2021

MECHANISM OF DNA DAMAGE CHECKPOINT (DDC)- MEDIATED REPRESSION OF HISTONE MRNAS AND ROLE OF ACETYL-COA IN HISTONE TRANSCRIPTION

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MECHANISM OF DNA DAMAGE CHECKPOINT (DDC)-MEDIATED REPRESSION OF HISTONE MRNAS AND ROLE OF ACETYL-COA IN HISTONE TRANSCRIPTION

A dissertation submitted in partial fulfillment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

to the faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES

of

ST. JOHN'S COLLEGE OF LIBERAL ARTS AND SCIENCES

at

ST. JOHN'S UNIVERSITY

New York

by

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Date submitted: 8/17/2021 Date approved: 8/17/2021

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ABSTRACT

MECHANISM OF DNA DAMAGE CHECKPOINT (DDC)- MEDIATED REPRESSION OF HISTONE MRNAS AND ROLE OF ACETYL-COA IN HISTONE TRANSCRIPTION

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My dissertation contains two projects. The first project is focused on the mechanism of DNA damage checkpoint (DDC)- mediated repression of histone mRNAs. The second project deals with the role of acetyl-CoA in histone transcription.

Proliferating cells coordinate histone and DNA synthesis to maintain correct stoichiometric amounts for chromatin assembly. Histone mRNA levels must be repressed when DNA replication is inhibited to prevent toxicity and genome instability due to free non-chromatinized histone proteins. In the first project we show that, unlike in mammalian cells, DNA replication stress does not trigger decay of histone mRNAs in the yeast *Saccharomyces cerevisiae*. However, histone mRNAs can be degraded by both 5' to 3' and 3' to 5' pathways as indicated by significantly elevated half-lives of histone mRNAs in strains with defects in deadenylation, decapping, and exonucleolytic degradation in 5' to 3' and 3' to 5' directions. Replication stress inhibits transcription of histone genes by removing the histone genes-specific transcription factors Spt10p and Spt21p, disassembling the preinitiation complexes and evicting RNA Pol II from histone promoters by a mechanism that is facilitated by checkpoint kinase Rad53p and histone chaperone Asf1p. In contrast, replication stress does not affect occupancy of Swi4p, the DNA binding subunit of the SBF complex, at the histone promoters, suggesting that Spt10p and Spt21p are responsible for the transcriptional downregulation of histone genes during replication stress.

In the second project, we determined the role of acetyl-CoA in histone transcription. Acetyl-CoA is a key metabolite linking catabolism and anabolism. This central position endows acetyl-CoA with an important regulatory and signaling role, and the acetyl-CoA level reflects the energetic and metabolic state of the cell. Acetyl-CoA also serves as a substrate for lysine acetyltransferases that catalyze the transfer of acetyl groups to lysines in a vast array of proteins, including histones. The level of nucleocytosolic acetyl-CoA regulates the global acetylation of chromatin histones and the transcription of many genes. Here we show that reduced synthesis of nucleocytosolic acetyl-CoA leads to reduced acetylation of chromatin histones in the promoters of histone genes, and decreased histone transcription. The globally altered chromatin structure triggers mitochondrial biogenesis and respiration and leads to increased synthesis of ATP.

Together, our data indicate that the depletion of the energy metabolite acetyl-CoA is compensated for by the induction of ATP synthesis.

DEDICATION

This thesis is dedicated to my father, Satish Bhagwat, for his unconditional love, encouragement, and support. He taught me to aim high, achieve that with perseverance and without complaint. He is the person I will always aspire to be.

ACKNOWLEDGMENTS

First and foremost, I am extremely grateful to my mentor Dr. Ales Vancura for his invaluable advice, continuous support, and patience during my Ph. D. study. His immense knowledge and plentiful experience have encouraged me in all the time of my academic research and daily life. I am thankful that you believed in me and helped me throughout the process. I would like to thank my helpful and encouraging lab mates, Dr. Luciano Galdieri, Dr. Tiantian Zhang, Dr. Pengli Bu, Pritpal Kaur, Shreya Nagar, and Riddhi Mehta. It is their kind help and support that has made my research stronger and my academic journey smooth.

I would like to sincerely thank my committee members Dr. Ivana Vancurova, Dr. Yong Yu, Dr. Yan Zhu, and Dr. Matteo Ruggiu for their valuable help throughout the course. Thank you very much for taking time out of your busy schedule to review my work and its progress over the period.

I am grateful to Dr. Yong Yu for being the best graduate director and helping me out from time to time in cases of crucial visa situations and making academic progress uninterrupted.

I would also like to thank all my friends and professors in the Department of Biology, St. John's University for making the entire journey smooth and productive.

Finally, I would like to express my sincere gratitude to my parents, Surekha and Satish Bhagwat, my husband, Satyajit Deshpande, and my family for their rock-solid support and love. Without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study.

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SECTION I

1.1Introduction

Proliferating cells need to maintain a delicate balance between histone and DNA synthesis to ensure correct stoichiometric amounts for chromatin assembly and to avoid genome instability (1, 2). Treatment of cells with genotoxic agents that damage DNA and inhibit DNA replication decreases histone mRNAs to avoid accumulation of free non-chromatinized histone proteins, which are toxic to the cell (3-5). While it is well established that DNA replication correlates with histone mRNA levels, and that activation of DNA damage checkpoint (DDC) or DNA replication checkpoint (DRC) represses histone mRNA levels (3-5), the corresponding mechanisms are not fully understood. The level of mRNA is determined by its synthesis and degradation rates; previous studies have suggested that DRC activation affects both histone transcription and histone mRNA stability (6-12).

Budding yeast possesses two genes encoding each of the four major core histones. The genes are organized into four loci, each containing two histone genes divergently transcribed from a central promoter. Two loci, *HHT1-HHF1* and *HHT2-HHF2*, encode identical H3 and H4 proteins. The other two loci, *HTA1-HTB1* and *HTA2-HTB2*, encode almost identical H2A and H2B proteins (Fig. 1). The expression of histone genes is activated by Spt10p, Spt21p, SBF, and MBF, and repressed by the HIR complex (1, 2, 13-15). Spt10p is a histone gene-specific transcription factor that binds to the upstream-activation sequences of all histones (14, 16, 17). During the S phase, Spt10p associates with Spt21p (18, 19). The protein level of Spt21p is cell cycle regulated; Spt21p is degraded during G1 and G2/M phases and accumulates only during S phase, when it binds to histone gene promoters and recruits Gcn5p HAT (15). Activation of histone gene promoters during

S phase also requires chromatin remodeling complex SWI/SNF (20). In addition, the activation of histone genes early in the S phase is mediated by SBF and MBF complexes (21). The SBF complex consists of Swi4p and Swi6p. The MBF complex consists of Mbp1p and Swi6p. Mbp1p and Swi4p proteins are the DNA-binding components of MBF and SBF, respectively, while Swi6p plays a regulatory role in both complexes. Outside of S phase, Spt10p facilitates recruitment of the HIR complex to the NEG elements in the histone promoters to establish their repression (1, 2, 15). The HIR complex and Spt10p recruit a myriad of additional factors, including histone chaperones Asf1p and Rtt106, chromatin boundary protein Yta7p, and chromatin remodeling complex RSC, which together repress histone genes transcription (1, 2). During S phase, Spt10p associates with Spt21p at the histone gene promoters and both Spt10p/Spt21p are required for normal transcription of histone genes (18). Outside of S phase, Spt10p facilitates recruitment the HIR complex and associated chromatin regulators to histone gene promoters to establish their repression (19). The protein level of Spt21p is cell cycle regulated; Spt21p is degraded during G1 and G2/M phases and accumulates only during S phase, when it binds to histone gene promoters and recruits Gcn5p HAT (18-20). In addition, activation of histone gene promoters during s phase also requires chromatin remodeling complex SWI/SNF (20, 23). Outside of S phase, the expression of histone genes is repressed by the HIR complex that binds to the NEG region in histone promoters (1, 2). The HIR complex recruits a myriad of additional factors, including histone chaperones Asf1p and Rtt106, chromatin boundary protein Yta7p, and chromatin remodeling complex RSC, which together repress histone genes transcription (1, 2).

The transcriptional repression of histone genes at the end of S phase or during replication stress induced by hydroxyurea (HU) treatment operates at all 4 histone loci and, except for *HTA2-HTB2*, requires the HIR complex, Rtt106p and Asf1p (22-24). Rtt106p is recruited to histone promoters in a histone-dependent manner, indicating that the repressive chromatin structure at the histone promoters requires free histones, which accumulate when DNA replication stops (24, 25).

In this study, we show that histone mRNAs are degraded by both 5' to 3' and 3' to 5' pathways; however, activation of DRC does not trigger decay of histone mRNA. Replication stress inhibits transcription of histone genes by removing Spt10p and Spt21p from histone promoters. The replication stress-induced disassembly of the preinitiation complex (PIC) at the histone promoters is facilitated by Rad53p and Asf1p.

1.2 Results

Genotoxic stress represses histone mRNA levels

To determine whether DDC/DRC activation represses mRNA levels of all histones, we utilized bleocin, 4-nitroquinoline 1-oxide (4-NQO), and hydroxyurea (HU). Bleocin belongs to the antibiotic bleomycin family and causes DNA double strand breaks (26). 4-NQO mimics the effect of UV light and forms DNA adducts (27). HU is an inhibitor of ribonucleotide reductase, decreases dNTP levels, and slows down progression of the replication fork. Treatment of wildtype cells with either chemical rapidly decreased mRNA levels of all histone genes (Fig. 2). This effect depended on Rad53p, since the repression of histone mRNA levels was significantly attenuated in *rad53*Δ*sml1*Δ cells (Fig. 3). This is in agreement with our previous results that showed histone mRNA levels reduced in Rad53p-dependent manner in *rad52*Δ cells (28). *RAD52* is required for DNA double-strand break repair and homologous recombination. Inactivation of *RAD52* makes cells unable to repair DNA strand breaks and activates DDC (29).

To systematically determine whether other checkpoint kinases are required for replication stress induced repression of histone mRNAs, we first determined histone mRNA levels in wild-type, *mec1*Δ*sml1*Δ, *tel1*Δ, *mec1*Δ*tel1*Δ [*RNR1*], *rad53*Δ*sml1*Δ, *chk1*Δ, and *dun1*Δ cells; *mec1*Δ and *rad53*Δ cells are viable only if harboring the *sml1*Δ mutation or overexpress *RNR1* gene (30). *rad53*Δ*sml1*Δ cells displayed highest mRNA levels for all histones (Fig. 4A). This is in agreement with a recent study demonstrating that under physiological conditions, Rad53p regulates histone mRNA levels by phosphorylating Spt21p (31). To assess the involvement of individual checkpoint kinases in HU-mediated repression of histone mRNAs, we calculated fractions of histone mRNAs

remaining after 30 min of HU exposure (Fig. 4B). In wild-type cells, the level of repression varied for individual histone genes, ranging from about 10% for *HTA2*, to about 60% for *HHT2*, perhaps suggesting that individual histone genes have partly unique modes of regulation. Cumulatively, the results show that all checkpoint kinases play a role in repressing histone mRNA levels in response to HU treatment, working either redundantly through the same mechanism or individually through different mechanisms (Fig. 4B).

Histone mRNAs are degraded by canonical 5' to 3' and 3' to 5' pathways

The level of mRNA is determined by its synthesis and degradation rates. mRNA degradation is an important factor in the regulation of gene expression, and complements transcriptional regulation by enabling cells to rapidly vary the levels of existing transcripts. Previous studies have suggested that DDC/DRC activation affects both histone transcription and histone mRNA stability in yeast (6-12).

Degradation of mRNA occurs by two general pathways, in 3' to 5' direction, or in 5' to 3' direction (Fig. 5) (32). Both pathways are initiated by deadenylation, which results in shortening of the 3' poly(A) tail. Deadenylation is carried out by the Pan2p/Pan3p complex as well as by the Ccr4p/Pop2p/Not complex. Subsequently, the cap structure (5'-m7GpppN) is removed by the decapping complex Dcp1p/Dcp2p, and mRNA is degraded in 5' to 3' direction by the major cytoplasmic enzyme Xrn1p. Interestingly, Xrn1p is a substrate of Rad53p (33). Alternatively, deadenylated mRNAs can be degraded from their 3' ends by the exosome, a multimeric complex with 3' to 5' exoribonuclease activity; Ski2p, Ski3p, and Rrp6p are subunits of the exosome (32).

To investigate the pathway responsible for histone mRNAs decay, we inhibited transcription with thiolutin and determined half-lives of histone mRNAs by following mRNA levels of individual histones by RT-qPCR. We used primers that amplify 3' end of the translated sequence and adjacent not-translated portion and can differentiate between the two genes encoding each histone. Our results showed that deadenylation carried out by both Pan2p/Pan3p and Ccr4p/Pop2p/Not complexes play a role in regulating histone mRNA decay, with Ccr4p/Pop2p/Not complex playing the more dominant role (Fig. 6A). Inactivation of the decapping complex Dcp1p/Dcp2p by *dcp2*Δ mutation also significantly stabilized histone mRNAs. After decapping, majority of histone mRNAs were degraded by the 5' to 3'exonuclease Xrn1p, as indicated by greatly stabilized histone mRNAs in *xrn1*Δ cells. In comparison, the cytosolic exosome played a lesser role in degradation of histone mRNAs than Xrn1p, as suggested by histone mRNA half-lives in *ski2*Δ cells. Ski2p is required for cytosolic function of the exosome (32). Conversely, inactivation of *RRP6*, required for the function of the exosome in the nucleus, affected stability of the histone mRNAs only slightly (Fig. 6A). We conclude that histone mRNAs are degraded by both 5' to 3' and 3' to 5' pathways, with Xrn1p making the major contribution in the 5' to 3' pathway.

Since inactivation of individual components of 5' to 3' and 3' to 5' decay pathways resulted in significantly elevated half-lives of histone mRNAs, we anticipated that the steady state levels of histone mRNAs would be correspondingly increased. However, this prediction turned out to be correct only for *xrn1*Δ, *dcp2*Δ, and, to a lesser extent, *ski3*Δ mutants. The steady-state histone mRNA levels were almost unchanged in *ccr4*Δ and *pan2*Δ cells, despite their significantly increased stability (Fig. 6B). We believe that this

result can be explained by transcriptional buffering, where a defect in mRNA decay pathway is compensated for by a decrease in transcription (34-37). This possibility is in agreement with the notion that Xrn1p is required for transcriptional buffering and *xrn1*Δ cells accumulate mRNAs (35). Whether Dcp1p is also required for transcriptional buffering has not been determined yet. Despite the significantly elevated histone mRNA levels in *xrn1*Δ and *dcp2*Δ cells, the level of histone H3 protein in these mutants was not correspondingly increased (Fig. 6C). These results suggest that the histone mRNAs in *xrn1*Δ and *dcp2*Δ cells are not translating and are probably stored in P bodies. This interpretation is in agreement with the observation that the size and number of P bodies are elevated in *xrn1*Δ and *dcp2*Δ cells (38).

DRC activation does not destabilize histone mRNAs

To determine whether activation of the DDC/DRC affects stability of histone mRNAs, we incubated the cells for 60 min in the presence of 200 mM HU and determined histone mRNA half-lives. In an alternative approach, we determined histone mRNA half-lives in *rad52*Δ cells (Fig. 7A). Regardless of whether DDC/DRC was activated by treating cells with HU or by using the *rad52*Δ mutation, DDC/DRC activation did not destabilize histone mRNAs and thus cannot account for the decreased histone mRNA levels in cells treated with genotoxic chemicals (Fig. 2). On the contrary, DDC/DRC activation resulted in somewhat increased half-lives of histone mRNAs (Fig. 7A). To confirm this result, in addition to primers that amplify 3' ends of histone mRNAs, we also used primers that amplify 5' ends of the translated sequences. However, these primers do not differentiate between the two transcripts of each histone and the reported halflives ($t_{1/2}$) represent

cumulative stabilities of the two mRNAs (*HTA1* and *HTA2*, *HTB1* and *HTB2*, *HHT1* and *HHT2*, and *HHF1* and *HHF2*). The mRNA stabilities measured with these primers confirm that DDC/DRC activation does not destabilize histone mRNAs (Fig. 7B).

DDC/DRC activation inhibits transcription of histone genes independently of SBF and MBF

Since DDC/DRC activation did not destabilize histone mRNAs, we next addressed whether DDC/DRC affects transcription of histone genes. The obvious candidates that link DDC/DRC and histone gene transcription are G1/S–specific transcription complexes SBF and MBF (1, 2). DDC/DRC activation induces Rad53p–dependent phosphorylation of Swi6p, resulting in downregulation of *CLN1* and *CLN2* transcription, and delayed G1 to S progression (39, 40). On the other hand, Rad53p phosphorylates and inactivates Nrm1p, a co-repressor of MBF, resulting in the activation of MBF targets (41, 42). In addition, a systematic phosphoproteomics screen identified Swi6p, Swi4p, and Mbp1p as direct targets of Rad53p (43).

To address the roles of MBF and SBF in DDC/DRC-mediated repression of histone genes, we determined histone mRNA levels during cell cycle in wild-type cells and *swi6*Δ cells in the absence or presence of HU (Fig. 8). Expression of all histone genes in wild-type cells peaked at 15-30 min after release from α-factor mediated arrest. The expression of histone genes in *swi6*Δ cells was somewhat delayed and attenuated in comparison with wild-type cells, peaking at 45 min. However, histone mRNAs in *swi6*Δ cells still displayed a cell cycle-dependent profile, suggesting that the inactivation of SBF and MBF complexes does not prevent accumulation of Spt21p at the promoters of histone genes and activation

of histone transcription during the S phase. Importantly, induction of DRC by HU in *swi6*Δ cells almost completely eliminated the accumulation of histone mRNAs during the S phase. Since both SBF and MBF complexes are inactivated in *swi6*Δ cells, we interpret these results to mean that DRC represses transcription of histone genes at least partly independently of SBF and MBF. The DRC-mediated repression of histone genes in the *swi6*Δ cells during S phase is also clear for *HTA2* and *HTB2* genes. Unlike the other three histone gene pairs, the *HTA2-HTB2* promoter does not contain the NEG sequence, required for the assembly of the Hir complex and repression of histone genes outside of the S phase (1, 2). This observation suggests that DRC represses histone genes independently of the Hir complex assembled at the NEG sequence.

DRC-triggered repression of histone mRNAs is attenuated in asf1Δ cells

Mutations that compromise the negative feedback regulation of histone transcription, such as *asf1*Δ or *HIR* mutations, render histone genes less responsive to HU-mediated repression (22, 24). To find out if other histone chaperones are required for HU-mediated repression of histone transcription, we determined *HTA1, HTA2*, and *HHT2* mRNA levels in wild-type, *asf1*Δ, *rtt106*Δ, *rtt109*Δ, *cac1*Δ, and *hir1*Δ cells before and after 30 min exposure to HU. The histone mRNA levels in *rtt106*Δ, *rtt109*Δ, *hir1*Δ, and *cac1*Δ cells were comparable or slightly elevated in comparison with wild-type cells (Fig. 9A). The replication stress-induced repression of *HTA1, HTA2*, and *HHT2* mRNAs was slightly attenuated in *rtt106*Δ, *rtt109*Δ, *hir1*Δ, and *cac1*Δ cells in comparison with wild-type cells as shown by the fractions of histone mRNAs remaining after 30 min of HU exposure (Fig. 9B). In *asf1*Δ cells, mRNA levels of individual histones were reduced to

varying degrees and the repression of histone genes was significantly attenuated (Fig. 9C and D). This is in agreement with the notion that *asf1*Δ mutation renders histone genes immune to HU repression (22). The requirement of Asf1p for HU-mediated repression of histone transcription appears to be independent of Asf1p's role in promoting H3K56 acetylation, since Rtt109p, the HAT responsible for H3K56 acetylation was not required for HU-mediated repression (Fig. 9D). In addition, *hir1*Δ and *rtt106*Δ mutants were competent for HU-mediated repression of histone transcription, indicating that the HIR and Rtt106p chaperones do not have redundant roles with Asf1p in HU-mediated repression of histone genes.

DRC activation removes preinitiation complex (PIC) and RNA Pol II from histone promoters in Rad53p- and Asf1p-dependent manner

To determine whether DRC activation affects the assembly of the PIC, we determined the occupancy of Spt15p, the yeast TATA-binding protein (TBP), and Rpb1p, the largest subunit of RNA Pol II, at the histone genes in wild-type, *rad53*Δ*sml1*Δ, *mec1*Δ*sml1*Δ, and *asf1*Δ cells before and after treatment with HU. The occupancies of RNA Pol II at histone genes were reduced to about 15-50% by 15 min HU treatment in wild-type cells (Fig. 10A). This is in agreement with the reduced histone mRNA levels in HU-treated cells (Fig. 2). The occupancies of RNA Pol II at histone genes in *rad53*Δ*sml1*Δ cells before HU treatment were somewhat elevated in comparison with wild-type cells, in agreement with elevated histone mRNA levels in *rad53*Δ*sml1*Δ cells (Fig. 4A). HU treatment reduced the RNA Pol II occupancy at histone genes in *rad53*Δ*sml1*Δ cells to 27-56%, slightly less significantly than in wild-type cells. The occupancies of RNA Pol II at

histone genes in *mec1*Δ*sml1*Δ cells before HU treatment were elevated in comparison with wild-type cells, and the HU treatment reduced the RNA Pol II occupancy in *mec1*Δ*sml1*Δ cells to 20-40%, similarly as in the wild-type cells. The occupancies of RNA Pol II at histone genes in *asf1*Δ cells before HU treatment were reduced in comparison with wild-type cells (Fig. 10A), mirroring reduced histone mRNA levels in *asf1*Δ cells (Fig. 9C). In contrast with wild-type and *mec1*Δ*sml1*Δ cells, the RNA Pol II occupancies at histone genes in *asf1*Δ cells were reduced only to about 57-83% by the HU treatment (Fig. 10A).

Since DNA damage may trigger stalling and arrest of RNA Pol II, leading to polyubiquitination and degradation of Rpb1p, we determined Rpb1p levels during the HU treatment in wild-type cells (Fig. 9B). The results showed that the 15 min incubation with 200 mM HU, the condition employed in our ChIP experiments, did not result in degradation of Rpb1p (Fig. 10B). This result suggests that the treatment with HU triggers removal of RNA Pol II from the histone genes, but not its degradation.

The occupancies of Spt15p at the histone promoters were similar in wild-type and *mec1*Δ*sml1*Δ cells before and after the HU treatment, which reduced Spt15p occupancy to about 20% (Fig. 10C). The occupancies of Spt15p in *rad53*Δ*sml1*Δ and *asf1*Δ cells were significantly reduced in comparison with wild-type cells; however, the occupancies were not significantly affected by the HU treatment.

DRC activation evicts Spt10p and Spt21p from histone promoters

Since Spt10p/Spt21p are the key factors responsible for cell cycle-dependent transcription of histone genes, we determined their occupancy at the histone promoters before and after HU treatment. Similarly to Spt15p and RNA Pol II, occupancies of Spt10p

and Spt21p at the histone promoters before HU treatment were significantly reduced in *asf1*Δ cells (Fig. 11A and B). The requirement of Asf1p for recruitment of Spt10p/Spt21p to histone promoters is not entirely surprising, since Asf1p is required for transcriptional activation of several inducible genes by disassembling promoter chromatin (44). These results suggest that Asf1p plays an important role not only in mediating transcriptional response of histones to replication stress imposed by HU, but also in assembly of the PICs at histone promoters in the absence of replication stress. It appears that the recruitment of Sp10p/Spt21p and Asf1p to histone promoters is mutually dependent. The Asf1p occupancy at histone promoters was reduced in *spt10*Δ and *spt21*Δ cells (15), and the occupancy of Spt10p and Spt21p was reduced in *asf1*Δ cells (Fig. 7A and B).

HU-mediated replication stress reduced the occupancies of Spt10p and Spt21p in both wild-type and *rad53*Δ*sml1*Δ cells. However, in *asf1*Δ cells, the Spt10p and Spt21p occupancies were not significantly affected by HU treatment (Fig. 11A and B). Cumulatively, these results indicate that the replication stress-induced removal of Spt10p from histone promoters requires Asf1p but not Rad53p (Fig. 11A). However, the replication stress-induced removal of Spt21p from histone promoters was attenuated in *rad53*Δ*sml1*Δ cells (Fig. 11B) as shown by the fractions of Spt21p remaining at the histone promoters after 15 min HU exposure (Fig. 11C). These results suggest that the HU-mediated replication stress inhibits histone transcription by two, perhaps parallel mechanisms. First, Spt10p is removed from the histone promoters in Asf1p-dependent manner, and second, Rad53p contributes to the removal of Spt21p.

Degradation of Spt10p and Spt21p during incubation with HU was not responsible for the reduced occupancy of both proteins at the histone promoters; while the Spt10p level

was unchanged during HU treatment, the Spt21p level slightly increased, probably reflecting accumulation of cells in the S phase (Fig. 11D). Cumulatively, these results suggest that the HU induced replication stress disassembles the PIC complex and evicts RNA Pol II from the histone genes by a mechanism that depends on Asf1p and Rad53p.

Since the DRC activation-triggered removal of Spt10p and Spt21p from histone promoters was quite unexpected, we also examined the occupancy of Swi4p, the DNA binding subunit of the SBF complex, at the histone promoters. Unlike Spt10p and Spt21p, the occupancy of Swi4p at histone promoters was not affected by HU treatment (Fig. 11E). This finding indicates that the removal of Spt10p and Spt21p from histone promoters is not a common response of transcription factors to DRC activation.

1.3 Discussion

In mammalian cells, replication-dependent histone mRNAs are not polyadenylated, but their sequence in the 3'-untranslated region forms a stem loop structure bound by a stem loop binding protein. At the end of S phase, or when the DNA replication is inhibited during the S phase, histone mRNAs are degraded in a stem loop-dependent manner by 5' to 3' and 3' to 5' pathways (45, 46). Unlike in mammalian cells, histone mRNAs in *S. cerevisiae* are polyadenylated. Early studies in budding yeast indicated that mRNA degradation contributes to disappearance of histone mRNAs at the end of S phase (6). This posttranscriptional control of histone mRNAs is likely facilitated by the 3' ends of histone mRNAs, since these sequences are capable of conferring cell cycle-specific regulation on heterologous mRNAs (7-9). In addition, inactivation of *RRP6*, component of the nuclear exosome, or *TRF4/TRF5*, components of the TRAMP complex, elevates histone mRNA levels (10, 11). TRAMP complex polyadenylates RNA substrates prior to their degradation by the nuclear exosome. Histone mRNA levels are also elevated in *lsm1*Δ mutant (12, 47); *LSM1* is a component of the Lsm1-7-Pat1 complex that stimulates decapping of mRNA. These results are consistent with our data (Fig. 6A) that indicate that histone mRNAs are regulated by degradation in 5' to 3' and 3' to 5' decay pathways. Inactivation of *PAN2, CCR4, DCP2, XRN1*, and *SKI2*, major players in both 5' to 3' and 3' to 5' decay pathways, resulted in significantly increased half-lives of histone mRNAs (Fig. 6A).

Histone mRNA levels correlate with DNA replication and must be suppressed when DNA replication is inhibited to prevent toxicity and genome instability due to free non-chromatinized histone proteins. One could argue that since half-lives of histone mRNAs in yeast are quite short under normal conditions (Fig. 4A) (48-50), further destabilization by

DRC activation would not provide sufficiently effective regulatory strategy. Our data appear to support this logic and show that activation of DDR/DRC does not destabilize histone mRNAs, but inhibits transcription of histone genes, as indicated by the disassembly of the PIC complex at the histone promoters after HU treatment (Fig. 10 and 11).

The three simplest and mutually not-exclusive mechanisms that explain the disassembly of the PIC complexes and removal of RNA Pol II from the histone promoters during DNA replication stress are:

(i) active disassembly driven by direct phosphorylation by one of the DRC kinases,

(ii) negative feedback regulation driven by free histone proteins that accumulate when DNA replication stops or slows down, or

(iii) degradation of RNA Poll triggered by DNA replication stress.

We believe that a combination of the first two possibilities is likely. The removal of RNA Pol II, Spt15p, and Spt21p after HU treatment was partly attenuated in *rad53*Δ*sml1*Δ cells, indicating the involvement of Rad53p. Rad53p phosphorylates several transcription factors that may account for the role of DRC in the assembly and disassembly of the PIC complexes at the histone promoters. Rad53p directly phosphorylates Mbp1p, Swi4p, Swi6p (39, 40, 43, 51), activates transcription of MBF targets by phosphorylating MBF repressor Nrm1p (41, 42), and downregulates Swi6p and transcription of SBF targets (39, 40). However, our data show that the transcription of histone genes in *swi6*Δ cells during S phase is repressed by HU (Fig. 8), indicating that SBF and MBF complexes are not responsible or at least not solely responsible for the DRC-mediated repression of histone genes. Another Rad53p target is Spt21p. Rad53p phosphorylates Spt21p in the absence of DRC activation and this phosphorylation reduces histone gene transcription

(31). This negative role of Rad53p on the transcription of histone genes in the absence of DRC activation is consistent with our data showing elevated levels of histone mRNAs and elevated occupancy of RNA Pol II at the histone genes in *rad53*Δ*sml1*Δcells (Fig. 2 and 10). However, since the removal of Spt21p from the histone promoters after HU treatment was only partly dependent on Rad53p (Fig. 11B), it is not clear whether this phosphorylation is responsible for removal of Spt21p from the histone promoters after DRC activation. Interestingly, proteome-wide screen identified Spt21p as a target of DNA damage induced phosphorylation (51) and a genome-wide study of changes in protein localization found that the localization pattern of Spt21p changes from nuclear foci to diffuse nuclear signal during DNA replication stress (52).

The second possible mechanism for the disassembly of the PIC complexes at the histone promoters during DNA replication stress is negative feedback regulation driven by free histone proteins that accumulate when DNA replication slows down. The model posits that the HIR complex binds to NEG regions and recruits Asf1p to histone promoters. During replication stress or at the end of S phase, free histones bind to Rtt106p. The histone-Rtt106p complex then binds to Asf1p at the histone promoters, causing transcriptional repression (24, 25). The recruitment of Asf1p to histone promoters requires Spt10p and Spt21p (15) and does not depend entirely on the HIR complex (24). Our data suggest that Asf1p is the only histone chaperone required for HU mediated transcriptional repression of histone genes and that the HIR complex and Rtt106p are redundant in this process (Fig. 9). The simplest interpretation of our results is that under conditions of HU-mediated replication stress, the free histones bind directly to Asf1p, which is recruited to the histone promoters by Spt10p/Spt21p. It is tempting to speculate that the recruitment of

histone-charged Asf1p leads to dissociation of Spt10p/Spt21p from the histone promoters, perhaps with the assistance of Rad53p.

The third possible mechanism for the removal of RNA Pol II from the histone promoters during DNA replication stress is degradation of Rpb1p, the largest subunit of RNA Pol II. Rpb1p polyubiqitination and degradation occur when elongating RNA Pol II arrests and cannot be restarted as a "mechanism of last resort" to make DNA accessible for DNA repair and/or continued transcription (53). Conditions leading to RNA Pol II stalling and/or arrest include DNA damage and various forms of transcription stress (53-56). RNA Pol II is also evicted from chromatin and degraded following HU-induced replication stress (57). While we cannot completely eliminate contribution of Rpb1p degradation to HU-induced transcriptional inhibition of histone genes, we believe that it does not represent the main mechanism for the following reasons. First, we have not detected Rpb1p degradation after 15 min HU treatment (Fig. 10B), while the occupancy of Rpb1p at the histone genes decreased to about 30-50%. Second, HU treatment removes Spt10p, Spt21p, and Spt15p from the histone promoters, thus disassembling the PIC complex and preventing recruitment of RNA Pol II to histone promoters, making Rpb1p degradation unnecessary for regulation of histone gene transcription.

Overall, our data suggest that the mechanism of repression of histone genes by HU requires Asf1p and is mediated by reduced occupancy of Spt10p and Spt21p that triggers disassembly of the PIC complexes at the histone promoters. However, the mechanism might be more complex given that Asf1p is found in complex with Rad53p and the two proteins dissociate in response to replication stress (58-60).

SECTION II

2.1 Introduction

Acetyl-CoA is a key energy metabolite that links metabolism with signaling, chromatin structure, and transcription. This central position endows acetyl-CoA with an important regulatory role: the level of nucleocytosolic acetyl-CoA reflects the energetic and metabolic state of the cell (74-76). Acetyl-CoA also serves as a substrate for lysine (K) acetyltransferases (KATs) that catalyze the transfer of acetyl groups to lysines in a vast array of proteins, including histones (Fig. 12). Fluctuations in the concentration of acetyl-CoA, reflecting the changes in the metabolic state of the cell, are translated into dynamic protein acetylations that regulate a variety of cell functions, including transcription and metabolic reprogramming. Histone acetylation is a dynamic modification that occurs on all four core histones; it affects chromatin structure and regulates gene expression by at least two mechanisms. First, acetylation of the lysine residues of the histone tails neutralizes positive charges and weakens interactions between histones and DNA and between neighboring nucleosomes. Second, bromodomain-containing proteins, such as Swi2p subunit of the Swi/Snf chromatin remodeling complex, bind acetyl-lysine motifs in the histone tails and facilitate transcription by repositioning or evicting nucleosomes (77). The level of nucleocytosolic acetyl-CoA regulates the global acetylation of chromatin histones and the transcription of many genes (78-80). The KAT complex SAGA and its catalytic subunit Gcn5p are likely responsible for coupling histone acetylation with acetyl-CoA levels (80). As transcriptional induction of histone genes during S phase in *Saccharomyces cerevisiae* is associated with the recruitment of Gcn5p to histone promoters (81),

we hypothesized that histone transcription is regulated by the acetylation status of chromatin histones in the promoters of histone genes. If this assumption is correct, then reduced levels of nucleocytosolic acetyl-CoA would result in decreased expression of histone genes. Since reduced histone expression globally affects chromatin structure (82), and increases mitochondrial activity and ATP synthesis (83), we tested whether reduced synthesis of nucleocytosolic acetyl-CoA reduces histone transcription and is compensated for by elevated respiration and ATP synthesis. Here we show that reduced synthesis of nucleocytosolic acetyl- CoA leads to reduced acetylation of chromatin histones in the promoters of histone genes, and decreased histone transcription. The globally altered chromatin structure triggers mitochondrial biogenesis and respiration, and leads to increased synthesis of ATP. Together, our data indicate that the depletion of the energy metabolite acetyl-CoA is compensated for by the induction of ATP synthesis.

Cells transcribe histones during S phase to maintain the balance between histone and DNA synthesis that is needed for chromatin assembly. The correct cell cycle-dependent transcription of histone genes is mediated by histone-specific transcription factors Spt10p/Spt21p (81, 84, 85). Spt21p accumulates only during S phase, when it is recruited to the promoters of histone genes through its interaction with Spt10p. Spt10p/Spt21p subsequently recruit KAT Gcn5p and an additional KAT that has not yet been identified. It is likely that the Spt10p/Spt21p-associated KATs then acetylate histones in the promoters of histone genes to activate histone transcription; however, this acetylation has not been demonstrated (81). Several other transcriptional regulators are recruited to histone promoters, including chromatin remodeling complex Swi/Snf (84-86). Since Swi/Snf binds to acetylated lysines in histones and frequently functions in concert with

SAGA, it is likely that Gcn5p/SAGA-mediated acetylation of histones in the promoters of histone genes facilitates recruitment of Swi/Snf to activate histone transcription.

2.2 Results

To determine whether the nucleocytosolic level of acetyl-CoA regulates the transcription of histone genes, we used the *acs2*^{ts} allele (78). *ACS2* encodes nucleocytosolic acetyl-CoA synthetase 2 responsible for the synthesis of acetyl-CoA from acetate and CoA (Fig. 12). Since yeast mitochondrial and nucleocytosolic pools of acetyl- CoA are distinct and isolated, Acs2p is the sole source of acetyl-CoA in the nucleocytosolic compartment. The *acs2*^{ts} mutant displays severe global histone hypoacetylation and transcriptional defects at restrictive temperatures. *acs2*^{ts} cells grow at the wild-type rate at 30°C, but are not able to grow at the restrictive temperature of 37°C (78, 87, Fig. 13). We found that histone mRNA levels are already slightly reduced at 30°C in *acs2*^{ts} cells in comparison with wild type cells, and significantly more reduced at the semipermissive temperature of 33°C (Fig. 14A). In *acs2*^{ts} cells at 33°C, mRNAs of individual histones were reduced to 25–35% of mRNA levels in wild-type cells at 30°C. This reduction in transcription of histone genes was also evident at the protein level of histone H3; the H3 protein level in *acs2*^{ts} cells was slightly reduced at 30°C and more significantly reduced at 33°C (Fig. 14B). We selected histone H3 for this analysis, because the antibody we used recognizes the C-terminal region of H3, which is not posttranslationally modified, and the signal obtained with this antibody represents the total H3 level.

To test whether the reduced histone mRNA levels are due to a defect in the assembly of the preinitiation complex (PIC) at the histone promoters, we performed chromatin immunoprecipitation (ChIP) to determine the degree of RNA Pol II recruitment to the histone promoters (Fig. 14C). In agreement with histone mRNA levels, the occupancy of RNA Pol II at the histone promoters in *acs2*^{ts} cells was significantly reduced

at 33°C, indicating that the decreased nucleocytosolic level of acetyl-CoA affects PIC assembly and transcriptional initiation. To test whether the decreased level of acetyl-CoA in *acs2*^{ts} cells at 33°C affects the acetylation of chromatin histones in the promoters of histone genes, we used ChIP to evaluate the occupancy of histone H3 acetylated at lysine 14 (acH3K14) in the histone promoters (Fig. 14D). To account for differences in nucleosome density due to differences in histone levels between WT and *acs2*^{ts} cells at 30°C and 33°C, we corrected the acH3 occupancy for histone H3 content, and generated values that represent acetylation per nucleosome (Fig. 14D). Histone H3 acetylation per nucleosome in the promoters of histone genes was significantly reduced in *acs2*^{ts} cells in comparison with WT cells at 33°C. Taken together, these results indicate that the reduced nucleocytosolic level of acetyl-CoA in *acs2*^{ts} cells at 33°C results in decreased transcription of histones due to hypoacetylation of chromatin histones in the promoters of histone genes.

2.3 Discussion

Decreased histone levels are associated with reduced chromatin nucleosome density and globally altered chromatin structure. From the perspective of nucleosomal architecture, genes can be classified as growth genes or stress genes (82). Growth genes are expressed at high levels and feature a nucleosome-free region, which allows for the unobstructed binding of transcription factors. The stress genes are expressed at lower levels and their promoters are dominated by delocalized nucleosomes. Consequently, stress genes are regulated by factors that affect chromatin structure, including histone levels. As the respiratory genes in *S. cerevisiae* belong to the stress category (88), decreased expression of histones triggers the transcription of respiratory genes and induces respiration (83). To test whether the decreased histone transcription in *acs2*^{ts} cells represents the mechanism responsible for elevated mitochondrial respiration and metabolism, we transformed WT and *acs2ts* cells with either a control plasmid or a low-copy-number plasmid encoding all four core histones. We found that even the slightly increased expression of histones from the low-copy-number plasmid suppressed the respiratory phenotypes of *acs2*^{ts} cells at 33ºC and significantly reduced oxygen consumption, ATP levels, mtDNA copy number, and mRNA levels of several genes encoding enzymes of the tricarboxylic acid cycle and oxidative phosphorylation pathway (Fig. 15A–D). On the basis of these results, we propose a model in which a reduced synthesis of nucleocytosolic acetyl-CoA results in reduced histone transcription, globally affected chromatin structure, and increased transcription of respiratory genes, mitochondrial respiration, and ATP synthesis (Fig. 16). This study identifies nucleocytosolic acetyl-CoA as an indicator of the energetic state of the cell and as a regulator of chromatin structure and mitochondrial metabolism.

MATERIALS AND METHODS

Yeast Strains and Media

All yeast strains are listed in Table 1. Wild-type (WT) strain is W303-1a Standard genetic techniques were used to manipulate yeast strains and to introduce mutations from non-W303 strains into the W303 background (70). Cells were grown in YPD medium (1% yeast extract, 2% Bacto peptone, 2% glucose), YEP medium (1% yeast extract, 2% Bacto peptone) containing 2% glucose, or under selection in synthetic complete medium (SC) containing 2% glucose and, when appropriate, lacking specific nutrients in order to select for a strain with a particular genotype.

The temperature sensitive allele *acs2*^{ts} was created by Takahashi et al. (78) and introduced into W303-1a background to construct strain LG419 (11). Plasmid pJH33 [*URA3 CEN ARS HTA1-HTB1 HHT2-HHF2*] was constructed by Blackwell et al. (13). Cells were grown at 30°C or 33°C in YPD medium (1% yeast extract, 2% Bacto peptone, 2% glucose) or under selection in synthetic complete medium (SC) lacking specific nutrients in order to select for a particular genotype.

Cell cycle arrest in G1 phase by α-factor

Cell cycle arrest in G1 phase by α-factor was carried out by adding α-factor to 10 µg/ml to cells exponentially growing in YPD medium. Following α-factor addition, the cultures were incubated for 3 h, and the arrest was monitored by examining cell morphology

Spotting assay

Cells were grown to log phase at 30°C, and 10-fold serial dilutions were spotted on the YPD plates and incubated at 30°C and 33°C for 48–72 h.

Western Blotting

Yeast cells were inoculated to an A_{600} = 0.1 and grown in YPD medium to an A_{600} = 1.0. Four A_{600} units (one A_{600} unit is equal to ~3×10^7 cells) were harvested and immediately boiled in SDS sample buffer. Denatured proteins were separated on a denaturing polyacrylamide gel and western blotting with antihistone H3 polyclonal antibody (ab1791; Abcam) at a dilution of 1:1000, anti-RNA Polymerase II Rpb1p monoclonal antibody (8WG16; 664912, BioLegend) at a dilution of 1:500, anti-myc monoclonal antibody (9B11; 2276S; Cell Signaling) at a dilution of 1:1000, and anti-Pgk1p monoclonal antibody (22C5D8; 459250; Invitrogen) at a dilution of 1:3000, anti-histone H4 polyclonal antibody (catalog number 2542; Cell Signaling) at a dilution of 1:750, anti-histone H2A polyclonal antibody (catalog number 07-146; Millipore) at a dilution of 1:750, and anti-histone H2B polyclonal antibody (catalog number 07-371; Millipore) at a dilution of 1:750. To confirm equivalent amounts of loaded proteins, the membranes were also probed with actin polyclonal antibody (catalog number A5060; Sigma) at a dilution of 1:500.

Real-time RT-qPCR

The procedures to extract total RNA from yeast cells and perform real-time reverse transcription quantitative PCR were as previously described (28, 71).

Primers *HTA1, HTA2, HTB1, HTB2, HHT1, HHT2, HHF1, HHF2* recognize individual histone mRNAs and amplify the 3' ends of the translated sequences and adjacent not-translated sequences. Primers *HTA1/HTA2, HTB1/HTB2, HHT1/HHT2*, and *HHF1/HHF2* were designed so that they measure expression of both genes for

that particular histone (*HTA1* and *HTA2*, *HTB1* and *HTB2*, *HHT1* and *HHT2*, and *HHF1* and *HHF2*)

The primers used are:

ACT1 (5'-TATGTGTAAAGCCGGTTTTGC-3' and 5'-

GACAATACCGTGTTCAATTGGG-3'),

RDN25 (5'-GGAATGTAGCTTGCCTCGGT-3' and 5'-

TTACGTCGCAGTCCTCAGTC-3'),

HTA1 (5'-ACGTTACCATTGCCCAAGGT-3' and 5'-

GTTTAGTTCCTTCCGCCTTCTT-3'),

HTA2 (5'-GCTATTGGGTAATGTTACCATCG-3' and 5'-

TGCTTTGTTTCTTTTCAACTCAGT-3'),

HTB1 (5'-TGGCTGCGTATAACAAGAAGTCT-3' and 5'-

CCAAAGGAAGTGATTTCATTATGC-3'),

HTB2 (5'-TGCTCTATACTCAAACCAACAACA-3' and 5'-

ATCTCTTCTTACCATCGACGGA-3'),

HHT1 (5'-AATATATAAACGCAAACAATGGC-3' and 5'-

GATTTTCTGGCAGCCTTAGAAG-3'),

HHT2 (5'-TTGAAGACACTAATCTGGCTGCT-3'

and 5'-GATGTCCCCCCAGTCTAAATG-3'),

HHF1 (5’-GAATCCGTCATCAGAGACTCTGTT-3’ and 5’-TGCTTGTTGTTACCGTTTTCTTAG-3’),

HHF2 (5’-TCTGTTACTTACACTGAACACGCC-3’ and 5’-AAACACCGATTGTTTAACCACC-3’).

HTA1/2 (5’-CGGTGGTAAAGGTGGTAAAGC-3’ and 5’-TGGAGCACCAGAACCAATTC-3’),

HTB1/2 (5’-CAAAGTTTTGAAGCAAACTCACCC-3’ and 5’-GCCAATTTAGAAGCTTCAGTAGC-3’).

HHT1/2 (5’GAAGCCTCACAGATATAAGCCAG3’ and 5’-ATCTTGAGCGATTTCTCTGACC-3’),

HHF1/2 (5’-CCAAGCGTCACAGAAAGATTCTA -3’ and 5’-ACCAGAAATACGCTTG ACACCA-3’).

CIT1 (5′-CAGCGATATTATCAACAACTAGCA-3′ and 5′-TAGTGGCGAGCATTCAATAGTG-3′),

CIT2 (5′-AGAGATTTAGCGAAATCTACCCC-3′ and 5′-CCTCTCATACCACCATATACCTGTT-3′)

IDH1 (5′-TGCTTAACAGAACAATTGCTAAGAG-3′ and 5′-AACACCGTCACCAGGTATCAA-3′),

QCR7 (5′-ACGTCTATTGCGAGAATTGGTG-3′ and 5′-AGCCCTAACTTCTTGTAACCTGC-3′),

ChIP assays

In vivo chromatin crosslinking and immunoprecipitation was performed essentially as described (71). For RNA Pol II and Spt15p-HA ChIP, the cells were crosslinked for 15 min. For Spt10p-myc and Spt21p-myc ChIP, the cells were crosslinked for 45 min. Immunoprecipitation was performed with the following antibodies: anti-RNA Polymerase II Rpb1p monoclonal antibody (8WG16; 664912, BioLegend), anti-myc monoclonal antibody (9B11; 2276S; Cell Signaling), and anti-HA monoclonal antibody (F-7; sc-7392X; Santa Cruz Biotechnology). The primers used for real-time PCR are as follows:

POL1 (5'-TCCTGACAAAGAAGGCAATAGAAG-3' and 5'-TAAAACACCCTGATCCACCTCTG-3'),

HTA1/HTB1 promoter (5'-ATAGTTAACGACCCAACCGC-3' and 5'-CTCCATTCCAATAGCTTCGCA3'),

HTA2/HTB2 promoter (5'-CGTCGCGTTTATGGCCCC-3' and 5'-GAGAACACCGCTTTATTAGGC-3'),

HHT1/HHF1 promoter (5'-GAACGCGGTTTCCAAATTCG-3' and 5'-GCAGAGCAAGGAAATGTGAGA-3'), and

HHT2/HHF2 promoter (5'-GCCAATAGTTTCACGCGCTT-3' and 5'-ACGTCCTGCCATACAAATGC-3').

Primers that recognize individual histone genes were used for RNA Pol II ChIP.

For anti-Histone H3 (Abcam ab1791), anti-acetyl-Histone H3 (Lys14) (Upstate Cell Signaling Solutions 07-353), and anti-RNA polymerase II monoclonal antibody (8WGI6, Covance). ChIP cells were crossed linked for 15 minutes. Acetylation per nucleosome was calculated as ratios of AcH3 to total H3. Each immunoprecipitation was performed at least

three times using different chromatin samples, and the occupancy was calculated using the *POL1* coding sequence as a negative control and corrected for the efficiency of the primers. *POL1*(5'-TCCTGACAAAGAAGGCAATAGAAG-3' and 5'-TAAAACACCCTGATCCACCTCTG-3'), *HTA1/HTB1* promoter (5'-ATAGTTAACGACCCAACCGC-3' and 5'-CTCCATTCCAATAGCTTCGCA3'), *HTA2/HTB2* promoter (5'-CGTCGCGTTTATGGCCCC-3' and 5'-GAGAACACCGCTTTATTAGGC-3'), *HHT1/HHF1* promoter (5'-GAACGCGGTTTCCAAATTCG-3' and 5'-GCAGAGCAAGGAAATGTGAGA-3'), and *HHT2/HHF2* promoter (5'-GCCAATAGTTTCACGCGCTT-3' and 5'-ACGTCCTGCCATACAAATGC-3').

Histone mRNA decay rates

The half-lives ($t_{1/2}$) of histone mRNAs were determined using transcriptional shut off with thiolutin as described (72, 73). Yeast cells were inoculated to an A_{600} = 0.1 and grown in YPD medium to an A_{600} = 0.8. Thiolutin was added to 8 µg/ml and culture samples were removed during 0 – 60 min incubation. Total RNA was isolated as described above and histone mRNA levels were determined by RT-qPCR using *RDN25* as a control. The half-lives of individual histone mRNAs were determined in Microsoft Excel from logarithmic plots of each remaining mRNA at different times after the transcriptional shut off.

Mitochondrial DNA isolation and quantification

Cells were grown to an A_{600} of 0.6 in YEP medium containing either 5% glucose or an alternative carbon source. Cells were harvested by centrifugation and lysed in a buffer containing 2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA with prechilled glass beads. The lysate was extracted with phenol and chloroform. RNA was digested with RNase, and total DNA was purified by phenol and chloroform. Relative mtDNA was quantified by real-time PCR using primers for *COX1* (5′CAACAAATGCAAAAGATATTGCAG-3′ and 5′-AATATTGTGAACCAGGTGCAGC-3′). The results were normalized with primers for *ACT1* (5′-TATGTGTAAAGCCGGTTTTGC-3′ and 5′-GACAATACCGTGTTCAATTGGG-3′).

Oxygen consumption

Cells were grown to an A_{600} of 0.6 in YEP medium containing either 2% glucose or an alternative carbon source, and 9×10^6 cells were harvested by centrifugation. Cells were resuspended in a buffer containing 10 mM HEPES and 25 mM K_2HPO_4 (pH 7.0) and incubated at 30°C in an oxygen consumption chamber (Instech Laboratories, Inc.) connected to a NeoFOX fluorescence-sensing detector using NeoFOX software (Ocean Optics, Inc.). Results were calculated as picomoles of O_2 per 10^6 cells per second and expressed as a percentage of values for wild-type cells.

ATP assay

Cells were grown to an A_{600} of 0.6 in YEP medium containing either 2% glucose or an alternative carbon source, and 9 X 10^7 cells were harvested by centrifugation and lyed in 5% trichloroacetic acid with prechilled glass beads. The cell lysate was neutralized to pH 7.5 with 10 M KOH and 2 M Tris-HCl (pH- 7.5). ATP levels were measured by using n Enliten ATP assay (catalog number FF2000; Promega) according to the manufacturer's instructions and normalized by the number of cells.

Statistical analysis

The results represent at least three independent experiments. Numerical results are presented as means ± SD. Data were analyzed by using an InStat software package (GrapPAD, San Diego, CA, USA). Statistical significance was evaluated by one-way Anova analysis, and $p<0.05$ was considered significant.

Table 1

Table1: Yeast strains used in the study

Strain	Genotype	Source/Ref.
W303-1a	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303-1 α	*MAT α ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303	*MATa/MAT α ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1ura3-1/ura3-1 can1-100/can1-100*	R. Rothstein
PB119	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 dun1::KAN*	28
SN159	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 tel1::HIS3*	28
SN136	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 chk1::HIS3*	28
SN117	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 mec1::HIS3 sml1::KAN*	28
LG606	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rad53::KAN sml1::HYG*	28
LG731	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rad52::TRP1*	28
DY5780	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6::TRP1*	61
FY2195	*MATa his3200 leu20 ura30 lys2-128 δ trp163 SPT10-MYC::kanMX*	18
MB141	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 SPT10-MYC::kanMX*	This study
FY2194	*MATa his3200 leu20 ura30 lys2-128δ trp163 SPT21-MYC::kanMX*	18
MB150	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 SPT21-MYC::kanMX*	This study
AD066	*MAT · ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 SPT15-3HA::URA3*	62
yRP1619	*MATa trp1 ura3-52 leu2-3, 112 cup1D::LEU2PM his4-539 pan2::URA3*	63
MB123	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 pan2::URA3*	This study
DY3462-4	*MATa his4-912d his4-912d-ADE2 lys2-128d can1 leu2 ura3 ccr4::URA3*	64
SM096	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 ccr4::URA3*	This study
yRP2859	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	65

	can1-100 dcp2::HIS3	
yRP1199	*MATα his4-539 leu2-3,112 trp1-Δ1 ura3-52 xrn1::URA3*	66
	MB115 *MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 xrn1::URA3*	This study
yRP1192	*MATα his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski2::LEU2*	66
MB133	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 ski2::LEU2*	This study
yRP1193	*MATα his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski3::TRP1*	66
MB109	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 ski3::TRP1*	This study
yRP1377	*MATα leu2-3 112 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG rrp6::URA3*	67
MB120	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rrp6::URA3*	This study
MB159	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rad53::KAN sml1::HYG SPT15-3HA::URA3*	This study
MB186	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rad53::HIS3 sml1::HYG SPT10-MYC::kanMX*	This study
MB189	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rad53::HIS3 sml1::HYG SPT21MYC::kanMX*	This study
MB163	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 mec1::HIS3 sml1::KAN SPT15-3HA::URA3*	This study
MB191	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 SPT15-3HA::URA3*	This study
MB195	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 SPT21MYC::kanMX*	This study
MB198	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 SPT10-MYC::kanMX*	This study
BY4691	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SWI4-myc::URA3* 68	
MZ576	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3*	69
MZ642	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rtt106*::*KAN*	69
MZ655	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rtt109*::*KAN*	69
MZ553	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 cac1*::*LEU2*	69
MZ700	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hir1*::*HIS3*	69
MB181	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 mec1::TRP1 tel1::HIS3 [2μ-LEU2-RNR1]*	This study
LG419	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100* pJH33 [*URA3 CEN ARS HTA1-HTB1 HHT2-HHF2*]	This study

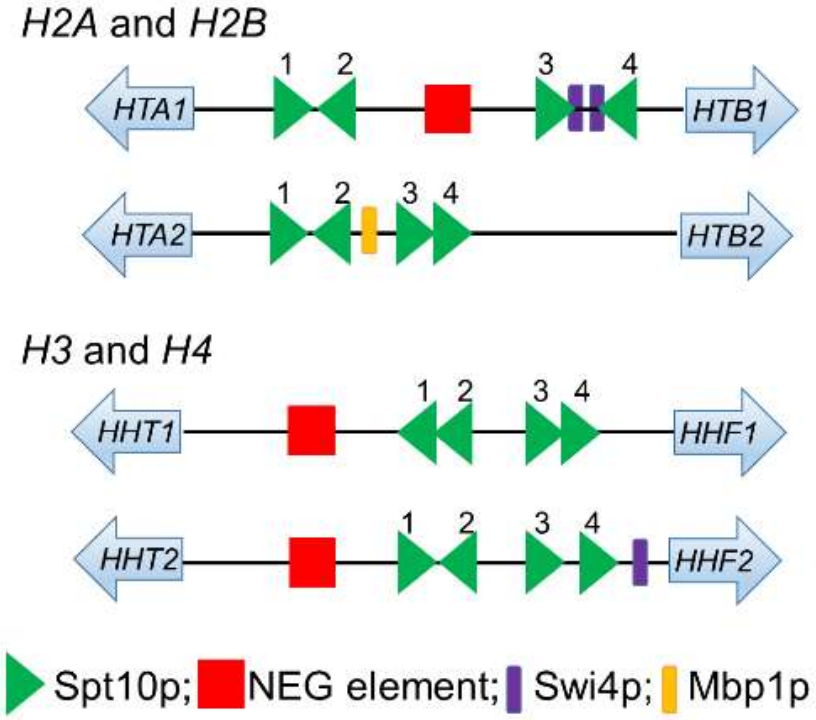

Figure 1. Organization of yeast histone genes.

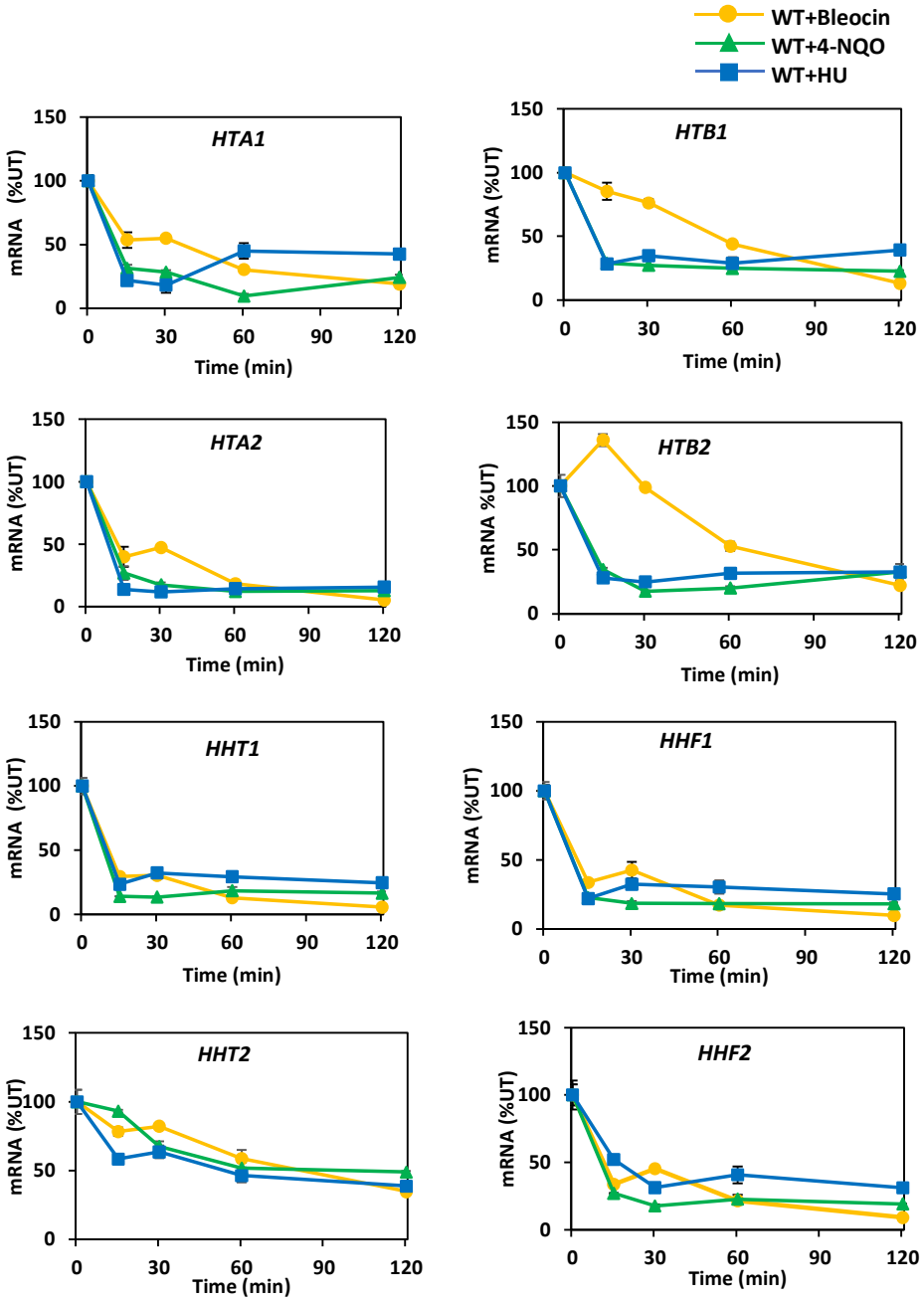

Figure 2. Genotoxic stress represses histone mRNA levels.
Time course of histone mRNA levels in wild-type cells (WT, W303-1a) treated with bleocin at 1.0 µg/ml, 4-NQO at 1.0 µg/ml, and HU at 200 mM. Histone mRNA levels were determined by RTqPCR and are expressed relative to the value for the wild-type grown in the absence of genotoxic chemicals.
The experiments were repeated three times, and the results are shown as means ± SD.

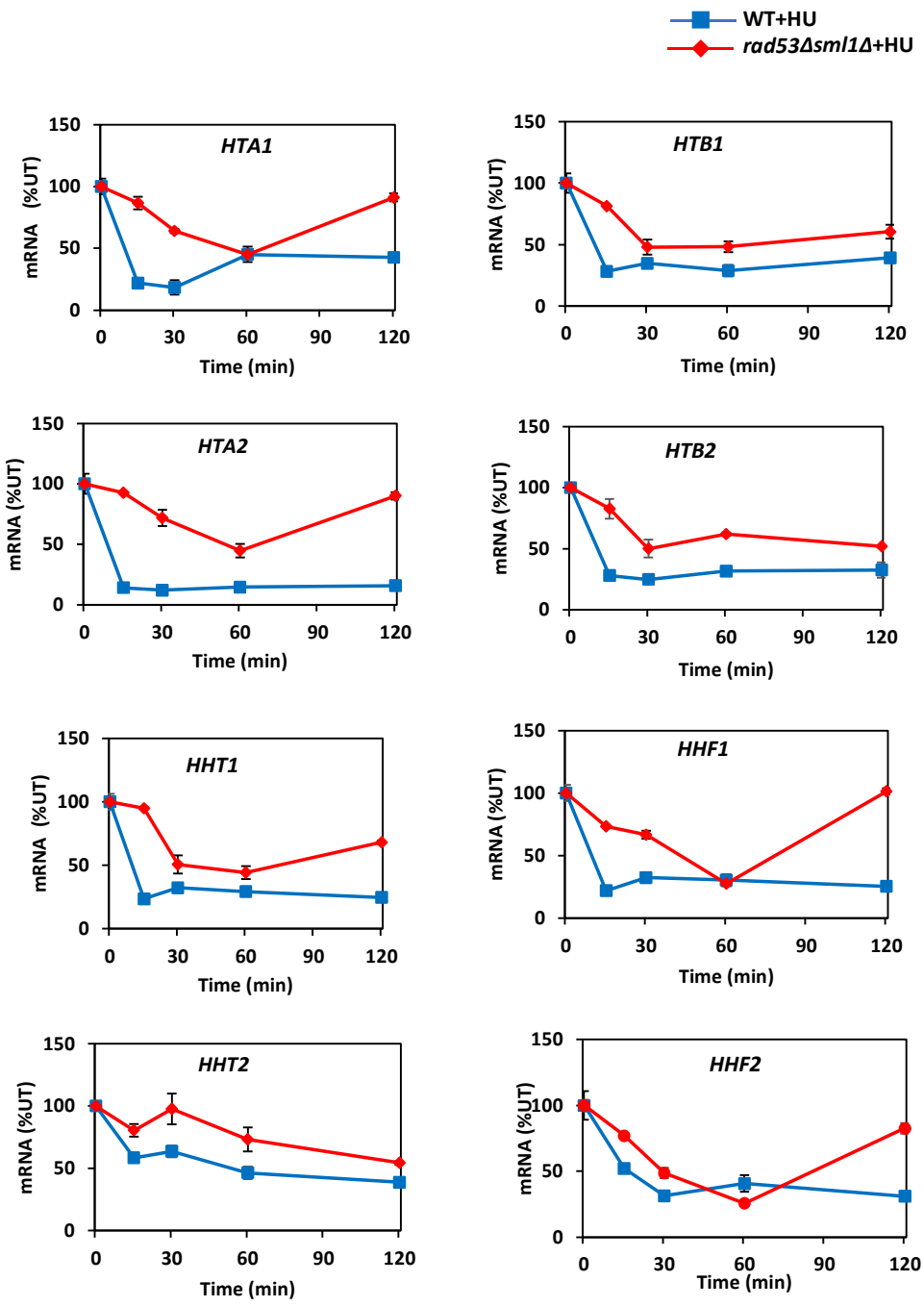

Figure 3. DDC-mediated repression of histone mRNAs requires Rad53p.

Time course of histone mRNA levels in wild-type cells (WT, W303-1a) and *rad53*Δ*sml1*Δ (LG606) cells treated with HU at 200 mM. Histone mRNA levels were determined by RTqPCR and are expressed relative to the value for the wild-type *rad53*Δ*sml1*Δ strain grown in the absence of genotoxic chemicals.
The experiments were repeated three times, and the results are shown as means ± SD.

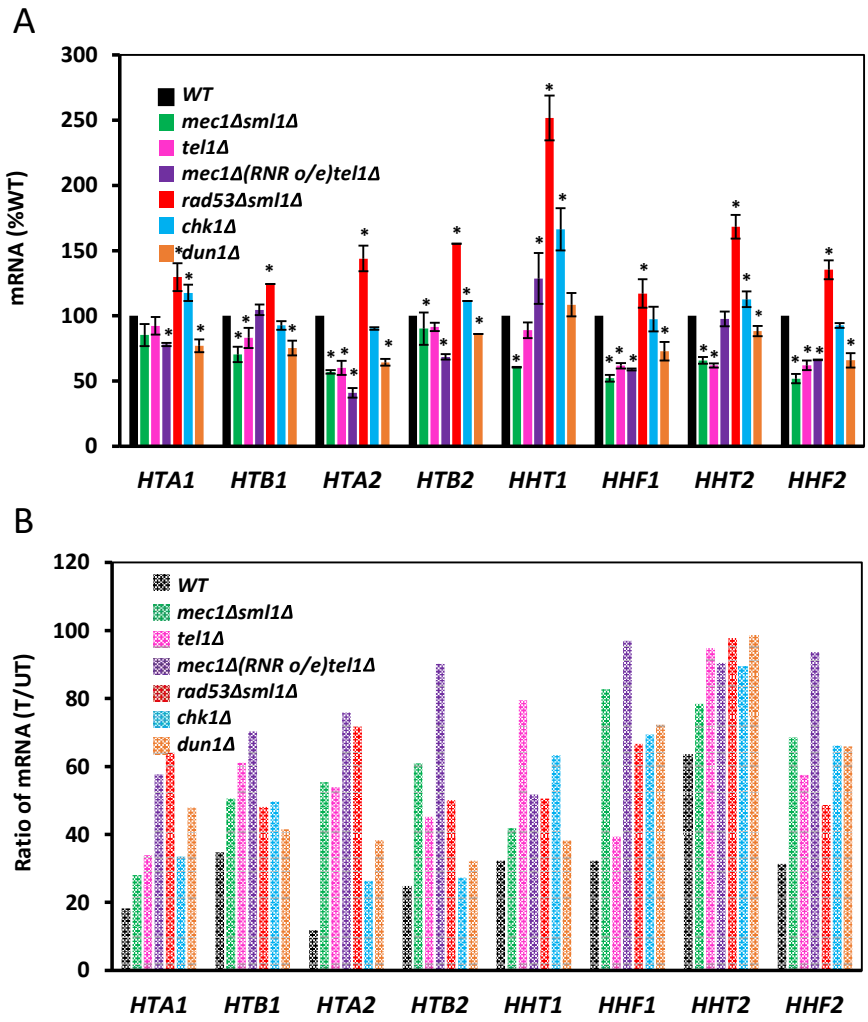

Figure 4. Genotoxic stress represses histone mRNA levels in checkpoint kinases-dependent manner.

(A) Histone mRNA levels in WT (W303-1a), *mec1*Δ*sml1*Δ (SN117), *tel1*Δ (SN159), *mec1*Δ*tel1*Δ [*RNR1* o/e] (MB181), *rad53*Δ*sml1*Δ (LG606), *chk1*Δ (SN136), and *dun1*Δ (PB119) cells. The experiments were repeated three times, and the results are shown as means ± SD.

Values that are statistically different ($p < 0.05$) from the WT cells are indicated by an asterisk. The results are expressed relative to the value for the wild-type strain.

(B) Mean percentage of mRNA levels remaining after 30 min HU treatment, calculated as a ratio of mRNA levels in treated (T)/untreated (UT) samples for each individual strain

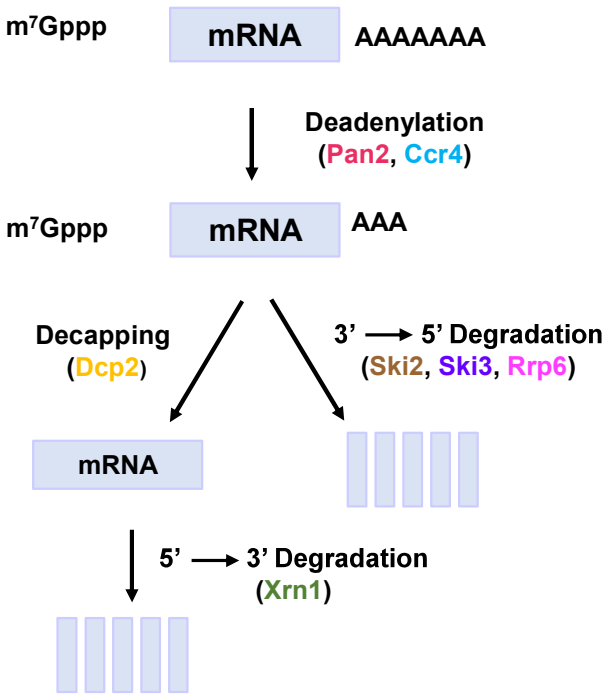

Figure 5. A model depicting the 5'→3' and 3'→5' pathways of mRNA decay and the corresponding enzymes.

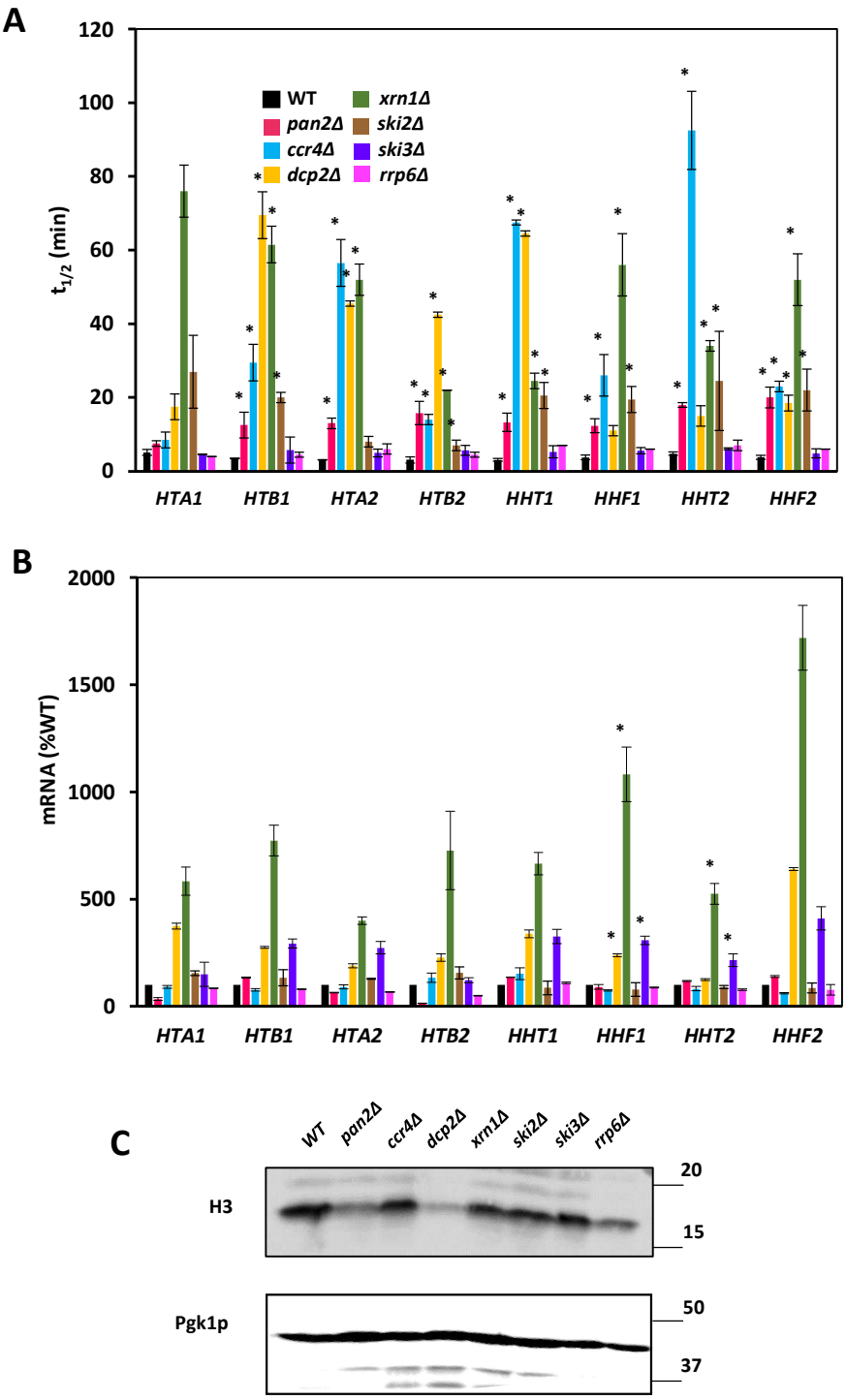

Figure 6. Histone mRNAs are degraded by both 5'→3' and 3'→5' pathways.

(A) Half-lives of histone mRNAs (t1/2), (B) steady-state histone mRNA levels, and (C) histone H3 protein levels in wild-type (WT, W303-1a), *pan2*Δ (MB123), *ccr4*Δ (SM096), *dcp2*Δ (yRP2859), *xrn1*Δ (MB115), *ski2*Δ (MB133), *ski3*Δ (MB109), and *rrp6*Δ (MB120) cells.

(A, B) The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($p < 0.05$) from the WT cells are indicated by an asterisk. (B) The results are expressed relative to the value for the wild-type strain. (C) Western blot was performed three times, and representative results are shown. Pgk1p served as a loading control.

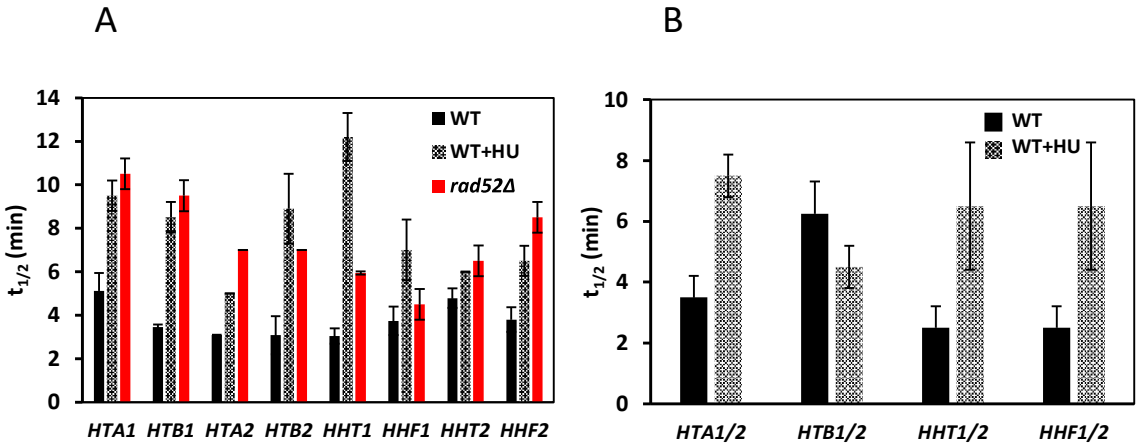

Figure 7. Histone mRNAs are not destabilized by DRC activation.
(A) Half-lives of individual histone mRNAs in wild-type (WT, W303-1a) cells before and after HU treatment (1 h) and in *rad52*Δ (LG731) cells were determined with primers that recognize individual histone mRNAs and amplify the 3' ends of the translated sequences and adjacent nottranslated sequences.
(B) Half-lives of histone mRNAs in wild-type (WT, W303-1a) cells before and after HU treatment (1 h) were determined with primers that amplify 5' ends of transcripts of both genes for particular histone (*HTA1* and *HTA2*, *HTB1* and *HTB2*, *HHT1* and *HHT2*, and *HHF1* and *HHF2*). The experiments were repeated three times, and the results are shown as means ± SD.

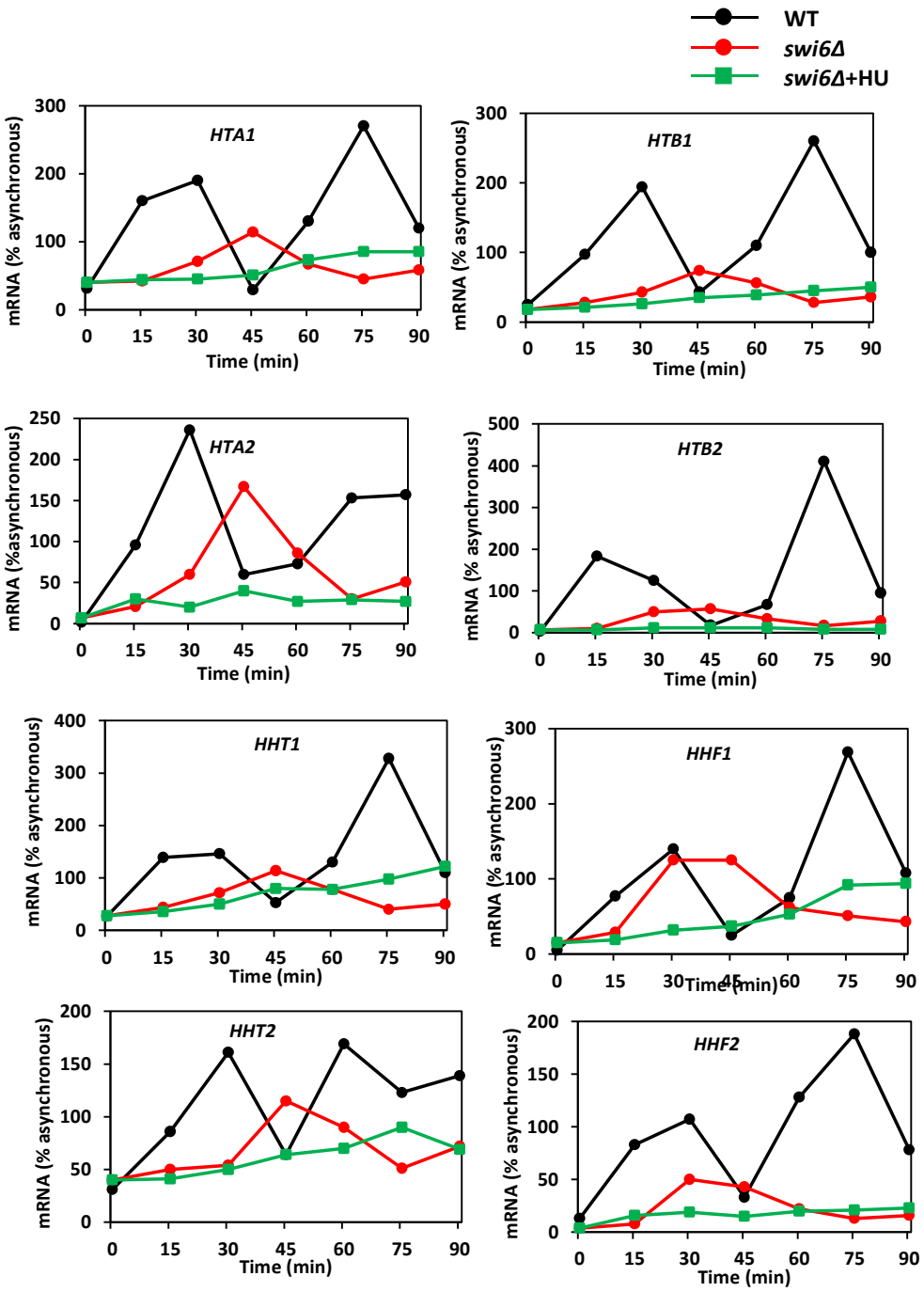

Figure 8. DRC activation inhibits transcription of histone genes independently of SBF and MBF.

Wild-type (WT) and *swi6*Δ (DY5780) cells were grown in YPD medium and synchronized with α-factor arrest and release in the absence (WT, *swi6*Δ) and presence (*swi6*Δ) of HU. mRNA levels are expressed relative to the values for the asynchronous WT cells.

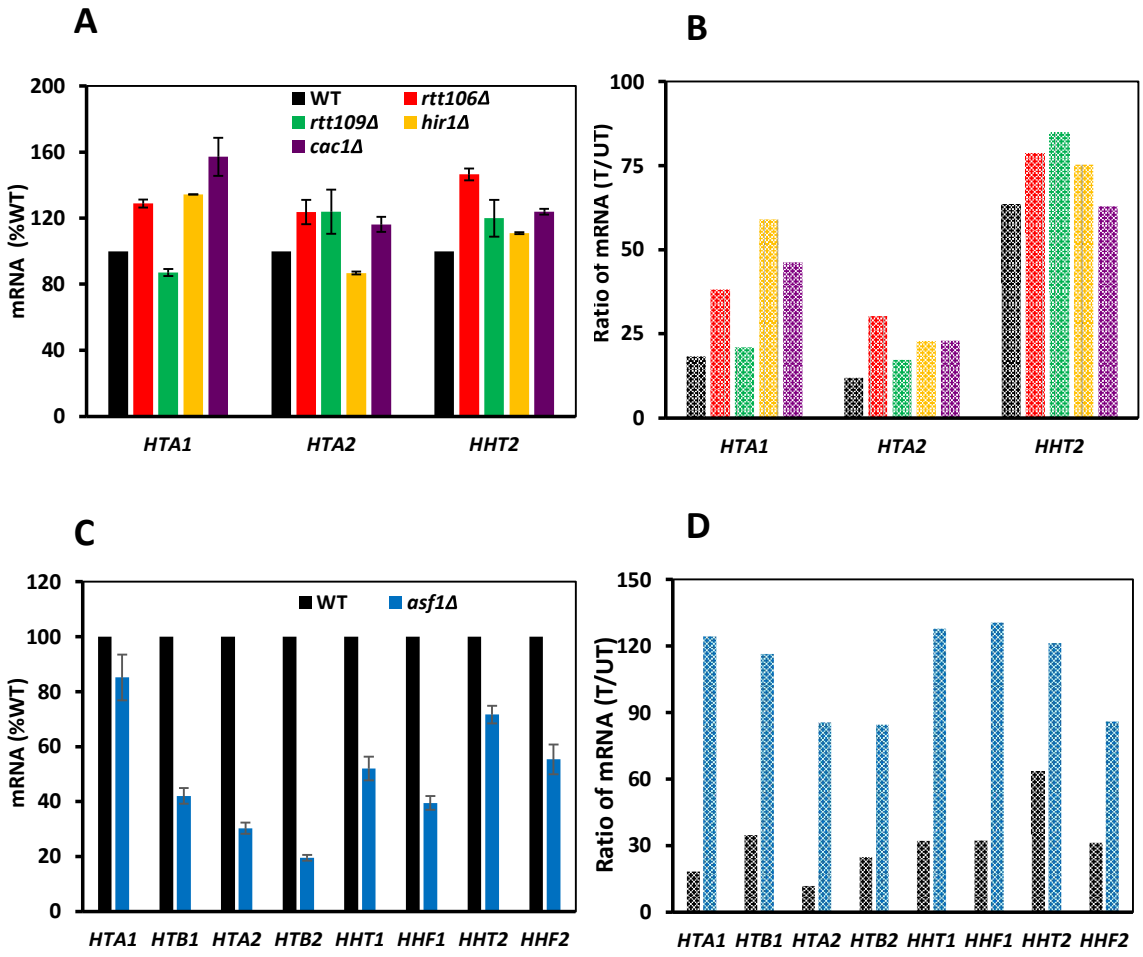

Figure 9. The DRC-triggered repression of histone mRNAs is attenuated in *asf1*Δ cells.

(A, C) Histone *HTA1*, *HTA2*, and *HHT2* mRNA levels in wild-type (WT, W303-1a), *rtt106*Δ (MZ642), *rtt109*Δ (MZ655), *hir1*Δ (MZ700), *cac1*Δ (MZ553), and *asf1*Δ (MZ576) cells. The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($p < 0.05$) from the WT cells are indicated by an asterisk. The results are expressed relative to the value for the WT strain. (B, D) Mean percentage of mRNA levels remaining after 30 min HU treatment, calculated as a ratio of mRNA levels in treated (T)/untreated (UT) samples for each individual strain.

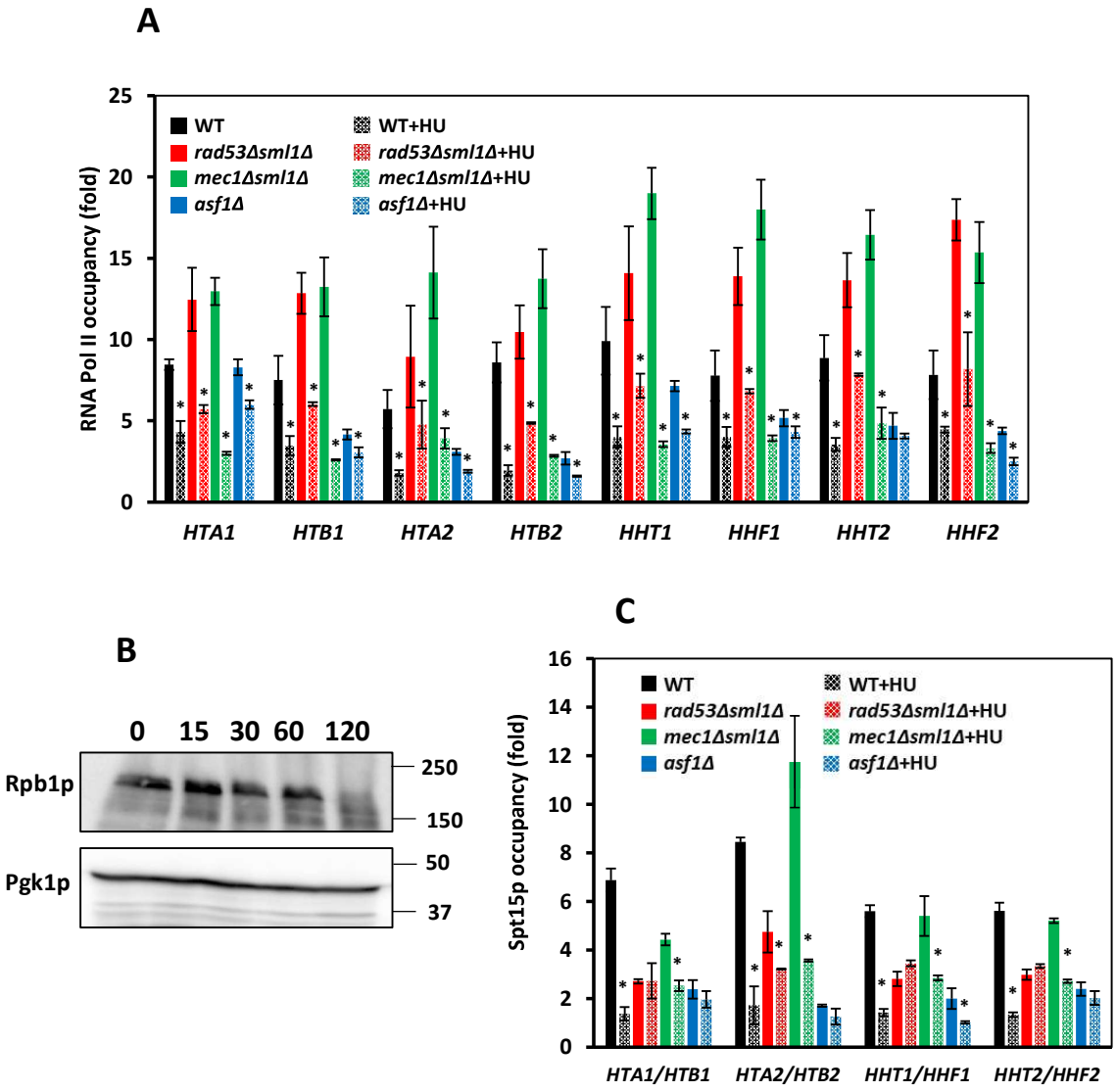

Figure 10. DRC activation removes RNA Pol II and Spt15p from histone genes.
(A, C) Occupancies of RNA Pol II and Spt15p at histone genes before and after treatment with 200 mM hydroxyurea (HU) for 15 min in wild-type (WT), *rad53*Δ*sml1*Δ, *mec1*Δ*sml1*Δ, and *asf1*Δ cells expressing *SPT15* tagged with three copies of the HA epitope (strains AD066, MB159, MB163, and MB191, respectively). Each immunoprecipitation was performed at least three times using different chromatin samples, and the occupancy at the indicated genes was calculated using the *POL1* coding sequence as a negative control. The data are presented as fold occupancy over the *POL1* coding sequence control and represent means ± SD. Values for the HU-treated samples that are statistically different ($p < 0.05$) from values for the untreated samples in the same strain are indicated by an asterisk. (B) Rpb1p protein levels in wild-type cells during treatment with 200 mM HU. Western blotting analyses were performed three times, and representative results are shown. Pgk1p served as a loading control.

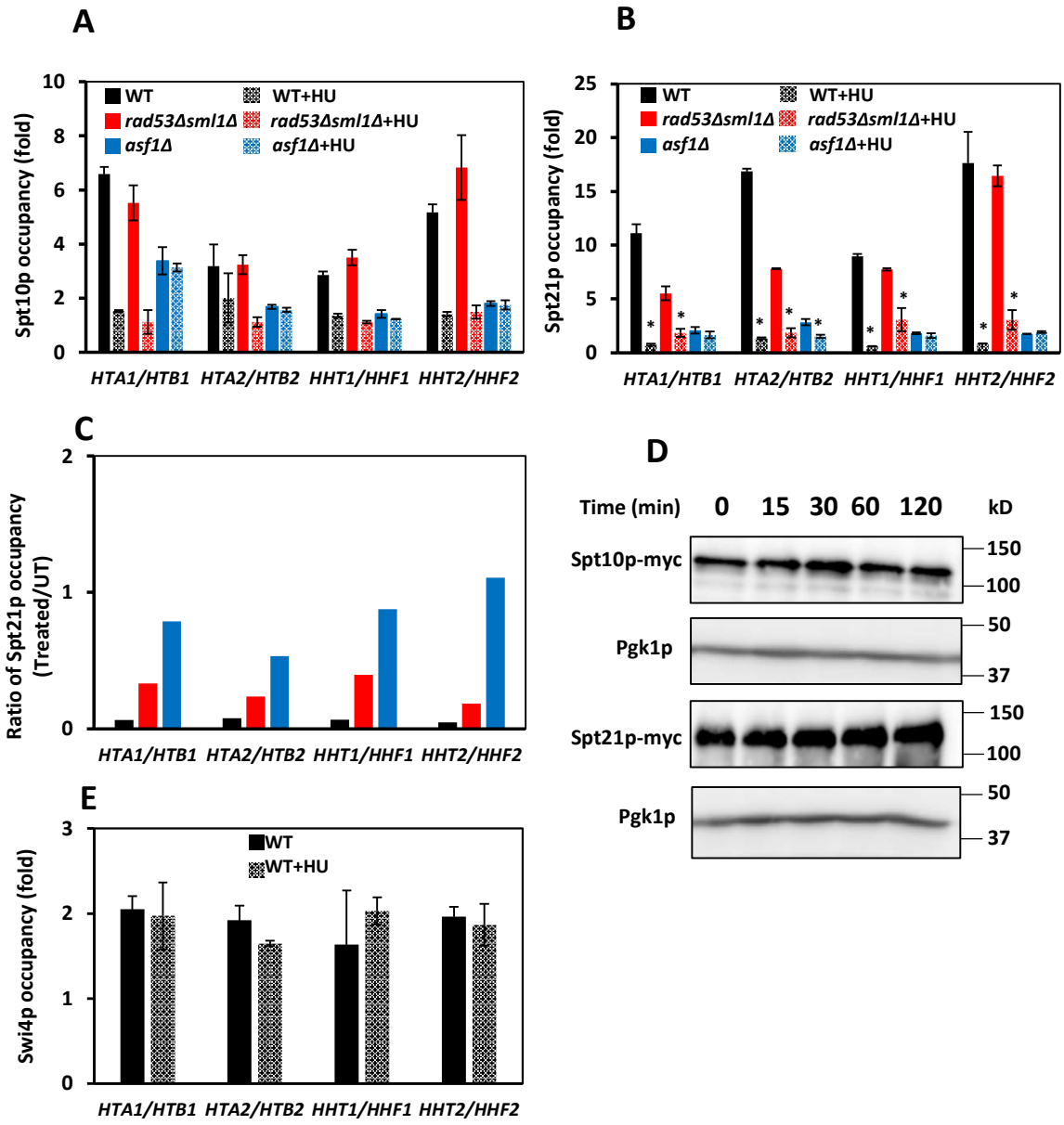

Figure 11. DRC activation evicts Spt10p and Spt21p from the histone promoters.
(A) Occupancy of Spt10p at the histone promoters before and after treatment with 200 mM hydroxyurea (HU) for 15 min in wild-type (WT), *rad53*Δ*sml1*Δ, and *asf1*Δ cells expressing *SPT10* tagged with myc epitope (strains MB141, MB186, and MB198, respectively). (B) Occupancy of Spt21p at the histone promoters before and after treatment with 200 mM hydroxyurea (HU) for 15 min in wild-type (WT), *rad53*Δ*sml1*Δ, and *asf1*Δ cells expressing *SPT21* tagged with myc epitope (strains MB150, MB189, and MB195, respectively). (C) Ratio of Spt21p occupancy at the histone promoters in treated (T)/untreated (UT) samples for wildtype (WT), *rad53*Δ*sml1*Δ, and *asf1*Δ cells. (D) Spt10p-myc and Spt21p-myc protein levels in wild-type cells during treatment with 200 mM hydroxyurea. Western blotting analyses were performed three times, and representative results are shown. Pgk1p served as a loading control.
(E) Occupancy of Swi4p at the histone promoters before and after treatment with 200 mM hydroxyurea (HU) for 15 min in wild-type (WT) cells expressing *SWI4* tagged with myc epitope (BY4691).

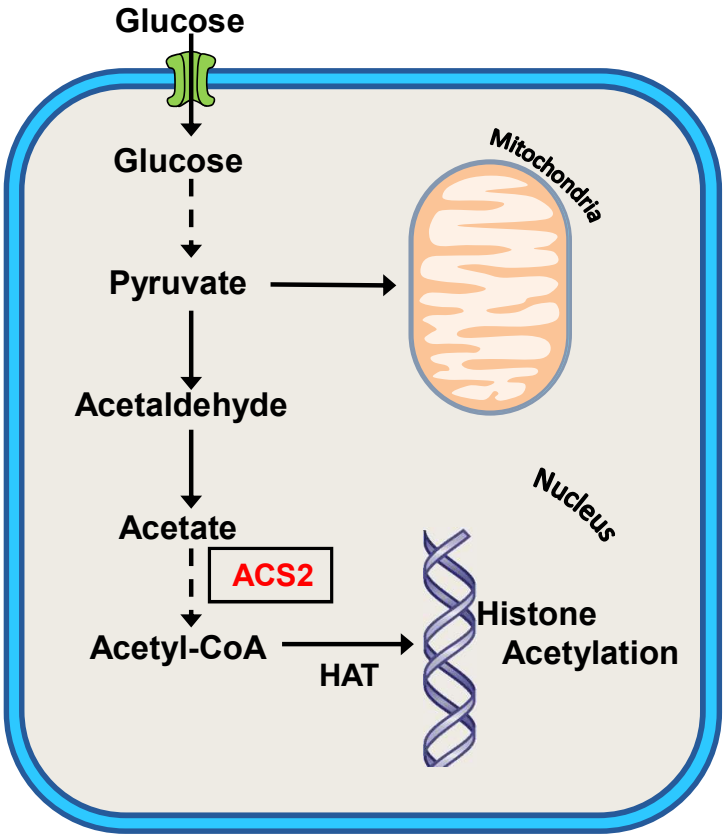

Figure 12. Role of Acetyl-CoA synthetase in determining nucleocytosolic pool of Acetyl-CoA

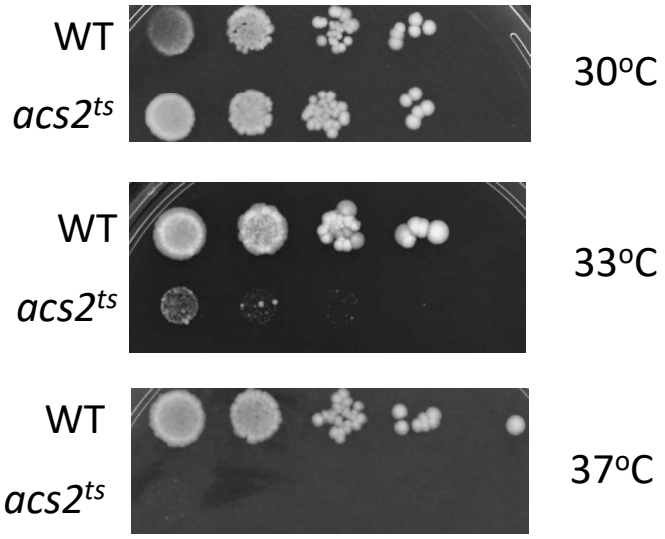

Figure 13. Temperature sensitivity of *acs2^{ts}* mutant.
Growth of WT and *acs2^{ts}* mutant at normal temperature and restrictive temperature. Tenfold serial dilutions of cells were spotted onto yeast extract-peptone-glucose plates and grown for 48 hours at 30°C , 33°C and 37°C

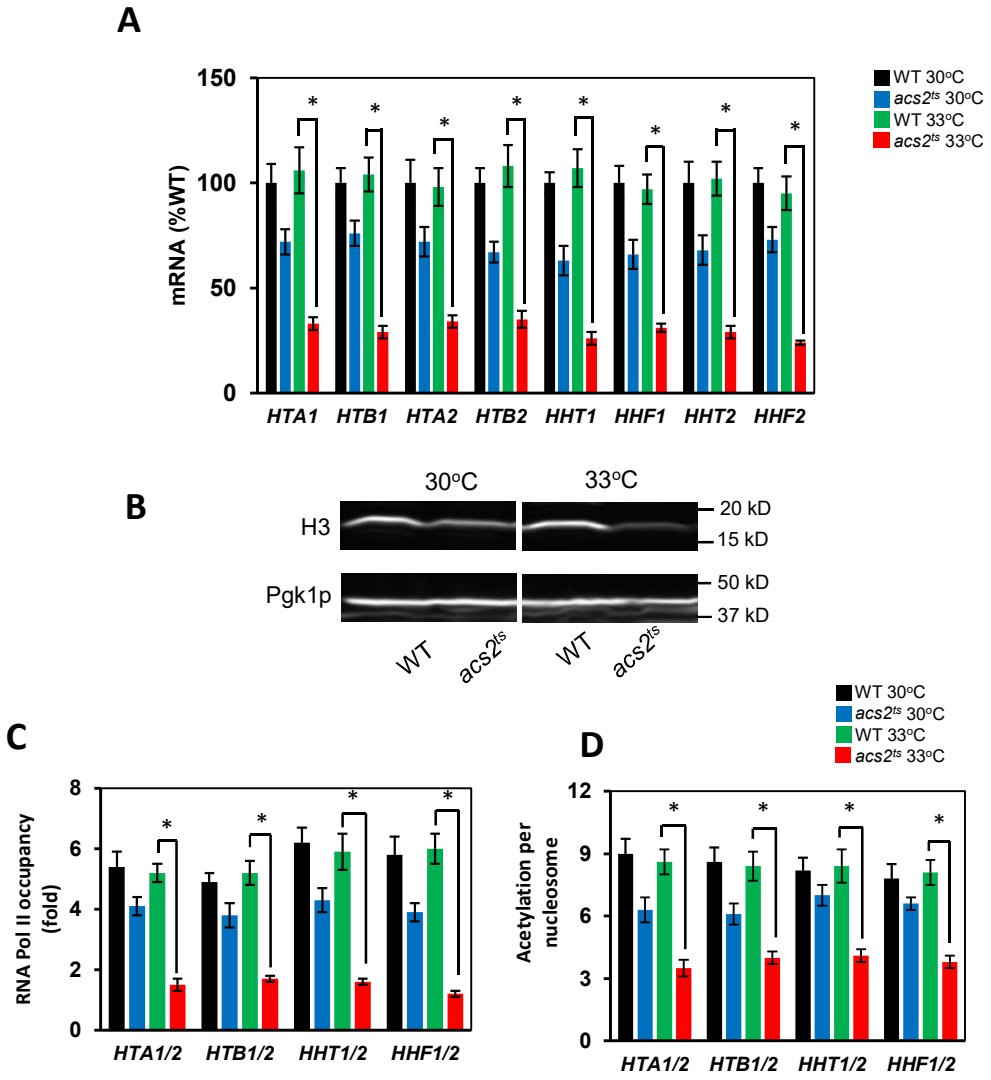

Figure 14. Acs2p inactivation downregulates transcription of histone.
(A) Relative histone mRNA levels; the results are shown relative to the value for the wild-type (WT) strain grown at 30°C. (B) Histone H3 protein levels; western blot analysis was performed three times, and representative results are shown. (C) Occupancy of RNA Pol II at histone promoters. (D) H3K14 acetylation in histone promoters. (A-D) WT and *acs2*^{ts} cells were grown in YPD medium at 30°C and 33°C.
(A, C, D) The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($p < 0.05$) from each other are indicated by a bracket and asterisk.

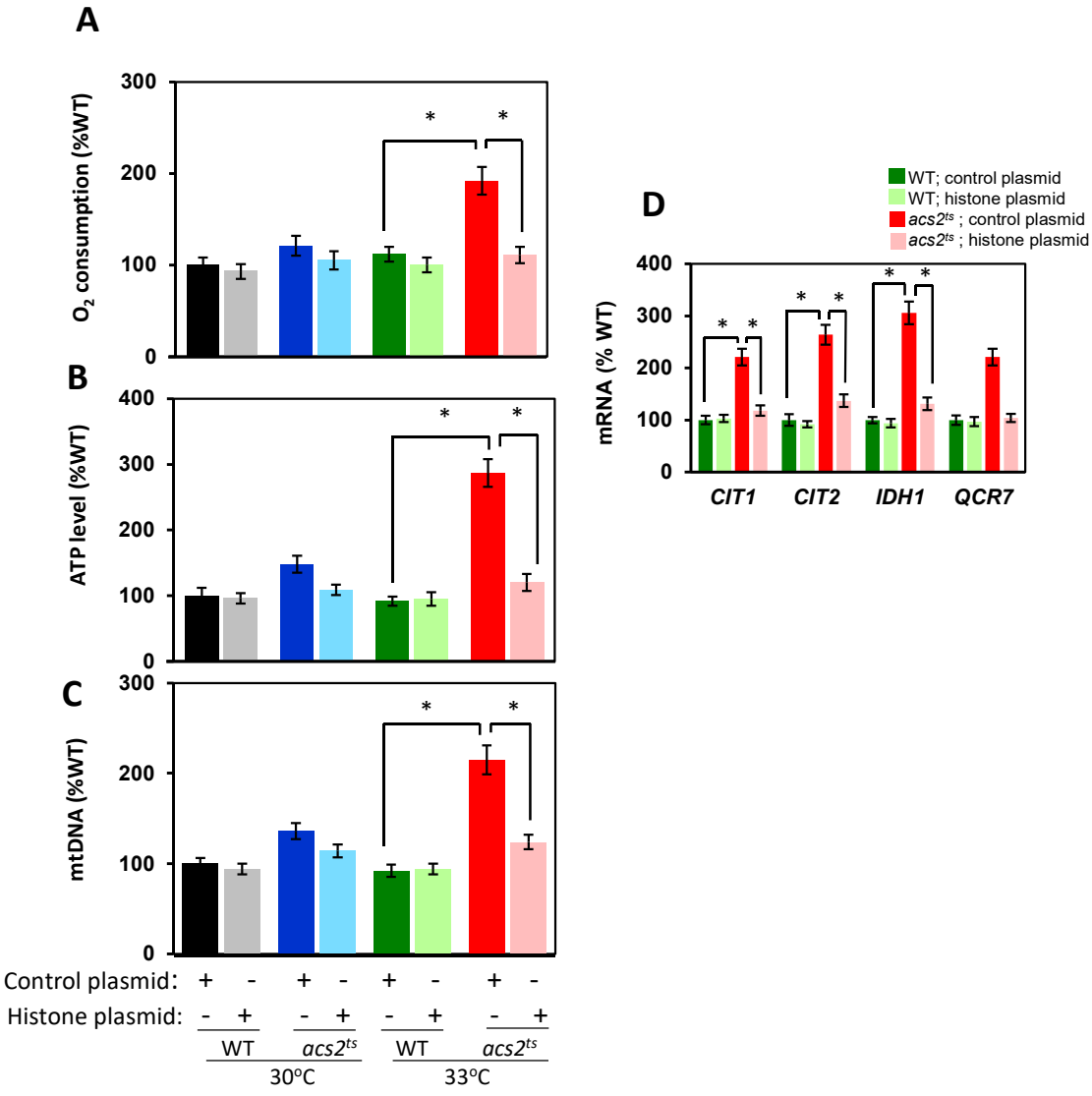

Figure 15. Acs2p inactivation elevates respiration.

(A) Relative oxygen consumption, (B) ATP levels, (C) mtDNA copy numbers, and (D) mRNA levels of respiratory genes in wild-type (WT) and *acs2*^{ts} cells containing either the control plasmid or a low-copy histone plasmid expressing all four core histone genes (plasmid pJH33; Blackwell et al., 2007). The cells were pre grown under selection in SC medium, inoculated to an A_{600} of 0.1 into YPD medium and grown for two generations at 33°C. (E, F, G, H) The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($p < 0.05$) from each other are indicated by a bracket and asterisk.

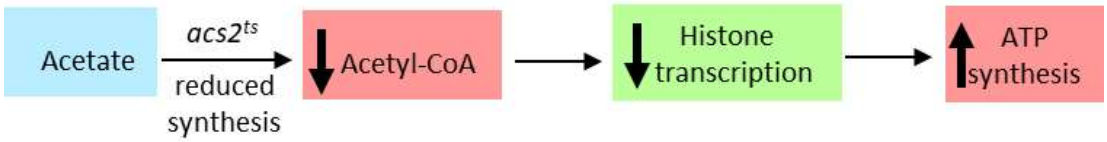

Figure 16. Model depicting the relationship between acetyl-CoA synthesis, histone transcription, and ATP synthesis.

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