Cell cycle-regulated oscillator coordinates core histone gene transcription through histone acetylation

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DNA replication occurs during the synthetic (S) phase of the eukaryotic cell cycle and features a dramatic induction of histone gene expression for concomitant chromatin assembly. Ectopic production of core histones outside of S phase is toxic, underscoring the critical importance of regulatory pathways that ensure proper expression of histone genes. Several regulators of histone gene expression in the budding yeast *Saccharomyces cerevisiae* are known, yet the key oscillator responsible for restricting gene expression to S phase has remained elusive. Here, we show that suppressor of Ty (Spt)10, a putative histone acetyltransferase, and its binding partner Spt21 are key determinants of S-phase–specific histone gene expression. We show that Spt21 abundance is restricted to S phase in part by anaphase promoting complex Cdc20-homologue 1 (APC^{Cdh1}) and that it is recruited to histone gene promoters in S phase by Spt10. There, Spt21-Spt10 enables the recruitment of a cascade of regulators, including histone chaperones and the histone-acetyltransferase general control nonderepressible (Gcn) 5, which we hypothesize lead to histone acetylation and consequent transcription activation.

Eukaryotic chromosomes are composed of chromatin, which in turn is composed of a fundamental repeated unit of a histone octamer and DNA, the nucleosome. Each histone octamer includes two H3-H4 histone dimers flanked on either side by H2A-H2B dimers. The four *r*eplication-*d*ependent (RD) core histones (H2A, H2B, H3, and H4) are among the most conserved eukaryotic proteins, and all characterized eukaryotic genomes carry more than one gene encoding each core histone protein. Budding yeast contains two copies of each RD histone gene, each arranged in opposite orientation to a gene encoding its partner within the nucleosome: *HHT1-HHF1* and *HHT2-HHF2*, the two gene pairs encoding core histones H3 and H4, and *HTA1-HTB1* and *HTA2-HTB2*, the two gene pairs encoding H2A and H2B (for recent reviews see refs. 1 and 2).

Histone gene expression is repressed outside of the synthetic (S) phase by a histone chaperone called the histone regulatory (HIR) complex, which is recruited to a poorly defined DNA sequence, the negative regulatory (NEG) region, present upstream of three of the four histone-gene pairs in yeast: *HTA1-HTB1*, *HHT1-HHF1*, and *HHT2-HHF2* (3–7). *HTA2-HTB2*, the fourth histone gene pair, does not have a NEG region and is regulated in a HIR-independent manner. At HIR-dependent promoters, HIR recruits other histone chaperones, Asf1 and Rtt106, as well as the chromatin boundary protein Yta7 (8, 9) and the remodel structure of chromatin (RSC) ATP-dependent chromatin-remodeling complex (10), which together assemble repressive chromatin, blocking recruitment of RNAPII (11, 12). In S phase, this repressive chromatin is overcome, allowing recruitment of RNAPII and activation of transcription. After recruitment of RNAPII, the AAA-ATPase Yta7 is important for efficient transcript elongation by evicting histones H3-H4 (11–13). Other chromatin remodelers also have roles in histone gene activation, including the SWI/SNF chromatin-remodeling complex, which activates NEG-dependent histone genes (14), and the histone-acetyltransferase (HAT) complex Rtt109-Vps75 (8).

Although the roster of histone chaperones and chromatin remodelers involved in histone gene regulation is impressive, the cell cycle oscillator responsible for restricting histone gene activation to S phase has remained elusive. In this context, we decided to revisit the functions of two poorly understood proteins that physically interact and are involved in histone gene regulation, suppressor of Ty (Spt)10 and Spt21 (15–21). Spt10 is a DNA-binding protein that localizes to the *u*pstream-*a*ctivation *s*equences (UAS) of histone genes (22, 23). Spt10 contains a putative HAT domain similar to that of general control nonderepressible (Gcn) 5 (19, 24), which was reported to be involved in acetylation of H3K56 at histone promoters in vivo (14). However, to date, HAT activity of Spt10 has not been demonstrated in vitro.

Spt21 is a protein of unknown function that physically interacts with Spt10 (19). Like Spt10, Spt21 influences histone gene transcription as *spt21Δ* mutants display dramatically reduced levels of *HTA2*, *HTB2*, and *HHF2* transcripts in logarithmically growing cells (15, 25). Here we show that Spt21 is a cell cycle oscillator that serves as a master regulator of S-phase–dependent histone gene expression. We demonstrate that Spt10 is required to establish repression by the recruitment of HIR and HIR-dependent regulators outside of S phase. Furthermore, the expression of Spt21 is cell cycle-regulated with levels peaking in S phase, when it is recruited to histone gene promoters by its partner protein Spt10. We use genetic and biochemical experiments to show that the abundance of Spt21 during G1 phase is

Significance

DNA replication and histone gene transcription are tightly linked and occur during the S phase of the eukaryotic cell cycle. Histone production outside of S phase is highly toxic, underscoring the importance of regulatory pathways that control histone gene expression. Although various histone regulators have been discovered, the molecular mechanisms responsible for the spatial and temporal control of histone gene expression have remained elusive. Here, we describe the discovery of Spt21 as a long-elusive cell cycle oscillator responsible for restricting histone gene transcription to the S phase of the eukaryotic cell cycle. We show here that Spt21, together with its partner protein Spt10, regulates histone gene transcription by enabling the recruitment of a roster of chromatin-remodeling proteins.

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The authors declare no conflict of interest.

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regulated by the anaphase-promoting complex/cyclosome (APC/C) associated with its activator protein Cdc20-homologue 1 (Cdh1). During S phase, Spt21 accumulates and recruits the Gcn5 HAT to histone gene promoters, where they influence histone gene transcription. Our data reveal an important cell cycle oscillator that links the cell cycle machinery and histone acetylation and explain how the timing of histone acetylation at histone gene promoters leads to gene activation.

Results

Spt10 and Spt21 Recruit HIR and Associated Proteins/Complexes. To explore the mechanism of Spt10-dependent activation of histone gene transcription, we first used affinity purification and mass spectrometry to discover proteins associated with Spt10. Specifically, we used a tandem affinity purification (TAP)-tagged version of Spt10 expressed either at its endogenous locus or from an inducible promoter (*GAL1-10*) and a *m*odified *ch*romatin immunoprecipitation (mChIP) protocol to identify proteins associated with Spt10 on chromatin (8, 11, 26). We discovered interactions between Spt10 and all HIR subunits (Hir1, Hir2, Hir3, and Hpc2), RSC, as well as most subunits of SWI/SNF (Fig. S1). We did not identify peptides for these Spt10-interacting proteins across several control purifications, and the recovery of peptides for interacting proteins scaled with the amount of Spt10 in our purifications. We performed a similar mChIP analysis with Spt21-TAP and detected SWI/SNF peptides, but only when Spt21 was overexpressed, consistent with its low abundance relative to Spt10 (Fig. S1). We also confirmed the Spt10/Spt21 interaction described previously (Fig. S1) (19).

Because we detected all four HIR subunits in our mChIP of Spt10-TAP, we next used chromatin immunoprecipitation (ChIP) to test whether *SPT10* is required for recruitment of HIR (Hir1-TAP), the HIR-dependent regulators RSC (Rsc8-TAP), SWI/SNF (Snf6), Rtt106-TAP, and Asf1-TAP, and the transcriptional activator Yta7 (Yta7-TAP) (8, 11, 13) to the promoter region of *HTA1-HTB1* (Fig. 1*A*) (11). Consistent with an important function for the Spt10–HIR interaction on chromatin, we observed a strong reduction in the recruitment of both negative regulators of histone gene expression—HIR, Rtt106, Asf1, and RSC–as well as activators of histone transcription–SWI/SNF and Yta7–to the *HTA1-HTB1* promoter in an *SPT10* deletion strain (Fig. 1*B*). We also saw less dramatic recruitment defects for HIR and associated proteins/complexes in an *spt21Δ* mutant (Fig. 1*B*). This difference likely reflects the dependence of Spt10 association with histone promoters on Spt21 only in S phase (19). We conclude that the Spt10–Spt21 complex forms a key scaffold for recruitment of important positive and negative regulators of histone gene expression to their regulatory regions.

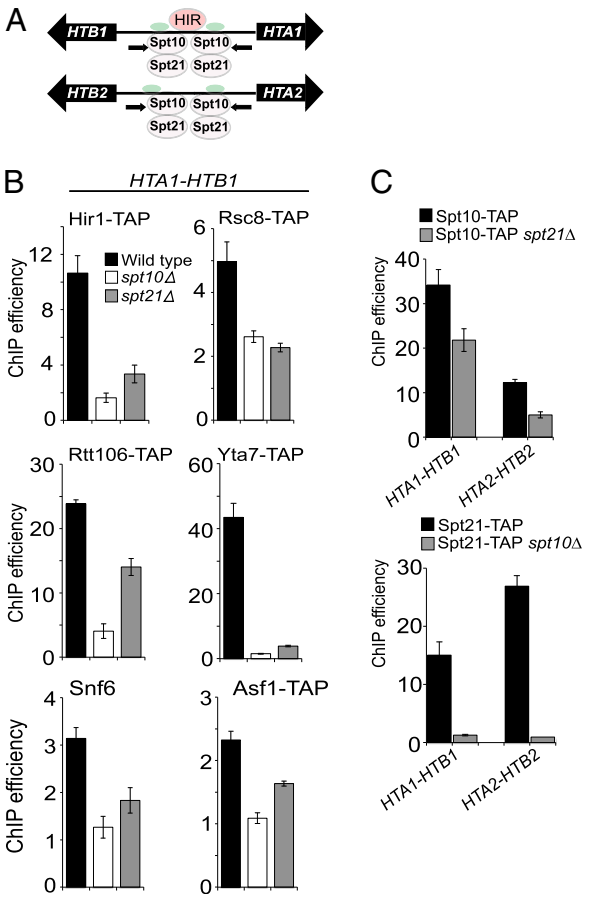

Fig. 1. The Spt10/Spt21 complex recruits chromatin-modifying proteins and complexes to the HTA1-HTB1 promoter. (*A*) Schematic representation of the HIR-dependent *HTA1-HTB1* and HIR-independent *HTA2-HTB2* histone gene promoters. UAS sequences (green circles) found in both regulatory regions (for a comprehensive review see ref. 2). Primer pairs used for ChIP assays are shown with black arrows (8, 10, 11). (*B*) Spt10 and Spt21 recruit histone regulators to regulatory regions of the *HTA1-HTB1* promoter. Hir1-TAP, Rsc8-TAP, Rtt106-TAP, Yta7-TAP, Snf6, and Asf1-TAP strains in the presence (wild type) or absence of *SPT10* (*spt10Δ*) or *SPT21* (*spt21Δ*) were cross-linked using formaldehyde. DNA copurifying with IgG-Sepharose and Snf6 antibody/Protein A ChIPs were analyzed for enrichment by qPCR, as indicated in the relevant panels. (*C*) Spt10 recruits Spt21 to the *HTA1-HTB1* and *HTA2-HTB2* regulatory regions. ChIP analyses were performed as described in *B* above. Error bars on the histograms represent SDs from the mean of at least three replicate qPCR reactions.

Spt21 Activates Histone Gene Expression During S Phase. To further explore the relationship between Spt21 and Spt10, we used ChIP to assay the dependence of Spt21 recruitment on Spt10 at HIR-dependent (*HTA1-HTB1*) and HIR-independent (*HTA2-HTB2*) promoters (Fig. 1*A*). Recruitment of Spt21-TAP to *HTA1-HTB1* and *HTA2-HTB2* was abolished in an *spt10*Δ strain (Fig. 1*C*, *Lower*), whereas recruitment of Spt10-TAP was reduced but not eliminated in a strain lacking *SPT21* (Fig. 1*C*, *Upper*), consistent with previous results (19).

We next assessed Spt10 and Spt21 protein levels in cells progressing synchronously through the cell cycle. We arrested Spt10-TAP and Spt21-TAP cultures in G1 phase with α-factor and released them into fresh medium to resume the cell cycle. Although Spt10-TAP levels remained constant through one synchronous cell cycle (Fig. 2*A*), Spt21-TAP protein levels were clearly cell cycle regulated, peaking in S phase just before the appearance of acetylation on lysine 56 on histone H3 (H3K56ac), a chromatin mark known to be enriched in S phase on newly synthesized histones (Fig. 2*A*) (27), consistent with an important activating role for Spt21 in S phase. Entry into S phase was significantly delayed in an *spt21*Δ strain compared with wild-type cells (Fig. 2*B*), a probable consequence of the mis-regulation of histone gene transcription.

HTA1 transcription was reduced during S phase (Fig. 2*C*, *Upper*), and *HTA2* expression was not activated in an *spt21* deletion strain (Fig. 2*C*, *Lower*), which supports a function for Spt21 in histone gene activation. Deletion of *SPT21* also caused a defect in *HTA1* repression during G2 and M phases (Fig. 2*C*, *Upper*), consistent with a failure to adequately establish the chromatin structure required to repress histone gene transcription outside of S phase, likely due to defective recruitment of Spt10 and HIR (Fig. 1*B*). Finally, we uncovered both biochemical and genetic evidence of the interdependence of Spt21 levels and H3K56ac (Fig. 2*A*): (*i*) accumulation of H3K56ac was delayed and its levels reduced in an *spt21*Δ strain throughout one cell cycle (Fig. S2*A*), which may reflect the delay in cell cycle progression seen in an *spt21Δ* mutant; and (*ii*) consistent with reduced levels of H3K56ac, deletion of *SPT21* suppressed the

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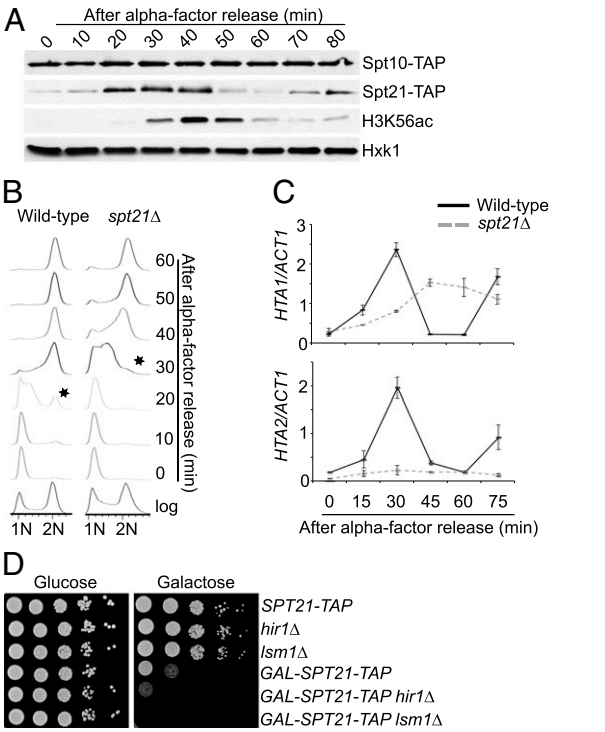

Fig. 2. Spt21 levels are cell cycle-regulated and important for histone gene transcription and S phase entry. (*A*) Spt21, but not Spt10 protein levels, are cell cycle-regulated and peak during S phase. Protein levels were monitored by Western blotting using anti-TAP antibody. Cells were synchronized in G1 phase using 5 μM α-factor and released into fresh medium to resume the cell cycle. Histone H3 acetylated at lysine 56 (H3K56ac; S and early G2 phase) was analyzed to monitor cell cycle. (*B*) Spt21 is important for timely S-phase entry. A *spt21* deletion mutant and an isogenic wild-type control strain were synchronized as in *A*, and FACS profiles were analyzed throughout the cell cycle as indicated. The asterisks mark the entry into S phase. (*C*) Mis-regulation of histone gene expression in the absence of *SPT21*. Cells were synchronized as described in *A*. cDNA was prepared and the ratio of *HTA1* or *HTA2* transcript to that of *ACT1* was determined using qPCR. Error bars in the experiments represent SDs from the mean of at least three replicate qPCR reactions. (*D*) Genetic interactions involving Spt21 overexpression and regulators of histone mRNA levels. The indicated strains were spotted in serial 10-fold dilutions onto glucose- or galactose-containing medium (to induce *SPT21* overexpression) and incubated at 30 °C for 2 d.

slow-growth phenotype seen during replication stress or high temperature in strains lacking both putative H3K56ac deacetylases *HST3* and *HST4* (28) (Fig. S2*B*). The elevated H3K56ac levels in an *hst3Δhst4Δ* double-mutant strain were restored close to wild-type levels in an *hst3Δhst4Δspt21Δ* triple mutant, likely due to reduced H3 levels (see H3 panel, Fig. S2*C*). Together, these results suggest that Spt10 sets up a repressive chromatin structure outside of S phase at the *HTA1-HTB1* promoter by recruiting HIR and associated proteins. To activate histone gene transcription, Spt10 recruits and stabilizes its S-phase–specific partner Spt21 at both *HTA1-HTB1* and *HTA2-HTB2* promoters.

Proper Regulation of Spt21 Is Critical for Normal Cell Cycle Progression. So far, our analysis implicates Spt21 in the coupling of S phase-specific histone gene expression and cell cycle progression. To test this further, we assessed the phenotypic consequences of constitutive Spt21 expression from the galactose-regulated promoter (*GAL1-10*). Overexpression of Spt21 caused increased *HTA1* and *HTA2* transcript levels relative to wild type (Fig. S3*C*) and a significant growth defect (Fig. 2*D*). To explore the relationship between these two phenotypes, we asked if the growth defect caused by *SPT21* overexpression was exacerbated by deletion of genes that normally repress histone levels through negative regulation of histone transcription (*HIR1*) or by promoting histone transcript degradation outside of S phase [*LSM1*, which encodes a component of Lsm1-7–Pat1 complex (29)]. Deletion of *HIR1* enhanced the toxicity of Spt21 overexpression, while deletion of *LSM1* was lethal in the presence of high Spt21 levels (Fig. 2*D*). These genetic results are consistent with a significant role for Spt21 in regulating histone gene expression, and emphasize the catastrophic consequences of a failure to ensure that high histone protein levels are restricted to S phase.

Our results suggest that a peak of Spt21 during S phase is important for appropriate histone gene expression. We next asked whether a canonical KEN box in Spt21 might be involved in its cell cycle-dependent degradation (Fig. 3*A*). The KEN box is a substrate recognition motif for the anaphase-promoting complex/Cdh1, a G1-specific E3 ubiquitin ligase composed of 13 distinct core proteins (APC/C^{Cdh1}) (30). We performed several experiments to ask if Spt21 is a substrate for APC/C^{Cdh1}. First, we saw that Cdh1 influenced Spt21 abundance; elimination of *CDH1* caused an increase in Spt21 levels relative to wild type, whereas overexpression of Cdh1 resulted in reduced levels of Spt21 (Fig. 3 *B* and *C*; Fig. S5*A*). Second, mutation of conserved residues in the KEN box of Spt21 known to be important for KEN-box function caused increased Spt21 levels during G1 phase (*spt21-ken*-TAP, Fig. 3*C*). *spt21-ken*-TAP protein levels were elevated only in G1 phase as revealed using synchronized cells throughout one cell cycle (Fig. S5*B*), consistent with the function of Cdh1 in degrading proteins exclusively during late M and G1 phases (30). Third, we observed a KEN-box–dependent physical interaction between Spt21-TAP and HA-Cdh1 (Fig. 3*D*). To avoid toxic effects of overexpressed Cdh1, expression was induced for not more than 30 min. Finally, G1 cells expressing the stabilized KEN-box mutant of Spt21 (*spt21-ken*-TAP) had higher levels of transcripts from all core histone genes (Fig. 3*E*), and combining the *spt21-ken* mutation with deletion of a component of the HIR complex required for repression of histone gene repression was lethal (Fig. 3*F*). Cell cycle experiments revealed that the increased levels of histone gene transcripts were most prominent in the G1 phase (Fig. S5*C*).

It is not only critical to restrict histone gene expression to S phase during an unperturbed cell cycle, it is also important for histone gene expression to be repressed upon genotoxic stress (31). We saw that both the *spt21-ken*-TAP mutant and the *cdh1Δ* mutant strain were comparably sensitive to hydroxyurea (HU), which inhibits DNA replication (Fig. 3*G*). We also observed a clear upregulation of *HTA2* transcript levels in a *spt21-ken*-TAP mutant under HU treatment (Fig. S5*D*), suggesting that sensitivity to HU might be due to increased histone levels. Also, a double mutant carrying both the *spt21-ken*-TAP allele and a deletion of *YTA7*, a key regulator of histone gene expression, which is also important for recovery from genotoxic stress (32), was inviable in the presence of HU (Fig. 3*G*). We conclude that Spt21 protein abundance is regulated and restricted to S phase by APC/C^{Cdh1} and that Spt21 degradation is also important to ensure that histone gene expression is not inappropriately activated during genotoxic stress. We note that how Spt21 is prevented from accumulating during G2 and M phases is currently unknown. However, because Spt21 does not possess a destruction box (D box), it is unlikely to be a direct target of the G2-M–specific form of the APC, APC/C^{Cdc20}.

Purified Spt21 Is Associated with HAT Activity in Vitro. Previous work has shown that Spt10 function in vivo is dependent on its putative HAT domain and on Spt21 (14, 19), suggesting that Spt21 may activate Spt10. So far, evidence for functionality of the Spt10 HAT domain is based on analysis of a mutant derivative of *SPT10*, *spt10-199*, carrying a mutation predicted to create a catalytically inactive HAT (Fig. S3*A*) (19). Consistent with a role for

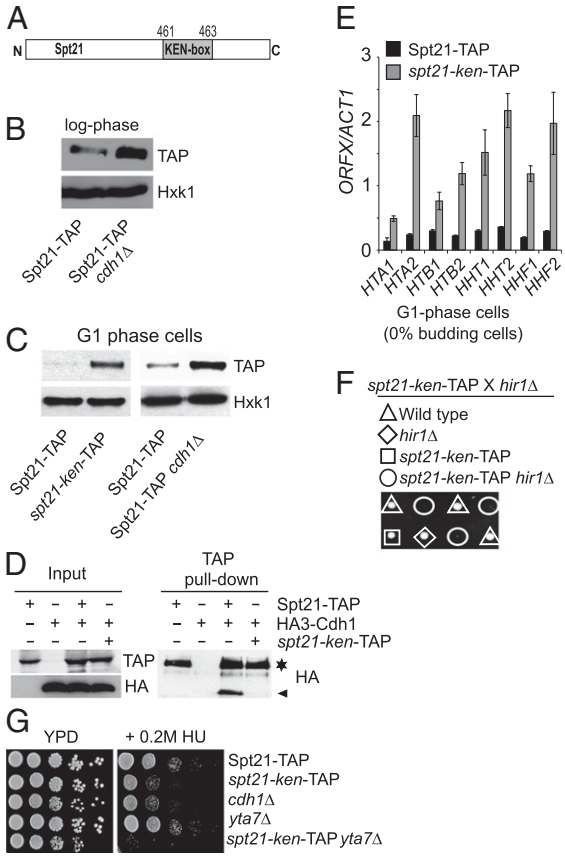

Fig. 3. APC/C^{Cdh1} targets Spt21 for degradation during G1 phase. (*A*) Schematic representation of Spt21 including the canonical KEN box. (*B*) Analysis of Spt21 levels in a *cdh1Δ* strain. Strain bearing Spt21-TAP ± *CDH1* were grown to log phase, and protein samples were taken at the indicated time and analyzed by Western blotting. (*C*) Increased Spt21 protein levels during G1 phase in the absence of *CDH1* or in a strain carrying a KEN-box mutation. Isogenic wild-type Spt21-TAP and *spt21-ken*-TAP mutant strains were arrested with α-factor as described in Fig. 2*A*. Spt21 protein levels were assayed by Western blotting with anti-TAP antibody. A similar experiment was performed using a Spt21-TAP *cdh1Δ* strain. In this case, G1-phase cells were isolated by centrifugal elutriation because the *cdh1* mutant strain is resistant to α-factor (the G1 phase fraction was confirmed by FACS analysis). (*D*) Spt21 physically interacts with Cdh1 in a KEN-box–dependent manner. Isogenic Spt21-TAP, *GAL*-HA3-Cdh1, Spt21-TAP, *GAL*-HA3-Cdh1, and spt21-ken-TAP *GAL*-HA3-Cdh1 strains were grown in galactose to induce Cdh1 expression. Copurifying proteins were analyzed by Western blotting using anti-HA antibody, which detects both the TAP and HA tags. The star marks Spt21-TAP and spt21-ken-TAP, and the arrowhead marks HA3-Cdh1. Input samples were incubated with anti-TAP and anti-HA antibodies, respectively. (*E*) Stabilization of Spt21 in G1 phase causes increased transcription of histone genes. Indicated strains were arrested in G1 phase. cDNA was prepared and levels of all histone gene pair transcripts were analyzed as described in Fig. 2*C* (for primers see ref. 11). Cells were in G1 phase as determined by budding index. Error bars in the experiments represent SDs from the mean of at least three replicate qPCR reactions. (*F*) Stabilized Spt21 shows genetic interaction with a *hir1* deletion mutant. Representative tetrads resulting from sporulation of a *spt21-KEN*-TAP HIR+/ *hir1Δ*/SPT21+ strain are shown. Spores were incubated for 2 d at 30 °C. (*G*) Stabilized Spt21 is sensitive to genotoxic stress and interacts genetically with *YTA7*. Indicated strains were spotted in serial 10-fold dilutions onto medium ±0.2 M HU and incubated for 2 d at 30 °C. Spt21-TAP in a *hir1Δ* or *yta7Δ* background behaved like wild type, indicating no interference of the TAP tag (Fig. S4 *A* and *B*).

Spt21 in activating Spt10, the elevated levels of *HTA1* and *HTA2* mRNA observed when Spt21 was overexpressed were completely suppressed in an *spt10-199* strain (Fig. S3*C*). We note that levels of Spt10-199-TAP protein are significantly reduced relative to Spt10-TAP, suggesting that the HAT domain mutation causes protein instability, making it difficult to attribute phenotypes specifically to HAT activity (Fig. S3*B*).

We next sought to directly assay HAT activity associated with Spt10 and any dependence on Spt21. To do this, we separately purified overexpressed Spt10-TAP and Spt21-TAP from yeast (Fig. S6*A*) and used these proteins in an in vitro HAT assay. Consistent with previous reports (14, 19), we did not detect HAT activity associated with purified Spt10 alone. Surprisingly, we detected a concentration-dependent HAT activity associated with purified Spt21-TAP in vitro against histone H3 and H4 substrates (Fig. S6*B*). We conclude that Spt21 is associated with a histone H3 and H4 HAT activity that does not appear dependent on Spt10, which is required for its association with histone gene promoters.

In Vitro HAT Activity of Spt21 Is Partly Dependent on Gcn5. As noted earlier, Spt21 does not possess a canonical HAT domain, and we wondered if the Spt21-associated HAT activity that we observed in vitro might be due to a copurifying HAT(s). Although we failed to detect other HATs copurifying with Spt21 in our mass spectrometry analysis, we used our ChIP assay to assess association of HATs with histone promoters and Spt10. We used tagged versions of Rtt109 (Rtt109-TAP), Hat1 (Hat1-TAP), NuA4 (Esa1-TAP), and Gcn5 (Gcn5-TAP) to assay their association with histone gene promoters. Only Gcn5, a HAT known to be involved in regulation of G1-specific promoters, was clearly detectable on *HTA1-HTB1* and *HTA2-HTB2* promoters (Fig. 4*A*). To determine if association of Gcn5 with histone gene promoters was dependent on Spt21, and because we could not detect Gcn5 associated with Spt21 in our standard mChIP analysis (Dataset S1), we next affinity-purified Gcn5-TAP in a strain in which the *spt21-ken* mutant was tagged with a HA tag. Using this strategy, we observed copurification of Spt21 with Gcn5 (Fig. 4*B*). Gcn5-TAP also interacts with Spt21-HA wild-type protein, although to a lesser extent than the *spt21-ken*-HA derivative (Fig. S7*A*). We also observed that Gcn5 recruitment to histone promoter chromatin was dependent on Spt21. Elimination of *SPT21* had the most dramatic effect on recruitment of Gcn5 to the *HTA2-HTB2* promoter, whereas recruitment to the *HTA1-HTB1* promoter was only slightly affected (Fig. 4*C*). This observation was corroborated by analysis of histone gene expression, which revealed that deletion of *GCN5* caused a minor but reproducible reduction in *HTA2* and *HTB2* mRNA levels, whereas expression of *HTA1* and *HTB1* were not significantly affected (Fig. 4*D*). Finally, using synchronized cells, we observed a defect in activation of *HTA2* transcription in *gcn5Δ* mutants during early S phase (Fig. S7*B*).

Because Gcn5 interacts with Spt21 and its deletion impacts the transcription of *HTA2* and *HTB2*, we wondered if the in vitro HAT activity that we observed for purified Spt21 might reflect its association with Gcn5. Indeed, we failed to detect H3 acetylation associated with Spt21 purified from a *gcn5Δ* mutant, consistent with the known role of Gcn5 as an H3K9 and H3K14 HAT (Fig. 4*E*) (33). However, H4 acetylation was still clearly present, which suggests that another H4-specific HAT (or HATs) may be present at low amounts in the purified Spt21 sample. Consistent with this possibility, the observed defect in histone gene transcription in a *gcn5Δ* mutant is relatively minor, making it likely that Gcn5 operates redundantly with another HAT to regulate histone transcription. In an attempt to test this possibility, we analyzed the stringently purified material used for in vitro assays by mass spectrometry. We failed to detect any copurifying HAT, including Gcn5 or Spt10, in our sample after analyzing biological replicate purifications (Dataset S2), indicating that interaction with Gcn5, which appears to be biologically meaningful (see above), and other HATs, may be below the detection limit of our method.

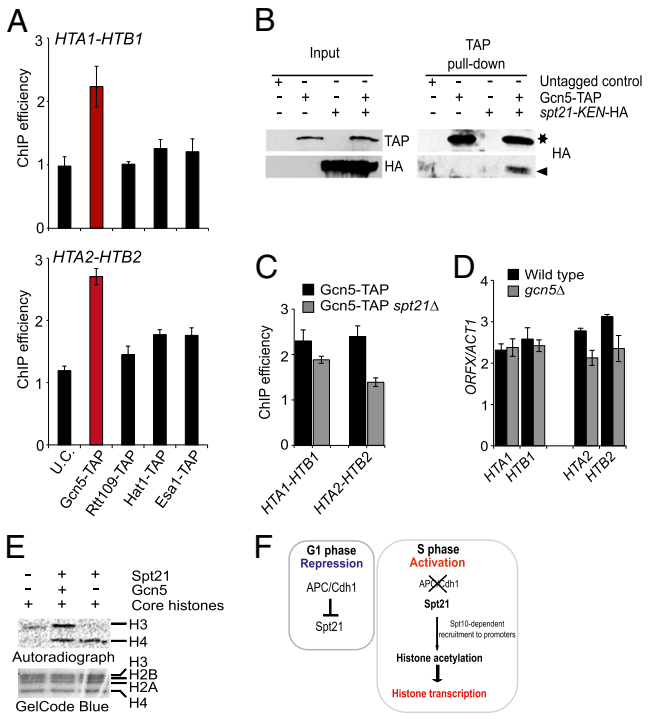

Fig. 4. HAT activity of Spt21 is partly dependent on Gcn5. (*A*) Gcn5-TAP but not other HATs associate with the *HTA1-HTB2* and *HTA2-HTB2* promoters in vivo. Gcn5-TAP, Rtt109-TAP, Esa1-TAP, Hat1-TAP, and control strains (U.C. means untagged control) were grown to midlog phase, and association with *HTA1-HTB1* and *HTA2-HTB2* promoters was assessed as described in Fig. 1*C*. (*B*) Spt21 physically interacts with Gcn5. Isogenic *GCN5*-TAP, *spt21-ken-HA*, or *GCN5*-TAP *spt21-ken*-HA strains were cultured overnight in glucose-containing medium YPD and grown to an OD_{600} nm of 0.8. Gcn5-TAP and copurifying *spt21-ken*-HA was detected by Western blotting using anti-HA antibody, which detects both the TAP and HA tags. The star marks Gcn5-TAP, and the arrowhead marks *spt21-ken*-HA. Input samples were incubated using either anti-TAP or anti-HA antibodies. (*C*) Spt21 recruits Gcn5 to the *HTA2-HTB2* promoter. Strains carrying Gcn5-TAP in the presence (wild type) or absence of *SPT21* (*spt21*Δ) were grown to midlog phase and cross-linked using formaldehyde. Association of Gcn5-TAP with the *HTA1-HTB1* and *HTA2-HTB2* promoters was examined as described in Fig. 1*B*. (*D*) Gcn5 influences *HTA2* and *HTB2* gene transcription. Isogenic wild-type and *gcn5*Δ mutants were grown to midlog phase, and histone transcript levels were assessed as described above (Fig. 2*C*). Error bars represent SDs from the mean of at least three replicate qPCR reactions. (*E*) Gcn5 is responsible for H3 HAT activity associated with Spt21. Overexpressed Spt21-TAP was purified from wild-type and *gcn5*Δ strains, and in vitro assays were performed as described in Fig. S6. (*F*) Model for Spt21 regulation and function as a histone gene regulator. See text for details.

Discussion

Here we show that Spt21 is a key cell cycle oscillator that serves as an important regulator of S-phase–dependent histone gene expression. We showed that Spt21 is recruited to histone loci by Spt10 where it may be required for acetylation of core histones. Spt10 has not been shown previously to have HAT activity, although it contains a putative HAT domain. We purified both Spt10 and Spt21 and detected a copurifying HAT activity specific for histone H3 and H4 substrates with Spt21 but not with Spt10. The in vitro HAT activity in our Spt21 preparations was surprising, given that an identifiable HAT domain is not present in Spt21. The identification of noncanonical HATs is not unprecedented. Rtt109, a known H3K56 HAT (34–36), also has no typical HAT domain detectable by primary amino acid sequence inspection; however, structural analysis revealed that Rtt109 is related to the CBP/p300 family of HATs from mammalian cells (37). We found that Gcn5 accounts for the H3 HAT activity that we detect associated with Spt21 in vitro. However, associated Gcn5 does not appear to explain the Spt21-associated H4 HAT activity, and we failed to detect other known HATs in our purified Spt21 samples using mass spectrometry. Furthermore, we failed to detect association of the two major H4 HATs, Hat1 or NuA4, with histone promoters (Fig. 4*A*). However, the relatively mild reduction in *HTA2* and *HTB2* transcript levels in a *gcn5Δ* mutant makes it likely that there are redundant HAT(s) involved in histone gene regulation. These unknown HATs might also acetylate a nonhistone protein, which might be important in histone gene activation. Although traces of Hat1 or NuA4 not detectable in our mChIP or Western blot-based assays may account for the observed in vitro activity, we cannot exclude the possibility that Spt21 is a previously unidentified H4 HAT that functions specifically on histone promoters during S phase. This idea is highly speculative at this point, and further work will be necessary to identify other Spt21-associated HATs.

We propose a model to describe histone gene activation in yeast where Spt10 recruits HIR to histone promoters outside of S phase, establishing repressive chromatin via HIR-dependent recruitment of Asf1, Rtt106, RSC, and Yta7. During G1 phase, the APC/C^{Cdh1} prevents Spt21 from accumulating and activating histone transcription prematurely (Fig. 4*F*). In S phase, when the APC/C^{Cdh1} is inactivated (30), Spt21 accumulates and is recruited to all histone gene promoters by Spt10, where it acetylates H3 and H4, in part by recruitment of Gcn5 and possibly directly or through recruitment of other unidentified HATs. Histone acetylation relieves HIR-dependent repression, permitting SWI/SNF recruitment and activation of histone gene expression and also activates *HTA2-HTB2* via an unknown mechanism (Fig. 4*F*).

The mechanism for degrading Spt21 during the G2 and M phases remains unknown. However, Spt21 has a functional homolog in fission yeast, Ams2, which is degraded by APC/C^{Cdh1} in G1 (38) and by SCF (Pof3) in G2 and M phases (39). The ortholog of Pof3 in budding yeast is the F-box protein Dia2 (40), which may regulate Spt21 in a similar manner. Together, our results also resolve the long-standing question of which DNA-binding factor recruits HIR and HIR-dependent factors to histone gene promoters; we show that Spt10 is required for HIR binding to promoters. We note that, although a significant sequence-based homolog for Spt21 in larger eukaryotes is not obvious, the human NPAT protein appears to be a functional homolog. Like Spt21, NPAT activates histone gene transcription. NPAT expression peaks in S phase and is important for S-phase entry (41). Interestingly, NPAT bears a putative KEN box in its amino acid sequence, but the biological function of this domain has not been explored. Despite these similarities, the importance of multiple regulatory components in histone gene regulation in both yeast and mammalian systems remains poorly understood, and the conserved elements of this important regulatory pathway may be most fruitfully dissected using the accessible yeast model.

Materials and Methods

Yeast Strains and Methods. Yeast strains (Dataset S3) and antibodies used in this study are described in *SI Materials and Methods*.

mChIP Affinity Purification for Mass Spectrometry and in Vitro HAT Assays. One-step mChIP affinity purification coupled to mass spectrometry analysis was performed as previously described (26) with minor modifications (*SI Materials and Methods*).

ChIP and Quantitative PCR Analyses of Histone Gene Transcription. ChIP, cDNA synthesis, and quantitative PCR (qPCR) to analyze histone mRNA levels were conducted as described (11).

TAP Pull-Down Assay. Two hundred milliliters of logarithmically growing cells (OD at 600 nm ~1) were harvested, frozen in liquid nitrogen, and processed as described in *SI Materials and Methods*.

In Vitro HAT Assays. In vitro HAT assays were performed using core histones (Millipore) and C14-labeled acetyl-CoA (60 mCi/mmol) and purified Spt10, Spt21, or both. Proteins were separated by SDS/PAGE and stained with GelCode Blue staining reagent before preparation for autoradiography. Gels were dried and exposed to a phosphoscreen for 4 d. Radioactivity was detected using a Typhoon scanner. Gels were dried and exposed to a phosphoscreen for 4 d.

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1. Kurat CF, et al. (2013) Regulation of histone gene transcription in yeast. *Cell Mol Life Sci* 71(4):599–613.
2. Eriksson PR, Ganguli D, Nagarajavel V, Clark DJ (2012) Regulation of histone gene expression in budding yeast. *Genetics* 191(1):7–20.
3. Hereford LM, Osley MA, Ludwig TR II, McLaughlin CS (1981) Cell-cycle regulation of yeast histone mRNA. *Cell* 24(2):367–375.
4. Osley MA, Gould J, Kim S, Kane MY, Hereford L (1986) Identification of sequences in a yeast histone promoter involved in periodic transcription. *Cell* 45(4):537–544.
5. Osley MA, Lycan D (1987) Trans-acting regulatory mutations that alter transcription of Saccharomyces cerevisiae histone genes. *Mol Cell Biol* 7(12):4204–4210.
6. Prochasson P, Florens L, Swanson SK, Washburn MP, Workman JL (2005) The HIR corepressor complex binds to nucleosomes generating a distinct protein/DNA complex resistant to remodeling by SWI/SNF. *Genes Dev* 19(21):2534–2539.
7. Xu H, Kim UJ, Schuster T, Grunstein M (1992) Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in Saccharomyces cerevisiae. *Mol Cell Biol* 12(11):5249–5259.
8. Fillingham J, et al. (2009) Two-color cell array screen reveals interdependent roles for histone chaperones and a chromatin boundary regulator in histone gene repression. *Mol Cell* 35(3):340–351.
9. Tackett AJ, et al. (2005) Proteomic and genomic characterization of chromatin complexes at a boundary. *J Cell Biol* 169(1):35–47.
10. Ng HH, Robert F, Young RA, Struhl K (2002) Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. *Genes Dev* 16(7):806–819.
11. Kurat CF, et al. (2011) Restriction of histone gene transcription to S phase by phosphorylation of a chromatin boundary protein. *Genes Dev* 25(23):2489–2501.
12. Lombardi LM, Ellahi A, Rine J (2011) Direct regulation of nucleosome density by the conserved AAA-ATPase Yta7. *Proc Natl Acad Sci USA* 108(49):E1302–E1311.
13. Zunder RM, Rine J (2012) Direct interplay among histones, histone chaperones, and a chromatin boundary protein in the control of histone gene expression. *Mol Cell Biol* 32(21):4337–4349.
14. Xu F, Zhang K, Grunstein M (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell* 121(3):375–385.
15. Dollard C, Ricupero-Hovasse SL, Natsoulis G, Boeke JD, Winston F (1994) SPT10 and SPT21 are required for transcription of particular histone genes in Saccharomyces cerevisiae. *Mol Cell Biol* 14(8):5223–5228.
16. Eriksson PR, Ganguli D, Clark DJ (2011) Spt10 and Swi4 control the timing of histone H2A/H2B gene activation in budding yeast. *Mol Cell Biol* 31(3):557–572.
17. Eriksson PR, et al. (2005) Global regulation by the yeast Spt10 protein is mediated through chromatin structure and the histone upstream activating sequence elements. *Mol Cell Biol* 25(20):9127–9137.
18. Fassler JS, Winston F (1988) Isolation and analysis of a novel class of suppressor of Ty insertion mutations in Saccharomyces cerevisiae. *Genetics* 118(2):203–212.
19. Hess D, Liu B, Roan NR, Sternglanz R, Winston F (2004) Spt10-dependent transcriptional activation in Saccharomyces cerevisiae requires both the Spt10 acetyltransferase domain and Spt21. *Mol Cell Biol* 24(1):135–143.
20. Natsoulis G, Dollard C, Winston F, Boeke JD (1991) The products of the SPT10 and SPT21 genes of Saccharomyces cerevisiae increase the amplitude of transcriptional regulation at a large number of unlinked loci. *New Biol* 3(12):1249–1259.
21. Natsoulis G, Winston F, Boeke JD (1994) The SPT10 and SPT21 genes of Saccharomyces cerevisiae. *Genetics* 136(1):93–105.
22. Mendiratta G, Eriksson PR, Clark DJ (2007) Cooperative binding of the yeast Spt10p activator to the histone upstream activating sequences is mediated through an N-terminal dimerization domain. *Nucleic Acids Res* 35(3):812–821.
23. Mendiratta G, Eriksson PR, Shen CH, Clark DJ (2006) The DNA-binding domain of the yeast Spt10p activator includes a zinc finger that is homologous to foamy virus integrase. *J Biol Chem* 281(11):7040–7048.
24. Neuwald AF, Landsman D (1997) GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem Sci* 22(5):154–155.
25. Chang JS, Winston F (2013) Cell-cycle perturbations suppress the slow-growth defect of spt10Delta mutants in Saccharomyces cerevisiae. *G3 (Bethesda)* 3(3):573–583.
26. Lambert JP, Mitchell L, Rudner A, Baetz K, Figeys D (2009) A novel proteomics approach for the discovery of chromatin-associated protein networks. *Mol Cell Proteomics* 8(4):870–882.
27. Masumoto H, Hawke D, Kobayashi R, Verreault A (2005) A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* 436(7048):294–298.
28. Celic I, et al. (2006) The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation. *Curr Biol* 16(13):1280–1289.
29. Herrero AB, Moreno S (2011) Lsm1 promotes genomic stability by controlling histone mRNA decay. *EMBO J* 30(10):2008–2018.
30. Peters JM (2006) The anaphase promoting complex/cyclosome: A machine designed to destroy. *Nat Rev Mol Cell Biol* 7(9):644–656.
31. Lycan DE, Osley MA, Hereford LM (1987) Role of transcriptional and post-transcriptional regulation in expression of histone genes in Saccharomyces cerevisiae. *Mol Cell Biol* 7(2):614–621.
32. Gradolatto A, et al. (2008) Saccharomyces cerevisiae Yta7 regulates histone gene expression. *Genetics* 179(1):291–304.
33. Kuo MH, et al. (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* 383(6597):269–272.
34. Collins SR, et al. (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* 446(7137):806–810.
35. Driscoll R, Hudson A, Jackson SP (2007) Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science* 315(5812):649–652.
36. Han J, et al. (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* 315(5812):653–655.
37. Tang Y, et al. (2008) Fungal Rtt109 histone acetyltransferase is an unexpected structural homolog of metazoan p300/CBP. *Nat Struct Mol Biol* 15(7):738–745.
38. Trickey M, Fujimitsu K, Yamano H (2013) Anaphase-promoting complex/cyclosome-mediated proteolysis of Ams2 in the G1 phase ensures the coupling of histone gene expression to DNA replication in fission yeast. *J Biol Chem* 288(2):928–937.
39. Takayama Y, et al. (2010) Hsk1- and SCF(Pof3)-dependent proteolysis of S. pombe Ams2 ensures histone homeostasis and centromere function. *Dev Cell* 18(3):385–396.
40. Jonkers W, Rep M (2009) Lessons from fungal F-box proteins. *Eukaryot Cell* 8(5):677–695.
41. Zhao J, et al. (2000) NPAT links cyclin E-Cdk2 to the regulation of replication-dependent histone gene transcription. *Genes Dev* 14(18):2283–2297.