antibody (Tomson et al. 2011). Blots were developed using Thermo Scientific West Pico Plus (34580) and images were collected on a BioRad ChemiDoc™ XRS+.

Northern hybridization analysis

Cells were grown at 30°C to OD₆₀₀ = 0.6 in YP media with 2% galactose. Isolation of RNA and northern analyses were performed as described (Swanson et al. 1991; Shirra and Arndt 1999), except that a 1.5% MOPS-formaldehyde-agarose gel was used. A region from the *FLO8* gene (+40 to +671) or the *SCR1* gene (−242 to +283) was amplified by PCR and the hybridization probe was made by random priming in the presence of [α -³²P-dATP] (Perkin-Elmer BLU512H) using the Klenow fragment (New England Biolabs M0210). Quantification was done using a .tif file generated by phosphorimaging (Amersham Typhoon) and analyzed by Image Lab 6.1 software (Bio-Rad). The *SCR1* signal was used for normalization.

Chromatin immunoprecipitation (ChIP) analysis

Cells were grown to 30°C to OD₆₀₀ = 0.6 in YP media with 2% galactose. With the exception of the sonication step, extracts were prepared as previously described (Shirra et al. 2005). Sonicated chromatin was prepared using a Bioruptor (Diagenode B01060010) with 25 cycles of 30s on and 30s off. Immunoprecipitations were performed using 350 μ l of extract and 1 μ l of 8WG16 antibody (Biolegend 664912). After overnight incubations with primary antibody, 15 μ l of Protein A beads (Cytiva 17528001) were added and incubation proceeded at room temperature for 2 h. Beads were washed and the DNA was obtained by reversing the crosslinking, as previously described (Shirra et al. 2005). DNA was purified using the QIAquick PCR Purification Kit (Qiagen #28106). Samples were analyzed by qPCR using a QuantStudio3™ Real-Time PCR System (Thermo Fisher) with SyGreen Blue Mix Lo-ROX (Genesee 17-505B). Input and immunoprecipitated DNA was amplified from the 5' region of the *FLO8* gene (+23 to +126; primer efficiency = 1.98) and normalized to DNA amplified near *TEL06R* (chromosomal coordinates, 269495 to 269598).

Data reproducibility

All growth phenotypes were observed at expected frequencies in genetic crosses. Dilution growth experiments were performed in biological triplicate. Western blots, northern analyses and ChIP experiments were performed in biological duplicate with each biological replicate analyzed twice at minimum. For ChIP experiments, the biological replicates were subjected to immunoprecipitation two independent times. Results shown in Figure 4 were also observed with *sup2-22* and *hda3 Δ* strains.

Results

Loss of Hda1C suppresses three different transcription-associated phenotypes of *rtf1* HMD mutants

In *S. cerevisiae*, the *Rtf1* HMD is required for H2Bub through its direct interaction with Rad6 (Van Oss et al. 2016). To identify factors that functionally interact with the HMD, we conducted a genetic

screen for suppressors of a previously characterized mutation, *rtf1-108-110A*, which replaces three consecutive residues within the HMD with alanine and greatly decreases global H2Bub levels *in vivo* (Tomson et al. 2011). Strains containing the *rtf1-108-110A* mutation exhibit transcription initiation from a cryptic TSS within the *FLO8* open reading frame (Tomson et al. 2011), as assayed by a *GAL1p-FLO8-HIS3* reporter construct (Cheung et al. 2008). *RTF1* strains carrying this reporter are His⁻ because transcription initiation at the canonical *FLO8* TSS gives rise to an mRNA in which the *HIS3* coding sequence is out of frame with respect to *FLO8*. However, in mutants defective in maintaining proper chromatin architecture, such as the *rtf1-108-110A* mutant, transcription initiation occurs at a cryptic TSS within the *FLO8* gene, creating a transcript from which *HIS3* is expressed (Figure 1A). We exploited this phenotype to isolate genetic suppressors of *rtf1-108-110A* with the initial intent of identifying genes that, when overexpressed from a plasmid, would suppress the His⁺ phenotype of *rtf1-108-110A* mutants containing the *GAL1p-FLO8-HIS3* reporter. Surprisingly, we identified two strains, with mutations in the same complementation group, in which suppression was not dependent on the presence of a plasmid (see Materials and Methods for details). These chromosomal mutations behaved similarly in all phenotypic tests, and we chose the *sup2-22* mutation for further analysis. As shown in Figure 1B, the *sup2-22* mutation suppressed the cryptic initiation phenotype caused by the *rtf1-108-110A* mutation and two other *rtf1* HMD loss-of-function mutations, *rtf1-102-104A* and *rtf1-E104K* (Tomson et al. 2011) (Figure 1B, compare sections 1 and 2 in the *rtf1* strains). The *sup2-22* mutation did not suppress the cryptic initiation phenotype of an *rtf1Δ* strain, indicating that suppression is allele-specific.

Bulk segregant analysis followed by whole-genome sequencing identified *sup2-22* as a frameshift mutation in the *HDA3* gene, which encodes a non-catalytic subunit of the Hda1C HDAC (Lee and Kim 2020). This mutation is predicted to eliminate 283 (out of 655) amino acids from the C-terminus of Hda3. The second, independently derived suppressor mutation identified in our screen, *sup2-23*, is a nonsense mutation at codon 198 of *HDA3*. Structural studies indicate that the regions deleted by the *sup2-22* and *sup2-23* mutations encompass a coiled-coil segment necessary for dimeric interactions between Hda2 and Hda3, which are required for the overall integrity and function of Hda1C (Wu et al. 2001a; Lee et al. 2009, 2021). We asked whether suppression of the *rtf1* mutations was specific to our original *hda3* mutations or whether loss of any subunit within Hda1C could confer suppression. Complete open reading frame deletions of *HDA1*, *HDA2*, or *HDA3* were created using one-step gene replacement with the *TRP1* gene (Moqtaderi and Geisberg 2013). Removal of any subunit of Hda1C, including the catalytic subunit Hda1, reversed the cryptic initiation phenotype of strains containing the *GAL1p-FLO8-HIS3* reporter and *rtf1* HMD point mutations (Figure 1B, sections 3 to 5); no differences in suppression were detected among the Hda1C mutants. Consistent with results obtained with the *sup2-22* mutation, the cryptic initiation phenotype of strains lacking the entire *RTF1* gene was not suppressed by deletion of individual Hda1C subunits. This result suggests that additional regions of *Hda1* or additional proteins that interact with *Rtf1* outside of the HMD are necessary to observe the suppression.

Another phenotype displayed by certain *rtf1* mutants is the inability to silence transcription near telomeres, as assayed using a *TELVR::URA3* reporter (Gottschling et al. 1990; Chu et al. 2007; Tomson et al. 2011; Van Oss et al. 2016). Strains containing this reporter and the wild-type *RTF1* gene grow in the presence of 5FOA,

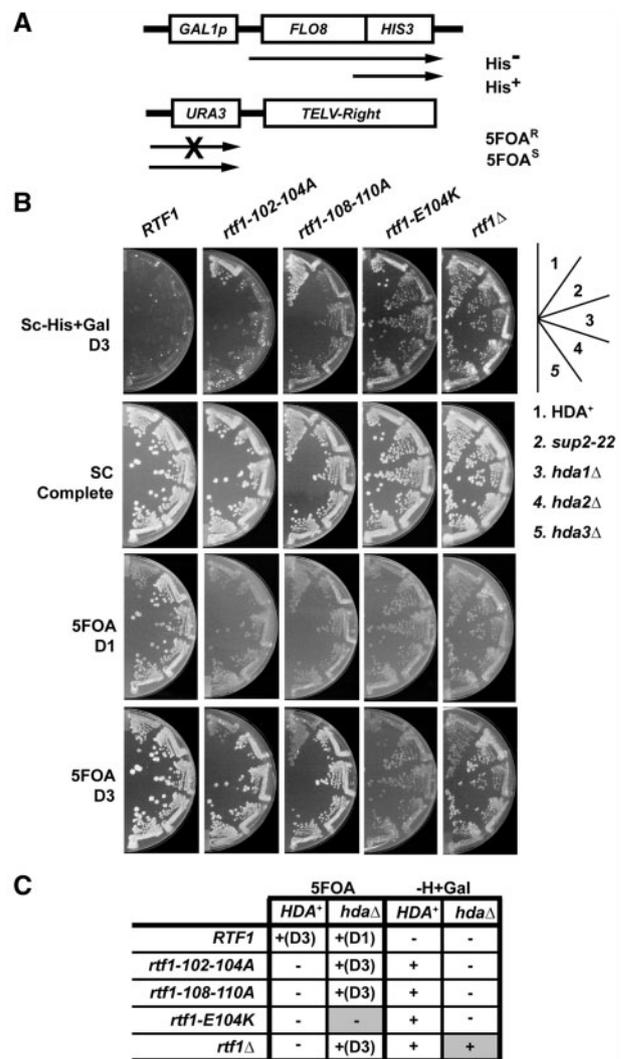


Figure 1 Deletion of any Hda1C subunit suppresses the cryptic initiation and telomeric silencing phenotypes of *rtf1* HMD mutants. (A) Schematic diagrams of the cryptic initiation and telomeric silencing reporters used in this study. (B) Suppression of the cryptic initiation and telomeric silencing defects of *rtf1* point mutations was assessed by replica plating to SC-His+Gal and 5FOA medium, respectively. SC complete medium was used as a control. Plates were imaged one (D1) or three (D3) days after replica plating and incubating at 30°C. (C) Table summarizing growth phenotypes shown in panel B (+ indicates growth; - indicates lack of growth). Gray boxes highlight where the phenotype differed from the other *rtf1* alleles. The following strains were used: KY1523, KY1496, KY2963, KY2934, KY2868, KY2792, KY1519, KY2957, KY2965, KY2898, KY1494, KY1502, KY2973, KY2974, KY2902, KY1526, KY2855, KY2999, KY3001, KY2867, KY1370, KY1520, KY2981, KY2983 and KY2943.

a drug that is toxic to cells expressing *URA3*. Strains, such as *rtf1-108-110A* mutant, that are defective for silencing of the *TELVR::URA3* reporter are 5FOA^S. Therefore, we used this reporter as a secondary readout for the suppression of the *rtf1* mutations. We originally noted that *RTF1* strains with the *sup2-22* allele grow better on 5FOA-containing media than wild-type strains (Figure 1B, see growth on 5FOA on day one). Consistent with our identification of *sup2-22* as a recessive mutation in *HDA3*, *hda1Δ* mutants were previously shown to have increased resistance to 5FOA, using a telomeric reporter (Rundlett et al. 1996; Hang and Smith 2011). The *sup2-22*, *hda1Δ*, *hda2Δ* and *hda3Δ* mutations suppressed the 5FOA sensitivity of two different *rtf1* HMD mutants, *rtf1-102-104A* and *rtf1-108-110A*, as well as the *rtf1Δ* allele. In

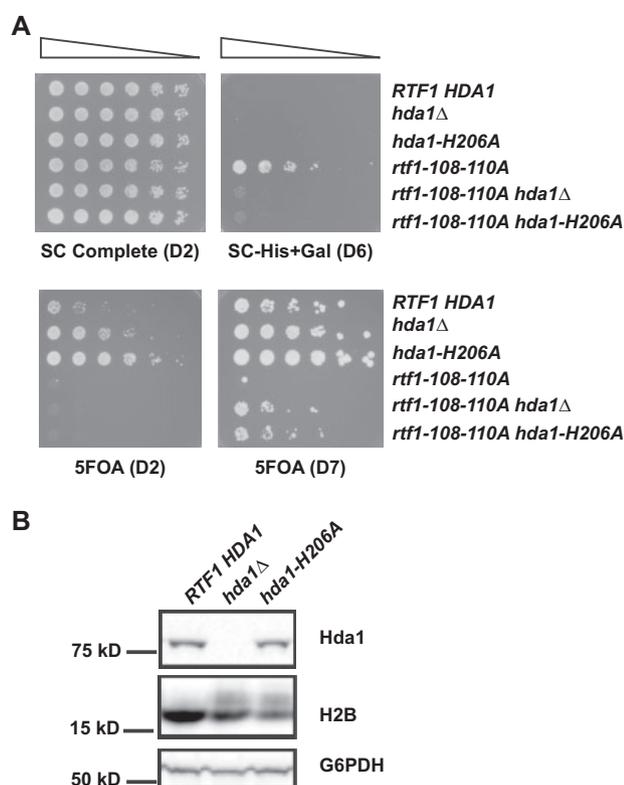


Figure 2 A substitution in the Hda1 catalytic domain suppresses an *rtf1* HMD mutant. (A) Cryptic initiation and telomeric silencing phenotypes of the indicated strains containing the *GAL1p-FLO8-HIS3* and *TELV::URA3* reporters were measured on SC-His+Gal (top panel) and 5FOA-containing media (bottom panel), respectively. (B) Western blot analysis of Hda1 and H2B levels in extracts prepared from a wild type strain, an *hda1Δ* strain, and an *hda1-H206A* strain. The following strains were used: KY1523, KY2963, KY3910, KY1494, KY2973 and KY3911

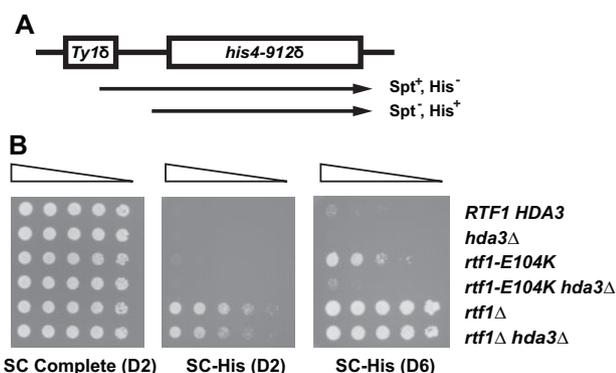


Figure 3 The Spt^- phenotype of *rtf1-E104K* is suppressed by *hda3Δ*. The Spt^- phenotype of *rtf1-E104K* at the *his4-912δ* locus (diagrammed at top) was detected on medium lacking histidine (SC-His). Plates incubated for two (D2) or six (D6) days at 30°C are shown. Reduced growth of the *rtf1-E104K hda3Δ* strain on the SC-His medium indicates suppression of the Spt^- phenotype. The following strains were used: KY3191, KY3186, KY1532, KY3178, KY3034 and KY3032.

contrast, the *hda* mutations did not suppress the 5FOA sensitivity of a different HMD mutant, *rtf1-E104K*, indicating allele-specific suppression for this phenotype as well (summarized in Figure 1C).

Our results demonstrate that absence of a functional Hda1C suppresses the cryptic initiation and telomeric silencing phenotypes of certain *rtf1* HMD mutants. To test if loss of Hda1C catalytic activity could similarly suppress the *rtf1-108-110A* mutation, we mutated an invariant histidine, H206, in the catalytic center of Hda1 and introduced this *hda1-H206A* mutation at the endogenous *HDA1* locus by allelic replacement (Lee et al. 2021). For both the cryptic initiation and telomeric silencing phenotypes, the *hda1-H206A* mutation largely phenocopied the *hda1Δ* mutation with respect to *rtf1* suppression (Figure 2A). Western blotting confirmed that the Hda1-H206A mutant protein was expressed at levels similar to wild type (Figure 2B).

In addition to effects on telomeric silencing and cryptic initiation from the internal *FLO8* promoter, some mutations that alter the Rtf1 HMD also confer a suppressor-of-Ty (Spt^-) phenotype (Warner et al. 2007; Tomson et al. 2011; Van Oss et al. 2016). At the *his4-912δ* allele, an insertion of a Ty1 δ element in the *HIS4* promoter renders cells unable to grow on media lacking histidine due to an upstream shift in transcription initiation (Winston 1992). However, mutations in many genes regulating transcription and chromatin, including *RTF1*, are able to grow in the absence of histidine because they restore initiation to the native *HIS4* TSS and produce a functional *HIS4* transcript (Stolinski et al. 1997). This could be considered another case of cryptic transcription since the native promoter is downstream of the promoter provided by the Ty element (Kaplan et al. 2003). Similar to results seen with the *GAL1p-FLO8-HIS3* reporter, the Spt^- phenotype of *rtf1-E104K*, but not *rtf1Δ*, was suppressed by deleting *HDA3* (Figure 3).

The identification of loss-of-function mutations in genes encoding an HDAC as suppressors of cryptic initiation was unexpected. Previous work has argued that increased acetylation of histones facilitates cryptic transcription (Rando and Winston 2012; Venkatesh and Workman 2015), likely by relaxing chromatin structure, which could allow spurious pre-initiation complex assembly and the initiation of new transcripts. Thus, mutations that inactivate an HDAC, which would increase histone acetylation, would have been unlikely to reverse the cryptic initiation phenotype of the *rtf1* mutant strains. Due to the unanticipated role of Hda1C identified with the *GAL1p-FLO8-HIS3* reporter, we focused on the coordination between Hda1C and Rtf1 with respect to cryptic initiation.

Loss of Hda1C does not restore H2Bub in *rtf1* strains

The *rtf1* alleles studied here are unable to stimulate H2Bub in vivo (Van Oss et al. 2016). One possibility is that suppression of cryptic initiation occurs because loss of Hda1C increases H2Bub levels. However, as shown by western blot analysis, the severe H2Bub defect of the *rtf1* mutants was not rescued by deletion of *HDA1* or *HDA2* (Figure 4A). We also asked if loss of Hda1C recovered H3 methylation marks dependent on a functional Rtf1 HMD and H2Bub (Warner et al. 2007; Piro et al. 2012; Van Oss et al. 2016). While slight changes in global levels of H3 K4me2, H3 K4me3 and H3 K79me2/3 were detected in strains lacking Hda3, we also observed a small increase in total H3 recovered from these strains (Supplementary Figure S1). When normalized to total H3 levels, the changes in H3 K4 or H3 K79 methylation marks were less than two-fold. Coupled with previous results showing that *set1Δ* and *dot1Δ* mutants do not express the *GAL1p-FLO8-HIS3* reporter (Quan and Hartzog 2010), these observations suggest that suppression of the cryptic initiation phenotypes of the *rtf1* mutants

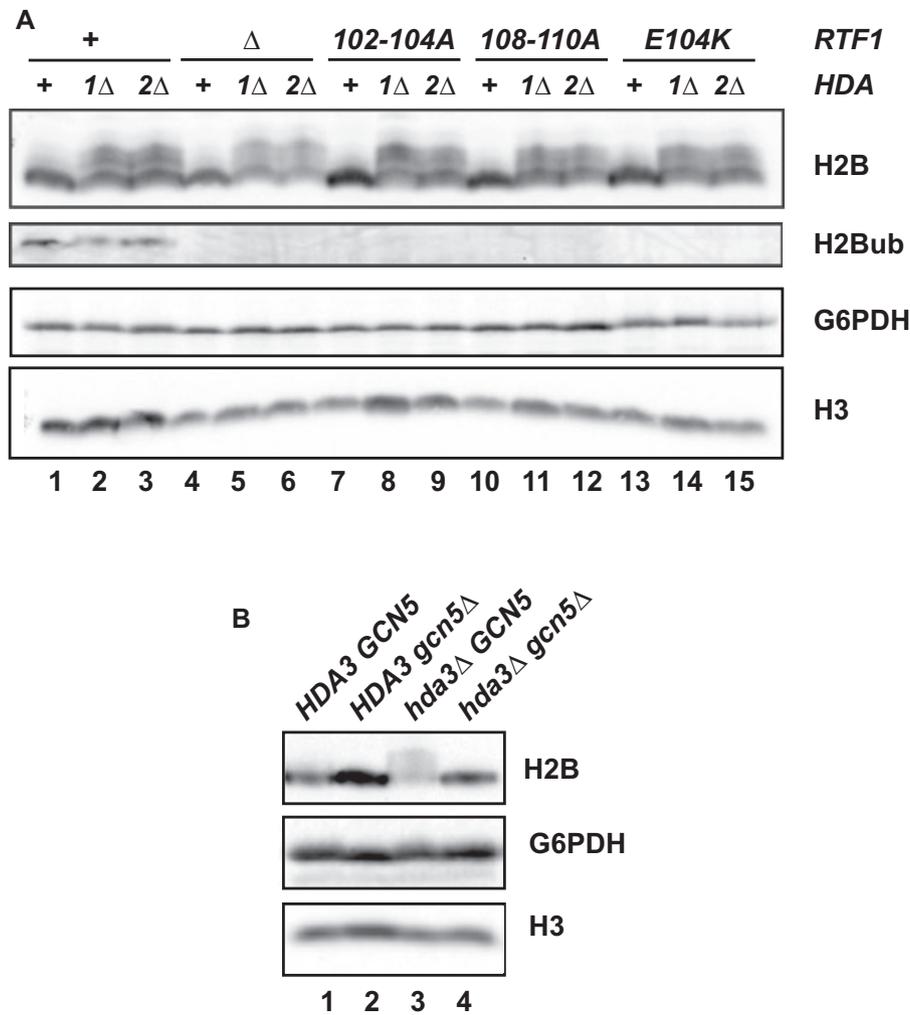


Figure 4 Loss of Hda1C does not restore H2Bub in *rtf1* mutants. (A) Western blot analysis of the indicated *rtf1* mutant strains in which Hda1C is intact (+) or the *HDA1* (1Δ) or *HDA2* (2Δ) genes have been deleted. Antibodies against total H2B or H2Bub were used to monitor levels of H2B K123 ubiquitylation. Longer exposures also failed to show a recovery of H2Bub in the *hda1Δ* and *hda2Δ* strains. The following strains were used: KY1523, KY2963, KY2934, KY2987, KY2981, KY2983, KY2792, KY2957, KY2965, KY1494, KY2973, KY2974, KY3002, KY2999 and KY3001. (B) Western blot analysis of the indicated strains. The following strains were used: KY433, KY3014, KY2861 and KY3011. In both panels, levels of G6PDH and H3 served as controls.

is unlikely due to the recovery of H3 K4 or H3 K79 methylation. However, we cannot exclude the possibility that, within a bulk population, a small percentage of *rtf1 hda3* double mutant cells have high levels of H3 methylation and these cells are most affected for cryptic initiation.

Strikingly, western blot analysis of total H2B levels showed bands that ran with slower mobility when Hda1C activity was absent, even in *RTF1* strains (Figures 2B, 4, A and B). Given the function of Hda1C, we suspected that these bands represented an enrichment of acetylated forms of H2B (Wu et al. 2001a,b) or possibly another modification that is dependent upon H2B acetylation. As the Gcn5 HAT was previously shown to contribute to H2B acetylation (Grant et al. 1997; Suka et al. 2001), we asked if the slower migrating forms of H2B were dependent on Gcn5. Indeed, in *gcn5Δ hda3Δ* strains, the slower migrating anti-H2B-reactive bands were no longer observed (Figure 4B, lane 4). Interestingly, we did not observe a shift in the mobility of the ubiquitylated form of H2B upon inactivation of Hda1C (Figure 4A), raising the possibility that H2B K123 ubiquitylation inhibits acetylation or an acetylation-dependent modification of H2B.

Loss of H2Bub leads to cryptic initiation dependent on Hda1C

Since the *HDA* mutations suppress the cryptic initiation phenotype of *rtf1* mutants that are severely defective in H2Bub, we assessed the cryptic initiation phenotype of other strains lacking H2Bub and the dependence on Hda1C for this phenotype. We analyzed strains expressing an H2B mutant protein that cannot be ubiquitylated, H2B K123R, as the only form of H2B and a strain lacking Rad6, the ubiquitin conjugase that targets H2B K123. In both cases, when *HDA3* is wild type, we observed growth on SC-His+Gal medium, indicating cryptic initiation from the *GAL1p-FLO8-HIS3* reporter (Figure 5A, panel 4, and Figure 5B, upper right), identifying a role for H2B K123 in controlling cryptic initiation using this reporter. Consistent with suppression of the *rtf1* mutations, *hda3Δ* also suppresses the cryptic initiation phenotype of *htb1-K123R* and *rad6Δ* strains (Figure 5, A and B). Together these results suggest that the role of Hda1C in supporting cryptic initiation in the *rtf1* mutants occurs downstream of the effects of losing H2Bub.

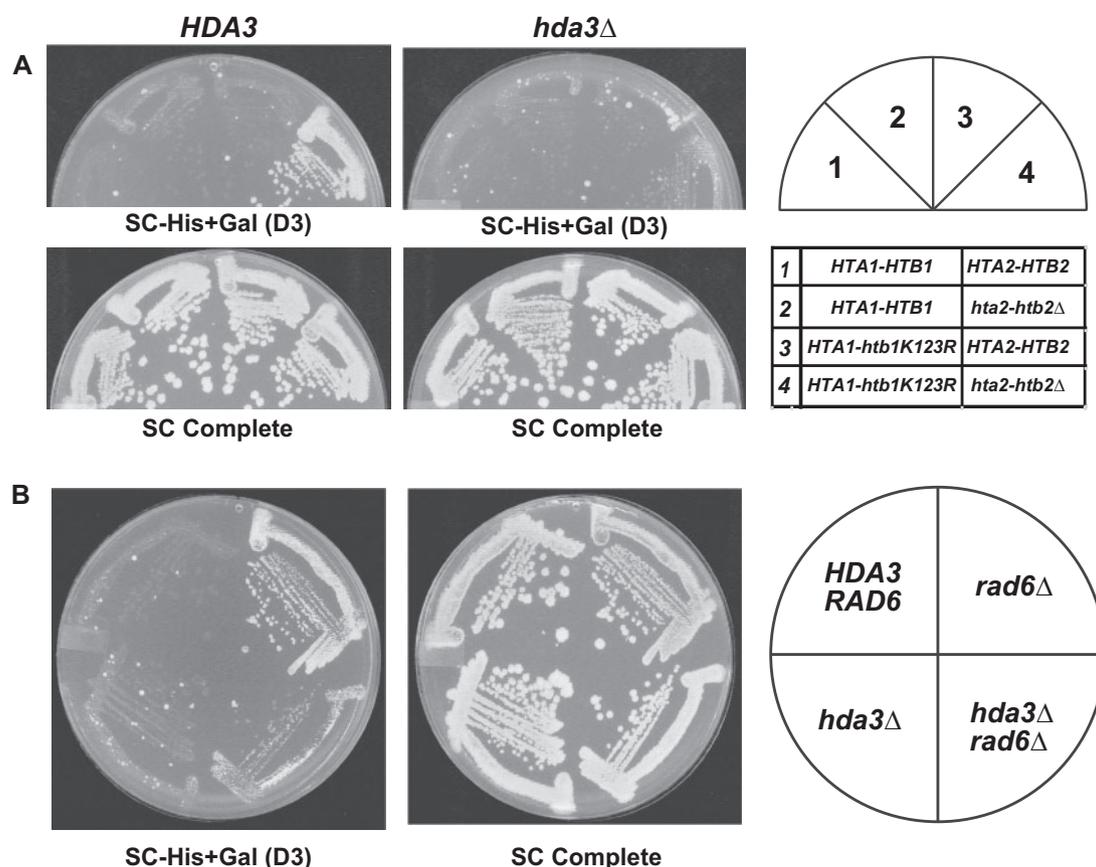


Figure 5 Deletion of *HDA3* suppresses defects in the H2Bub pathway. (A) A strain carrying the H2B K123R substitution as the only source of H2B (section 4) exhibits cryptic initiation as measured with the *GAL1p-FLO8-HIS3* reporter when *HDA3* is present (left), but not when *HDA3* has been deleted (right). The following strains were used: KY1523, KY2878, KY2929, KY2876, KY2868, KY2921, KY2924 and KY2910. (B) A strain in which *RAD6* has been deleted exhibits cryptic initiation when *HDA3* is present (top right quadrant), but not when *HDA3* has been deleted (bottom right quadrant). The following strains were used: KY1523, KY2832, KY2918 and KY2868. For both panels, plates were imaged three days after replica plating and incubation at 30°C.

The genetic interactions between Rtf1 and Hda1C are distinct from those involving other HDACs and chromatin regulators

Contrary to our results with Hda1C, previous studies showed that other HDACs suppress cryptic initiation; that is, in their absence, increased levels of cryptic transcription are detected (Cheung et al. 2008; Silva et al. 2012). Given our unexpected result, we asked whether mutations in genes encoding other HDACs, in particular Rpd3S (*rco1*Δ) (Carrozza et al. 2005; Keogh et al. 2005) or Set3C (*set3*Δ) (Pijnappel et al. 2001; Kim et al. 2012), would also suppress the transcription defect in *rtf1-102-104A* strains. By using a deletion of *RCO1*, we specifically targeted the Rpd3S complex, which has been previously connected to regulation of intragenic cryptic transcription. As expected (Carrozza et al. 2005; Cheung et al. 2008; Silva et al. 2012; McDaniel et al. 2016), a strain lacking the Rpd3S subunit Rco1 and containing the *GAL1p-FLO8-HIS3* reporter grew on SC-His+Gal media [Figure 6A, SC-His+Gal (D3)]. In fact, the double mutant, *rco1*Δ *rtf1-102-104A*, grew better than either single mutant [Figure 6A, SC-His+Gal (D2)], suggesting the mutations act in different pathways to affect cryptic transcription. In addition, *rp3*Δ strains, lacking both the Rpd3S and Rpd3L complexes, showed similar results (Supplementary Figure S2), though strains lacking Rpd3 grow more slowly. Deletion of *SET3*, which eliminates activity from both the Hos2 and Hst2 HDACs in Set3C, did not lead to a cryptic initiation phenotype as measured by the *GAL1p-FLO8-HIS3* reporter, in agreement with

previous reports that *set3*Δ did not elicit transcription from the cryptic promoters in the native *FLO8* and *STE11* genes (Kim and Buratowski 2009). Furthermore, unlike *hda3*Δ, *set3*Δ did not suppress the cryptic initiation phenotype of *rtf1-102-104A*. Therefore, suppression of cryptic transcription in this *rtf1* mutant by *hda3*Δ is not a common feature of HDAC mutants.

Next, we investigated if the effect of Hda1C was specific to strains defective for H2Bub. Many strains with mutations in genes encoding chromatin and transcription factors show cryptic initiation on the *GAL1p-FLO8-HIS3* reporter (Cheung et al. 2008; Quan and Hartzog 2010; Silva et al. 2012). These include strains lacking or mutated in the H3 K36 methyltransferase Set2, the chromatin remodeling enzyme Chd1, and the histone chaperones Spt6 and Spt16. In contrast to *rtf1* mutants, *hda3*Δ did not suppress the cryptic initiation phenotypes of strains lacking Set2 or Chd1 or containing mutations in *SPT6* or *SPT16* (Figure 6, B and C). In fact, *set2*Δ *hda3*Δ and *spt16-197* *hda3*Δ double mutants grew better on the SC-His+Gal medium than *set2*Δ or *spt16-197* single mutant strains. These results show that the genetic interactions we identified between mutants lacking H2Bub and Hda1C are specific. They also suggest that loss of Hda1C is not simply suppressing cryptic initiation by elevating Pol II transcription from the *GAL1* promoter and impeding internal initiation events. In support of this, levels of the full-length *FLO8* transcript produced from the reporter construct are unchanged by deletion of *HDA3* (Supplementary Figure S3, A–C), and Pol II occupancy at the *FLO8*

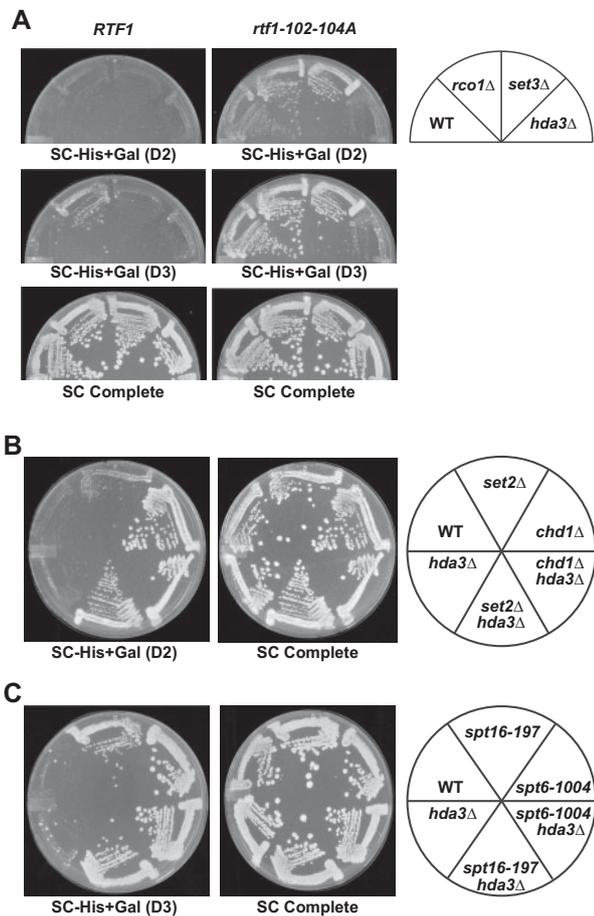


Figure 6 Genetic interactions between *rtf1* mutations and mutations in genes encoding Hda1C subunits are specific. (A) Mutations that disrupt other HDACs do not suppress the cryptic initiation phenotype of an *rtf1* HMD mutant. Strains mutated for the Rpd3S (*rco1Δ*) or Set3C (*set3Δ*) HDACs and containing either *RTF1* or *rtf1-102-104A* were replica plated to SC-His+Gal or SC Complete media and scanned after two (D2) or three (D3) days of incubation at 30°C. For both the *RTF1* and *rtf1-102-104A* panels, WT indicates the presence of wild-type *RCO1*, *SET3*, and *HDA3* genes. The following strains were used: KY1523, KY3057, KY3042, KY2868, KY2792, KY3053, KY3038 and KY2898. (B, C) Deletion of *HDA3* does not suppress the cryptic initiation phenotypes of strains defective in other chromatin regulators. Strains with the indicated genotypes were replica plated to SC-His+Gal or SC Complete media and scanned after two (D2) or three (D3) days of incubation at 30°C. The following strains were used: (B) KY1523, KY3200, KY3198, KY3199, KY3201 and KY2868 and (C) KY1523, KY3073, KY3084, KY3082, KY3093 and KY2868.

5' region is similar in wild-type strains and *hda3Δ* mutants (Supplementary Figure S3, A and D).

Sas3 and H3 K14 suppress cryptic initiation in an *rtf1* mutant

Since loss of Hda1C suppresses the cryptic initiation phenotype of *rtf1* mutants, we hypothesized that deletion of a HAT that works in opposition to Hda1C might exacerbate the cryptic initiation phenotype of the *rtf1* mutants and reverse the effect of inactivating Hda1C. Based on our western blot results showing that deletion of *GCN5* reversed the H2B mobility shift caused by *hda3Δ* (Figure 4B), we investigated if *Gcn5* was the relevant HAT. *Gcn5* is the catalytic subunit of several HAT complexes (Lee and Workman 2007), any of which might be involved in regulating cryptic initiation. If *Gcn5* antagonized Hda1C, then the *rtf1-102-104A hda3Δ gcn5Δ* triple mutant would be expected to phenocopy

the *rtf1-102-104A* single mutant and grow on Sc-His+Gal medium. Instead, the triple mutant was strongly His⁺ (Supplementary Figure S4, right panel, section 4). In addition, on its own, the *gcn5Δ* mutation suppressed the cryptic initiation phenotype of the *rtf1-102-104A* mutation (Supplementary Figure S4, right panel, section 3). These results suggest that suppression of *rtf1-102-104A* by *hda3Δ* is not easily explained by increased acetylation of a *Gcn5*-dependent target protein.

Because the *rtf1* HMD mutants exhibit telomeric silencing defects and Hda1C also plays a role in silencing, we asked whether deletion of two HATs previously implicated in transcriptional silencing would affect the cryptic initiation phenotype of the *rtf1* mutants. *SAS2* was first discovered in a screen for genes affecting transcriptional silencing at the mating-type locus and *SAS3* was subsequently uncovered by its similarity to *SAS2* (Reifsnnyder et al. 1996). *Sas2* and *Sas3* are the catalytic subunits of the HATs *SAS* and *NuA3*, respectively (John et al. 2000; Shia et al. 2005). While deletion of *SAS2* had little effect, deletion of *SAS3* exacerbated the cryptic initiation phenotype of the *rtf1* mutants, as indicated by enhanced growth on SC-His+Gal medium (Figure 7A). Interestingly, *sas3Δ* on its own did not confer a cryptic initiation phenotype. Notably, *Sas3* and *Hda3* appear to oppose each other because deleting both proteins in the context of *rtf1-108-110A* gave a cryptic initiation phenotype nearly identical to the *rtf1* allele alone (Figure 7B). These results suggest that the acetylation state of one or more shared targets of Hda1C and *Sas3* modulates the cryptic initiation phenotype of the *rtf1* mutants.

One target of *Sas3* is H3 K14 (Taverna et al. 2006). We took advantage of an integrated synthetic histone mutant library (Dai et al. 2008) to create strains in which the only copy of H3 had a lysine-to-alanine substitution at position 14 (*synH3-K14A*). Unlike *sas3Δ* strains, the H3 K14A mutant showed cryptic initiation using the *GAL1p-FLO8-HIS3* reporter (Figure 7C, compare row 5 to row 1 control). The distinct phenotypes of the *sas3Δ* and H3 K14A mutants may be due to residual acetylation of H3 K14 in the *sas3Δ* strain, as previous studies showed that H3 K14 is also acetylated by *Gcn5* (Church et al. 2017). However, like the *sas3Δ* mutation, the H3 K14A substitution strongly enhanced the cryptic initiation phenotype of the *rtf1-102-104A* mutant (Figure 7C). Deleting *HDA3* only partially reversed the *rtf1-102-104A synH3-K14A* cryptic initiation phenotype. Unlike the *rtf1-102-104A hda3Δ synH3* control strain, which carries a synthetic version of wild-type H3, the *rtf1-102-104A hda3Δ synH3-K14A* remained His⁺. These results suggest that *Sas3* represses the cryptic initiation phenotype of *rtf1* mutants and Hda1C opposes *Sas3* in this process. One possible target for their action is H3 K14. However, because *hda3Δ* partially suppresses the cryptic initiation phenotype of the *synH3-K14A* mutant, other Hda1C targets that control chromatin accessibility must exist.

Discussion

In this study, we discovered a connection between *Rtf1*, a component of the *Paf1* transcription elongation complex necessary for *H2Bub*, and *Hda1C*, an HDAC complex, in controlling transcription at three loci that are sensitive to chromatin architecture. Previous studies have shown that deletion of certain HDACs, including *Rpd3S* or *Set3C*, causes cryptic initiation (Carrozza et al. 2005; Keogh et al. 2005; Li et al. 2007a,b; Kim et al. 2012). This is consistent with the expectation that increased histone acetylation promotes a permissive chromatin environment. In contrast, our study shows that loss of Hda1C activity suppresses the

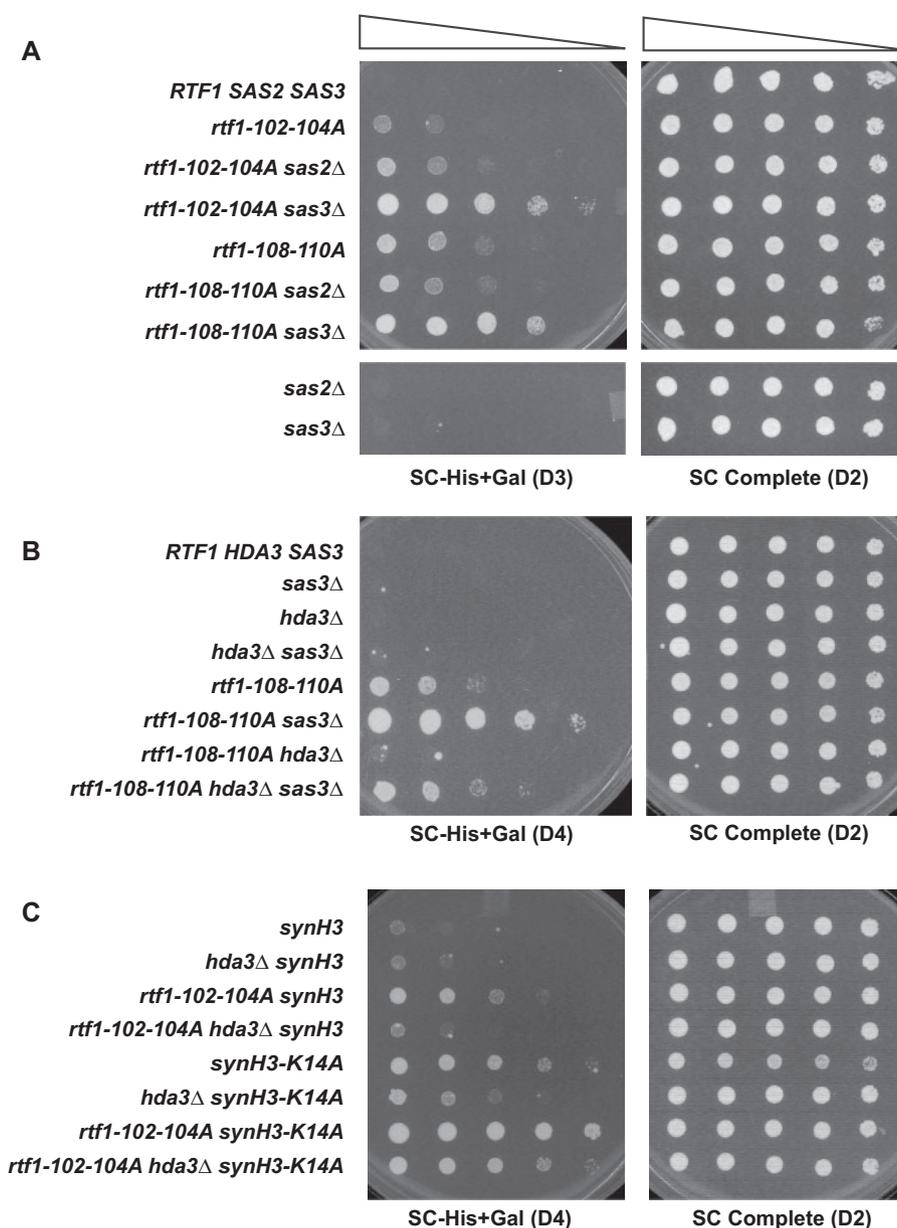


Figure 7 Mutation of SAS3 or H3 K14 enhances the cryptic initiation phenotype of *rtf1* HMD mutants. (A, B) Strains with the indicated genotypes were grown as described in the Materials and Methods and serial dilutions were spotted onto SC-His+Gal and SC Complete media and allowed to grow for two (D2), three (D3) or four (D4) days at 30°C before imaging. (C) Strains deleted for *HHT1-HHF1* and carrying a synthetic copy of *HHT2-HHF2* (*synH3*), either wild-type or H3 K14A, were used in genetic crosses to create double and triple mutants with *rtf1-102-104A* and *hda3Δ*. Strains were grown on the indicated media for two to four days at 30°C. The following strains were used: (A) KY1491, KY2792, KY3366, KY3371, KY1490, KY3369, KY3372, KY3362 and KY3364; (B) KY3423, KY3431, KY3427, KY3438, KY3425, KY3434, KY3429 and KY3441; (C) KA255, KA256, KA257, KA258, KA295, KA296, KA297 and KA298.

cryptic initiation phenotype of strains deficient in or completely lacking in H2Bub. The interplay between H2Bub and Hda1C in controlling cryptic transcription initiation appears specific, because deletion of *HDA3* did not reduce cryptic initiation in strains lacking several other chromatin-related proteins and mutations in genes encoding other HDACs did not suppress the cryptic initiation phenotype of an *rtf1* mutant defective in H2Bub.

We and others have shown that mutations in *RTF1* and genes encoding other proteins required for H2Bub cause cryptic initiation within the *FLO8* gene (Cheung et al. 2008; Fleming et al. 2008; Tomson et al. 2011; Silva et al. 2012). While H2Bub is required for H3 K4me2/3 and H3 K79me2/3 *in vivo*, previous studies found that strains lacking Set1 or Dot1, the methyltransferases

responsible for H3 K4me and H3 K79me, respectively, did not exhibit cryptic initiation at the *GAL1p-FLO8-HIS3* reporter (Quan and Hartzog 2010). This suggests that H2Bub functions independently of these downstream modifications in preventing cryptic initiation. H2Bub has been shown to stabilize nucleosomes, particularly in the middle and ends of genes (Chandrasekharan et al. 2009; Batta et al. 2011), and loss of H2Bub in *S. pombe* up-regulates noncoding RNAs, particularly antisense transcripts (Pagé et al. 2019; Murawska et al. 2020; Sansó et al. 2020). Recent work using optical tweezers has confirmed the increase in stability of nucleosomes containing H2Bub, and this increase in stability is an energetic barrier to passage by Pol II (Chen et al. 2019). One possibility based on our findings is that Hda1C may contribute to the

instability of nucleosomes lacking H2Bub by deacetylating a histone or other target.

Deletion of Hda1C did not suppress the cryptic initiation phenotype of strains completely lacking Rtf1. This suggests that the absence of other functional domains within Rtf1, outside of the HMD, contributes to cryptic initiation in a way that is not suppressed by mutations in genes encoding Hda1C. In addition to the HMD, Rtf1 has separable domains required for its interactions with the Pol II elongation complex, Chd1 and other Paf1C subunits (Simic et al. 2003; Warner et al. 2007; Mayekar et al. 2013; Wier et al. 2013; Vos et al. 2020). The N-terminus of Rtf1 is important for proper recruitment of Chd1 to certain open reading frames (Warner et al. 2007), and we found that deletion of *CHD1* causes a cryptic initiation phenotype that cannot be suppressed by *hda3Δ*. This observation suggests that Chd1 and Hda1C affect cryptic initiation through different pathways or that the effect of Chd1 is upstream of the effect of Hda1C. Therefore, while a complete deletion of Rtf1 can affect transcription and chromatin through several mechanisms, our data suggest that only the effect on H2Bub is suppressed by loss of Hda1C.

What is the target of Hda1C that creates a permissive condition for initiation of transcription within the coding sequence? Cells lacking Hda1C have increased acetylation at several positions on H2B, H3 and H4, including H3 K14 (Carmen et al. 1996; Rundlett et al. 1996; Wu et al. 2001a,b; Yang and Seto 2008; Islam et al. 2011; Ha et al. 2019; Lee and Kim 2020). H3 K14Ac has been previously shown to help maintain proper levels of H3 K4me3 by inhibiting the Jhd2 histone demethylase (Maltby et al. 2012). Furthermore, in a positive feedback loop, methylation of H3 K4, a modification dependent on H2Bub, promotes H3 K14 acetylation by Sas3 as part of the NuA3 HAT complex, through the binding of the Yng1 subunit of NuA3 (Taverna et al. 2006; Martin et al. 2017). Our results demonstrate that, in the context of an *rtf1* mutation, the absence of Sas3 or H3 K14 enhances cryptic initiation, supporting the idea that an appropriate level or pattern of histone acetylation marks is needed to prevent cryptic initiation. While Hda1C and Sas3 appear to work in opposition in controlling cryptic initiation, the role of Hda3 in promoting cryptic initiation in *rtf1* mutants cannot be solely through deacetylation of H3 K14, as we observed partial suppression of the cryptic initiation phenotype of an H3 K14A mutant by *hda3Δ*.

The enrichment of Hda1C on the bodies of active genes and its interactions with RNA and Pol II argue for a role in controlling histone acetylation levels during transcription elongation (Govind et al. 2010; Ha et al. 2019; Lee and Kim 2020). Moreover, a recent study showed that Hda1C reduces H4 acetylation within the coding regions of genes undergoing high levels of transcription (Ha et al. 2019; Lee and Kim 2020). With the *GAL1p-FLO8-HIS3* reporter used here, high levels of transcription driven by the inducible *GAL1* promoter are required to detect cryptic initiation in the *rtf1* HMD mutants as has been observed for other mutants (Cheung et al. 2008; Silva et al. 2012). Surprisingly, by a mechanism not understood, *hda1Δ* cells, with higher levels of H4 acetylation, exhibit higher histone occupancy levels, especially on longer genes where the effect of *hda1Δ* on H4 acetylation is more evident (Ha et al. 2019). Increasing histone occupancy within gene bodies could be one mechanism by which loss of Hda1C counteracts the disruption in chromatin structure caused by loss of H2Bub.

In summary, our results suggest an interplay between the H2Bub pathway and Hda1C in controlling chromatin accessibility during transcription elongation. Unexpectedly, a functional Hda1C permits cryptic initiation in cells lacking H2Bub. While

cryptic initiation is most frequently observed under mutant conditions, it is possible that the dynamic removal of H2Bub during transcription elongation could give rise to cryptic transcripts in wild-type cells, albeit at low levels. In this scenario, the activity of Hda1C could regulate the output of these cryptic transcripts. Whether these transcripts would have biological consequences, as has been reported for some internally initiated transcripts (McKnight et al. 2014; Tamarkin-Ben-Harush et al. 2017), is unclear. Future work will be needed to elucidate the histone or non-histone target(s) through which Hda1C impacts cryptic initiation in opposition to H2Bub and delineate whether these targets are functioning directly or indirectly in this process. Recent technical and computational advances in detecting transcript initiation events within genes bodies (Pelechano et al. 2013; Wei et al. 2019) offer the opportunity to study this and additional examples of crosstalk between epigenetic regulators in maintaining chromatin structure during transcription elongation.

Reagent and data availability

All strains and detailed protocols are available upon request. Sequencing data are available at NCBI as part of the BioProject: PRJNA634539, BioSample accessions: SAMN14999039, SAMN14999040. Supplemental Table S1 and Supplemental Figures S1-S4 are available via figshare: <https://doi.org/10.25387/g3.15040245>.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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