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Reduced Histone Expression or a Defect in Chromatin Assembly Induces Respiration

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Regulation of mitochondrial biogenesis and respiration is a complex process that involves several signaling pathways and transcription factors as well as communication between the nuclear and mitochondrial genomes. Here we show that decreased expression of histones or a defect in nucleosome assembly in the yeast *Saccharomyces cerevisiae* results in increased mitochondrial DNA (mtDNA) copy numbers, oxygen consumption, ATP synthesis, and expression of genes encoding enzymes of the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). The metabolic shift from fermentation to respiration induced by altered chromatin structure is associated with the induction of the retrograde (RTG) pathway and requires the activity of the Hap2/3/4/5p complex as well as the transport and metabolism of pyruvate in mitochondria. Together, our data indicate that altered chromatin structure relieves glucose repression of mitochondrial respiration by inducing transcription of the TCA cycle and OXPHOS genes carried by both nuclear and mitochondrial DNA.

Mitochondria are essential and multifunctional organelles of eukaryotic cells. They contain enzymes of the tricarboxylic acid (TCA) cycle, β-oxidation, and oxidative phosphorylation (OXPHOS) and are thus responsible for the majority of ATP synthesis. Mitochondria also generate precursors for anabolic processes, including the synthesis of nucleotides, fatty acids, steroids, and amino acids. In addition, mitochondria are key players in metabolic regulation and are central to a number of physiological and pathophysiological processes such as apoptosis, cancer, degenerative diseases, and aging (1, 2). Mitochondrial biogenesis is a complex process that requires the replication of mitochondrial DNA (mtDNA) as well as the synthesis and assembly of mitochondrial proteins and lipids in preexisting mitochondrial structures. The control of this process requires the coordinated transcription of a large number of nuclear and mitochondrial genes (2).

The preferred sources of carbon and energy for the yeast *Saccharomyces cerevisiae* are fermentable sugars such as glucose (3–5). When yeast cells are grown in liquid cultures in rich media containing glucose under aerobic conditions, the cells metabolize glucose predominantly by glycolysis, producing pyruvate. The majority of this pyruvate is converted to acetaldehyde and subsequently to ethanol in the cytosol; only a small fraction of acetaldehyde is converted to acetate (6–8). This acetate is subsequently converted to acetyl coenzyme A (acetyl-CoA) by nucleocytosolic acetyl-CoA synthetase 2 (Acs2p) (9). Since glucose represses the TCA cycle and respiration in *S. cerevisiae*, only a small fraction of glycolytically produced pyruvate is translocated into mitochondria and converted to acetyl-CoA by the pyruvate dehydrogenase complex (6, 7).

After glucose is exhausted, yeast cells switch metabolism from fermentation to respiration during the diauxic shift and utilize ethanol as a carbon source (10–13). Yeast mitochondria respond to these changes in nutritional conditions. The abundance and activity of mitochondria increase, and mitochondria enlarge during the diauxic shift (14, 15). Mitochondrial biogenesis and activity are controlled by the protein kinase A (PKA), TOR, Sch9p, Snf1p, and Mec1p/Rad53p signaling pathways (11, 16–19). The transition from fermentation to oxidative metabolism and the upregulation of mitochondrial biogenesis and activity require several transcription factors, including Hap2/3/4/5p, Cat8p, Rtg1/3p, and Hcm1p (19–23). However, despite the central position of mitochondria in cell metabolism and physiology, the signaling mechanisms and coordinated transcriptional regulation by which mitochondrial abundance and activity are aligned with nutritional and environmental conditions are not well understood.

Mitochondrial function is monitored by the retrograde (RTG) signaling pathway. RTG signaling is triggered by mitochondrial dysfunction; however, it is also required for the activation of mitochondrial metabolism when cells are grown in nonfermentable carbon sources (21). The key event in the RTG pathway is the translocation of the Rtg1/3p transcription factor from the cytoplasm to the nucleus, with concomitant activation of the first four genes of the TCA cycle (19). Upregulation of peroxisomal citrate synthase, encoded by the *CIT2* gene, is a hallmark of RTG signaling activation (24).

Recent evidence revealed that metabolism and chromatin structure are tightly linked and that histones act as metabolic sensors of acetyl-CoA (25–27). On the other hand, histone acetylation and chromatin structure regulate the expression of metabolic genes (28, 29). In this study, we demonstrate that a decreased expression of histones or altered nucleosome structure of chromatin in budding yeast results in the upregulation of mitochondrial activity, redirection of metabolism from fermentation to respiration, and significantly increased ATP synthesis.

MATERIALS AND METHODS

Yeast strains and media. All yeast strains used in this study are listed in Table 1. Standard genetic techniques were used to manipulate yeast

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TABLE 1 Yeast strains used in this study

Strain	Genotype	Source or reference
W303-1a	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303-1α	*MAT*α *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303	*MAT***a**/*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
JHY200	*MAT***a** *leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 hta1-htb1*::*Nat hta2-htb2*::*HPH hht1-hhf1*::*KAN hht2-hhf2*::*KAN* pJH33[*URA3 CEN ARS HTA1-HTB1 HHT2-HHF2*]	30
LG329	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hta1-htb1*::*Nat hta2-htb2*::*HPH hht1-hhf1*::*KAN hht2-hhf2*::*KAN* pQQ18*[CEN LEU2 HTA1-HTB1 HHT2-HHF2]*	31
LG341	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hta1-htb1*::*Nat hta2-htb2*::*HPH hht1-hhf1*::*KAN hht2-hhf2*::*KAN* pQQ18[*HTA1-HTB1 HHT2*(*K9R K14R K18R*)*-HHF2*]	31
LG345	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hta1-htb1*::*Nat hta2-htb2*::*HPH hht1-hhf1*::*KAN hht2-hhf2*::*KAN* pQQ18 [*HTA1-HTB1 HHT2-HHF2*(*K5R K8R K12R*)]	31
YJL127C	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 spt10*::*KAN*	Open Biosystems
MZ672	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 spt10*::*KAN*	31
DY5780	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1*	32
BY108	*MAT*α *TRP1 ura3 leu2 lys2 ade2 swi4*::*HIS3*	33
MZ544	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi4*::*HIS3*	This study
DY2348	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi2*::*HIS3*	32
NWY009	*MAT*α *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS bar1*::*LEU2 cac2*::*KAN*	34
MZ576	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3*	This study
YNL206C	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 rtt106*::*KAN*	Open Biosystems
MZ642	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rtt106*::*KAN*	This study
YLL002W	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 rtt109*::*KAN*	Open Biosystems
MZ655	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rtt109*::*KAN*	This study
YJB8420	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 cac1*::*LEU2*	35
JWC113	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 cac1*::*LEU2*	This study
SY577	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*URA3 hir1*::*HIS3*	36
MZ700	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hir1*::*HIS3*	This study
BY551	*MAT***a** *ura3-52 lys2-801*^{a} *ade2-107*^{0} *his3Δ200 leu2-Δ1 SCB-lacZ mbp1*::*TRP1*	37
AD016	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 mbp1*::*TRP1*	This study
JFY006	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hht1-hhf1*::*LEU2 hht2-hhf2*::*kanMX3trp1*::*hht1-K56R HHF1*::*TRP1 asf1*::*his5*$^{+}$	38
LG635	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hht1-hhf1*::*LEU2 hht2-hhf2*::*kanMX3 trp1*::*hht1-K56R HHF1*::*TRP1*	This study
YEF473A	*MAT***a** *trp1-63 leu2-1 ura3-52 his3-200 lys2-801*	39
DCB200.1	*MAT***a** *trp1-63 leu2-1 ura3-52 his3-200 lys2-801 HHT1*::*TRP1 KAN-GAL1p-HHT2*	39
DY7250	*MAT*α *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 GALp*::*CDC20*::*ADE2 SPT15-HA*$_3$::*URA3*	40
LG564	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1 hir1*::*HIS3*	This study
LG658	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hht1-hhf1*::*LEU2 hht2-hhf2*::*TRP1 hht1-K56R HHF1*::*TRP1 hir1*::*HIS3*	This study
YKL109W	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 hap4*::*KAN*	Open Biosystems
LG579	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 hap4*::*KAN*	This study
LG625	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 spt10*::*KAN hap4*::*KAN*	This study
LG633	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1 hap4*::*KAN*	This study
LG576	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 hap4*::*KAN*	This study
YOL067C	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 rtg1*::*KAN*	Open Biosystems
FY2191	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 lys2-128δ spt10Δ201*::*HIS3* pFW217[*URA3 CEN ARS SPT10*]	41
TZ299	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 rtg1*::*KAN*	This study
LG693	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 spt10*::*HIS3 rtg1*::*KAN*	This study
TZ441	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1 rtg1*::*KAN*	This study
TZ371	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 rtg1*::*KAN*	This study
YER178W	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pda1*::*KAN*	Open Biosystems
TZ354	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 pda1*::*KAN*	This study
LG699	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 spt10*::*HIS3 pda1*::*KAN*	This study
TZ438	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1 pda1*::*KAN*	This study
TZ401	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 pda1*::*KAN*	This study
YGL080W	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 mpc1*::*KAN*	Open Biosystems
TZ341	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 mpc1*::*KAN*	This study
LG696	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 spt10*::*HIS3 mpc1*::*KAN*	This study
TZ435	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1 mpc1*::*KAN*	This study
TZ412	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 mpc1*::*KAN*	This study
YOR065W	*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cyt1*::*KAN*	Open Biosystems
LG533	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 cyt1*::*KAN*	This study
LG580	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 spt10*::*Kan cyt1*::*KAN*	This study
LG567	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 swi6*::*TRP1 cyt1*::*KAN*	This study
LG570	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100 asf1*::*HIS3 cyt1*::*KAN*	This study

strains and to introduce mutations from non-W303 strains into the W303 background (42). Cells were grown at 28°C in YEP medium (1% yeast extract, 2% Bacto peptone) containing 5% glucose or under selection in synthetic complete (SC) medium containing 5% glucose and, when appropriate, lacking specific nutrients in order to select for a particular genotype.

mtDNA 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 5% 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, glucose, and ethanol assays. For ATP assays, cells were grown to an A_{600} of 0.6 in YEP medium containing either 5% glucose or an alternative carbon source, and 9×10^7 cells were harvested by centrifugation and lysed 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 an Enliten ATP assay (catalog number FF2000; Promega) according to the manufacturer's instructions and normalized by the number of cells. Glucose and ethanol concentrations were assayed in culture supernatants by using a glucose (HK) assay kit (catalog number GAHK-20; Sigma) and an ethanol assay kit (catalog number MAK076; Sigma), respectively.

Real-time RT-PCR. Real-time reverse transcription-PCR (RT-PCR) was performed as described previously (43), using primers for *ACT1* (5′-TATGTGTAAAGCCGGTTTTGC-3′ and 5′-GACAATACCGTGTTCAATTGGG-3′), *CIT1* (5′-CAGCGATATTATCAACAACTAGCA-3′ and 5′-TAGTGGCGAGCATTCAATAGTG-3′), *ACO1* (5′-TGTTCGTGGTCTTGCGACA-3′ and 5′-CGTTTCCACATTCTGCTTGTAGT-3′), *IDH1* (5′-TGCTTAACAGAACAATTGCTAAGAG-3′ and 5′-AACACCGTCACCAGGTATCAA-3′), *IDH2* (5′-GGCTACTGTAAAGCAACCTTCAATC-3′ and 5′-GGAAATTTCAGGTCCGATACCA-3′), *KGD1* (5′-AACCTGCCGGTATAGTTCAAGA-3′ and 5′-CCCTCTTCCGACAATTTTGA-3′), *LSC1* (5′-ACCGTTTCAAAAGCTTCTCTCA-3′ and 5′-AAAGATCACCTTGGTGTCCTTG-3′), *SDH1* (5′-CTCCAAGTTGACTTTGCTCAGAA-3′ and 5′-ACGCGGAACCGTTTACAGA-3′), *FUM1* (5′-CAGAACTGAAACCGATGCATT-3′ and 5′-CGCCACCAATCTTGAAGTTT-3′), *MDH1* (5′-TAAACGTGCGTTTTCCTCTACAG-3′ and 5′-GAAGCAAAGACAATGGTTGTCC-3′), *COR1* (5′-CTAACCAGTTCAAGAGGTCTTTGG-3′ and 5′-TGTGTGAGCGGAAGGATTATG-3′), *QCR7* (5′-ACGTCTATTGCGAGAATTGGTG-3′ and 5′-AGCCCTAACTTCTTGTAACCTGC-3′), *CYT1* (5′-TCAAAGGACCCTCTCGAAAAGT-3′ and 5′-AGCAACACCCGCTGTAACG-3′), *COX1* (5′-CAACAAATGCAAAAGATATTGCAG-3′ and 5′-AATATTGTGAACCAGGTGCAGC-3′), *CIT2* (5′-AGAGATTTAGCGAAATCTACCCC-3′ and 5′-CCTCTCATACCACCATATACCTGTT-3′), *HAP4* (5′-CCGCAAAGACTTTTCTACACAGG-3′ and 5′-TGTTATGATGGTTGGTATTTGGG-3′), *RTG1* (5′-GCAAGCGCAGAGATAAGATCA-3′ and 5′-TTGCTACCAGAATTGCCGTAA-3′), *RPO41* (5′-AACATCCCTGTTACAAAGACGG-3′ and 5′-TGTTACAAGGGGGTCTTCAGAT-3′), *MTF1* (5′-CTTGACCTCTACCCTGGTGTTG-3′ and 5′-GACCCTTCGAATTTTGCATTC-3′), *COX2* (5′-GTGGTGAAACTGTTGAATTTGAATC-3′ and 5′-CATCAGCAGCTGTTACAACGAA-3′), and *COX3* (5′-AGACATCAACAACATCCATTTCAT-3′ and 5′-CCATGCATTGTTAATGCTAGTGA-3′).

Western blotting. Cells were grown in YEP medium containing 5% glucose to an A_{600} of 0.6. Four A_{600} units (one A_{600} unit is equal to $\sim 3 \times 10^7$ cells) were harvested and boiled immediately in SDS sample buffer. Denatured proteins were separated on a 15% denaturing polyacrylamide gel. Western blotting with anti-histone H3 polyclonal antibody (catalog number ab1791; Abcam) at a dilution of 1:1,000, 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 was carried out as described previously (43, 44). 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.

Statistical analysis. The results represent data from at least three independent experiments. Numerical results are presented as means ± standard deviations (SD). Data were analyzed by using an InStat software package (GraphPad, San Diego, CA). Statistical significance was evaluated by one-way analysis of variance (ANOVA), and a P value of <0.05 was considered significant.

RESULTS

Decreased histone expression or defect in chromatin assembly increases mitochondrial respiration. The diauxic shift is associated with extensive transcriptional reprogramming that includes the upregulation of genes involved in the TCA cycle and the OXPHOS pathway. Previously, we reported that a subset of the genes that are induced during the diauxic shift is also upregulated when chromatin histones are globally hypoacetylated (11, 31). One of the mechanisms that globally regulates the acetylation of chromatin histones is the nucleocytosolic concentration of acetyl-CoA available to histone acetyltransferases (9, 28, 45). To test whether histone acetylation is involved in the regulation of mitochondrial activity, we determined relative mitochondrial DNA (mtDNA) copy numbers and oxygen consumption in strains that express nonacetylatable versions of histones H3 and H4 (Fig. 1A). We used strains expressing histone H3 or H4 with mutations that change acetylatable lysine into nonacetylatable arginine residues within the N-terminal tails (H3-K9R/K14/R/K18R and H4-K5R/K8R/K12R); we refer to these histone mutants as H3-3KR and H4-3KR, respectively. The mtDNA copy number and oxygen consumption were slightly, but reproducibly, upregulated in these mutants, suggesting that global histone hypoacetylation increases the expression of mitochondrial genes and promotes mitochondrial respiration (Fig. 1A).

We previously showed that one of the mechanisms by which global histone hypoacetylation in H3-3KR and H4-3KR affects gene expression is by altering nucleosome positioning and thus making regulatory regions of promoter DNA accessible to transcription factors (31). The set of genes that was upregulated in the H3-3KR and H4-3KR mutants was more significantly upregulated in *spt10*Δ cells (31). Spt10p is a transcription factor specific for histone genes, and *spt10*Δ cells display reduced expression of histones and globally altered chromatin structure (46, 47). To determine whether reduced expression of histone genes results in increased mitochondrial respiration, we measured mtDNA copy numbers, oxygen consumption, and cellular ATP levels in *spt10*Δ, *swi6*Δ, *swi4*Δ, *mbp1*Δ, and *swi2*Δ cells (Fig. 1B to D). Spt10p, Swi6p, Swi4p, Mbp1p, and Swi2p are transcription factors and regulators involved in the expression of histone genes (48, 49). We

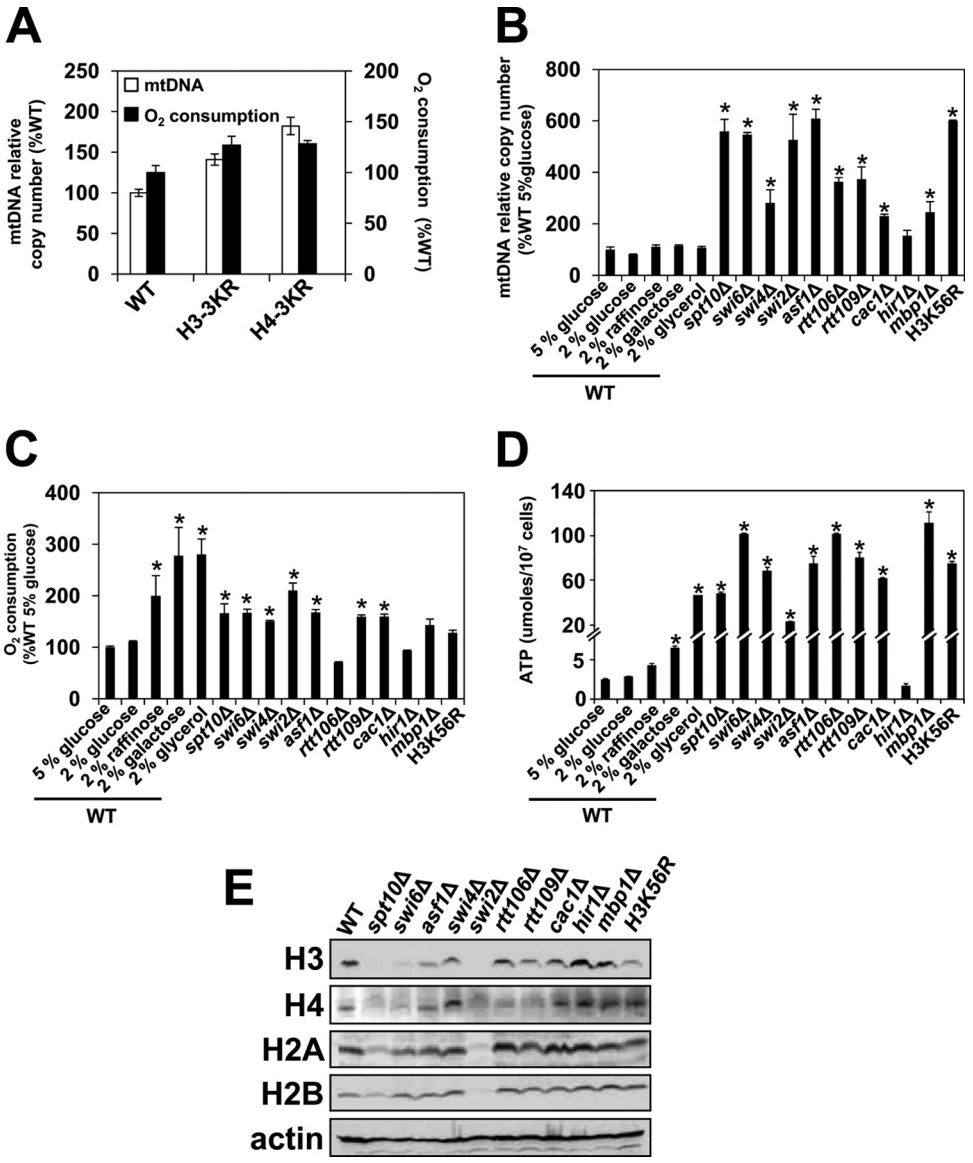

FIG 1 Decreased histone expression or a defect in nucleosome assembly induces mitochondrial respiration. Unless indicated otherwise, cells were grown in YEP medium containing 5% glucose. (A) Relative mtDNA copy numbers of WT (LG329), H3-3KR (LG341), and H4-3KR (LG345) cells. (B to E) Relative mtDNA copy numbers (B), cellular oxygen consumption (C), cellular ATP levels (D), and levels of histone proteins (E) in the wild-type (WT) (W303-1a), *spt10*Δ (MZ672), *swi6*Δ (DY5780), *swi4*Δ (MZ544), *swi2*Δ (DY2348), *asf1*Δ (MZ576), *rtt106*Δ (MZ642), *rtt109*Δ (MZ655), *cac1*Δ (JWC113), *hir1*Δ (MZ700), *mbp1*Δ (AD016), and H3K56R (LG635) strains. For panels A to D, the experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) from those of wild-type cells are indicated by an asterisk. For panels A to C, the results are expressed relative to the value for the wild-type strain grown in 5% glucose. For panel E, Western blot analysis was performed three times, and representative results are shown.

also included *asf1*Δ, *rtt106*Δ, *rtt109*Δ, *cac1*Δ, and H3K56R mutants in our analysis, since the corresponding genes and acetylation of lysine 56 of histone H3 are involved in chromatin assembly (50, 51). The data show that decreased expression levels of histone genes or altered chromatin assembly results in increased mtDNA levels, increased oxygen consumption, and elevated ATP levels (Fig. 1B to D). To ensure fully glucose-repressed conditions, the cells were grown in medium containing 5% glucose. The values for oxygen consumption and cellular ATP levels for most of the mutants grown in 5% glucose are comparable to the values for wild-type cells grown in glycerol.

Next, we compared the total levels of histone proteins in the mutants that display increased mitochondrial respiration. While the total levels of all four core histones were significantly decreased in *spt10*Δ, *swi6*Δ, and *swi2*Δ cells in comparison with wild-type cells, the levels of only some histones were slightly reduced in *swi4*Δ, *asf1*Δ, *rtt109*Δ, *mbp1*Δ, and H3K56R cells. We found no difference in the histone levels in *rtt106*Δ and *cac1*Δ cells and, as expected, increased histone levels in *hir1*Δ cells (Fig. 1E). Asf1p is a histone chaperone that stimulates the efficient acetylation of H3 on lysine 56 (H3K56) by Rtt109p. Acetylated H3K56 binds to Rtt106p, which interacts with Cac1p, the large subunit of chromatin assembly factor 1 (CAF-1) (50, 52). Since *asf1*Δ, *rtt109*Δ, *rtt106*Δ, and *cac1*Δ mutations result in defects in nucleosome as-

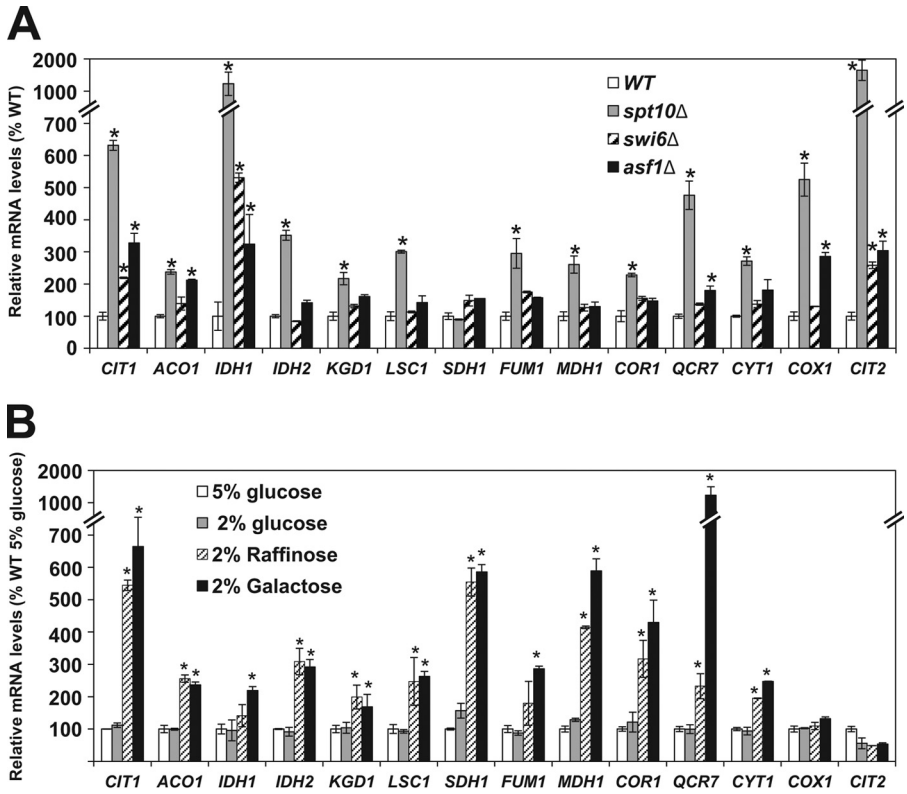

FIG 2 Derepression of the TCA cycle and OXPHOS genes. (A) Relative mRNA levels in the wild-type (WT) (W303-1a), *spt10*Δ (MZ672), *swi6*Δ (DY5780), and *asf1*Δ (MZ576) strains. (B) Wild-type (W303-1a) cells were grown in YEP medium containing the indicated carbon sources, and the relative mRNA levels were determined. The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) from those of wild-type cells are indicated by an asterisk. The results are expressed relative to the value for the wild-type strain grown in 5% glucose.

sembly (50–52), it seems likely that the primary mechanism through which they induce mitochondrial respiration involves altered chromatin structure rather than decreased levels of histone proteins. The levels of histones H3 and H4 were slightly reduced in H3K56R cells (Fig. 1E), probably due to the reduced histone gene dosage in H3K56R cells (strain LG635). Thus, the increase in mitochondrial respiration in the H3K56R strain is likely due to the combined effect of the reduced histone gene dosage and the defect in nucleosome assembly.

In addition to the significantly elevated oxygen consumption and ATP synthesis, we also found increased expression levels of genes encoding enzymes of the TCA cycle and OXPHOS pathway in the *spt10*Δ, *swi6*Δ, and *asf1*Δ mutants (Fig. 2A). While the mtDNA copy numbers, oxygen consumption, and cellular ATP levels were comparable in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells, *spt10*Δ cells displayed a significantly higher level of induction of the TCA cycle and OXPHOS pathway genes than did *swi6*Δ and *asf1*Δ cells (Fig. 2A). All the upregulated genes have predicted binding sites for the transcription complex Hap2/3/4/5p (22), and most of them are upregulated in a strain overexpressing *HAP4* (53). Interestingly, the genes with the greatest changes in expression levels (*CIT1*, *IDH1*, *IDH2*, *QCR7*, and *CIT2*) are also regulated by the Rtg1/3p transcription factor (54–57). Moreover, a microarray analysis showed that the Hap2/3/4/5p complex also regulates the expression of *RTG1* (55). It is possible that the altered chromatin structure in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells also permits increased recruitment of the Hap2/3/4/5p complex to the *RTG1* promoter, resulting in an increased transcription level of *RTG1* and synergistic activation of the TCA cycle and OXPHOS pathway genes by the Hap2/3/4/5p and Rtg1/2p complexes. This notion is supported by the increased expression of *CIT2* in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells. *CIT2* encodes the peroxisomal form of citrate synthase, and its induction is a hallmark of activation of the retrograde pathway (19). These results suggest that increased mitochondrial respiration in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells is associated with the induction of the retrograde (RTG) pathway and are in contrast with the induction of mitochondrial respiration in wild-type cells grown on raffinose or galactose, which is not associated with increased *CIT2* expression and the induction of the retrograde pathway (Fig. 2B).

The carbon source regulates oxygen consumption, cellular ATP levels, and expression of the TCA cycle and OXPHOS pathway genes in wild-type cells (Fig. 1C and D and 2B); however, it does not affect the mtDNA copy number in wild-type cells (Fig. 1B). In contrast, reduced expression of histones or a defect in chromatin assembly also elevate the mtDNA copy number (Fig. 1B).

mtDNA copy number and mitochondrial respiration are regulated by expression of histone genes. To confirm that decreased histone expression elevates mtDNA copy numbers by a different approach, we used a strain in which the sole histone H3 gene is under the control of the *GAL1* promoter (*GAL-H3*) (39). This allows transcription of histone H3 on galactose and its repression on glucose. After glucose was added to the *GAL-H3*

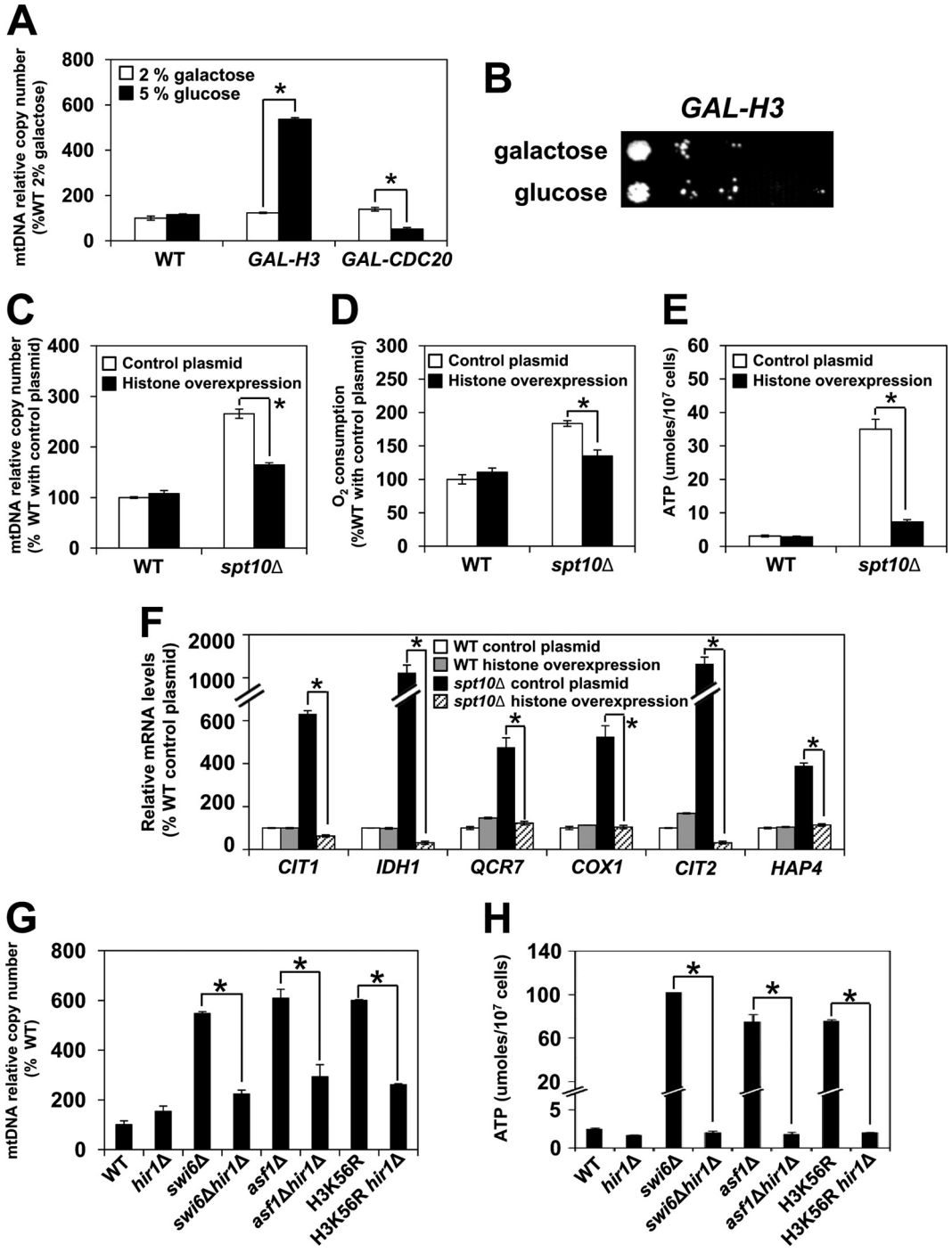

FIG 3 mtDNA copy number and mitochondrial respiration are regulated by expression of the histone genes. (A) Relative mtDNA copy numbers of the wild-type strain (WT) (W303-1a) (shown for comparison with previously reported results) and strains with the sole histone H3 gene (DCB200.1) or the *CDC20* gene (DY7250) under the control of the *GAL1* promoter. The DCB200.1 and DY7250 strains are shown as *GAL-H3* and *GAL-CDC20*, respectively. Cells were grown in YEP medium containing 2% galactose to an A_{600} of 0.6 at 30°C. Each culture was split in two, glucose was added to half of each culture to a final concentration of 5%, and cells were grown for an additional 16 h. (B) Viability of the *GAL-H3* strain is not affected by the addition of glucose. *GAL-H3* cells were grown as described above for panel A. Cells were harvested and washed, and 10-fold serial dilutions of the cells were spotted onto YEP plates containing 2% galactose. The plates were incubated for 2 days at 30°C, and typical results from three independent experiments are shown. (C to F) Relative mtDNA copy numbers (C), cellular oxygen consumption (D), cellular ATP levels (E), and relative mRNA levels (F) of wild-type (W303-1a) and *spt10*Δ (MZ672) cells containing either the control plasmid or a high-copy-number plasmid expressing all four core histone genes (plasmid pFB1156) (38). The cells were pregrown under selection in SC medium, inoculated to an A_{600} of 0.1 into YEP medium containing 5% glucose, and grown for two generations at 30°C. (G and H) Relative mtDNA copy numbers (G) and cellular ATP levels (H) of the wild-type (W303-1a), *hir1*Δ (MZ700), *swi6*Δ (DY5780), *swi6*Δ *hir1*Δ (LG564), *asf1*Δ (MZ576), *asf1*Δ *hir1*Δ (SY577), H3K56R (LG635), and H3K56R *hir1*Δ (LG658) strains. For panels A and C to 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 an asterisk. For panels A, C, D, F, and G, the results are expressed relative to the value for the wild-type strain.

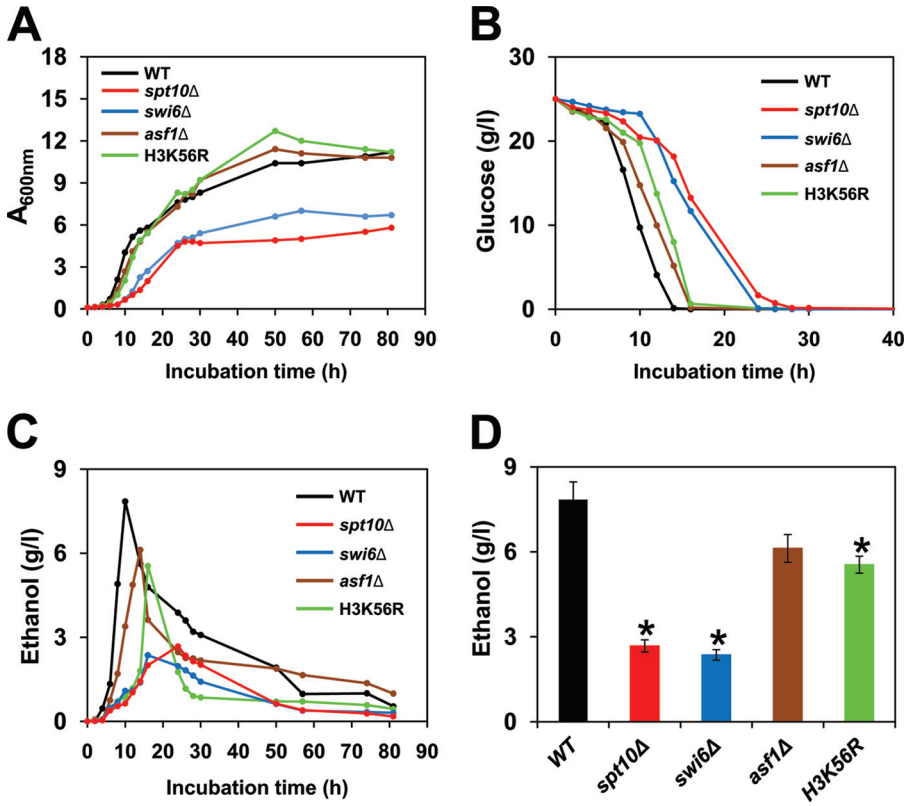

FIG 4 Altered chromatin structure in *spt10*Δ, *swi6*Δ, *asf1*Δ, and H3K56R cells results in a switch from fermentation to respiration. (A to C) Growth curve (A_{600}) (A) and glucose (B) and ethanol (C) concentrations during growth of the wild-type (WT) (W303-1a), *spt10*Δ (MZ672), *swi6*Δ (DY5780), *asf1*Δ (MZ576), and H3K56R (LG635) strains. (D) Highest concentrations of ethanol in culture medium reached during growth. For panels A to C, the experiments were performed three times, and representative results are shown. For panel D, the experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) from those of wild-type cells are indicated by an asterisk.

strain, the mtDNA copy number was significantly elevated (Fig. 3A), providing additional evidence that histone depletion results in increased mtDNA copy numbers. As the viability of *GAL-H3* cells remained unchanged after glucose addition (Fig. 1B), the increased mtDNA copy number cannot be attributed to decreased cell viability. To eliminate the possibility that the increase in the mtDNA copy number is a consequence of the inactivation of an essential gene, we used a strain in which the *CDC20* gene is under the control of the *GAL1* promoter. *CDC20* is an essential gene required for the activation of the anaphase-promoting complex/cyclosome and progression through mitosis (58). After the *GAL-CDC20* strain was transferred to glucose, the mtDNA copy number did not increase, indicating that the increase of the mtDNA copy number in *GAL-H3* cells is due to the depletion of histone H3 and is not a general response to a depletion of an essential protein (Fig. 3A).

In addition, we tested whether the ectopic expression of extra histones could decrease the respiratory phenotype of *spt10*Δ cells. A high-copy-number plasmid encoding all four core histones significantly reduced the mtDNA copy numbers, oxygen consumption, ATP levels, and mRNA levels of several genes encoding enzymes of the TCA cycle and OXPHOS pathway in *spt10*Δ cells, restoring all values to near-wild-type levels (Fig. 3C to F). Accordingly, deletion of *HIR1*, encoding a subunit of the HIR complex that represses histone gene transcription (59, 60), in the *swi6*Δ, *asf1*Δ, and H3K56R mutants decreased the mtDNA copy numbers and ATP levels of the corresponding strains (Fig. 3G and H). The effect of the *hir1*Δ mutation on *spt10*Δ cells could not be tested, as *hir1*Δ and *spt10*Δ mutations are synthetically lethal. Deletion of *HIR1* increases the expression of histone genes (61), and our results suggest that increasing the histone expression in *swi6*Δ, *asf1*Δ, and H3K56R cells by introducing the *hir1*Δ mutation suppresses both elevated mtDNA copy numbers and mitochondrial respiration, as indicated by lower ATP levels (Fig. 3G and H).

Altered chromatin structure in *spt10*Δ, *swi6*Δ, *asf1*Δ, and H3K56R cells results in a metabolic shift from fermentation to respiration. An interesting feature of budding yeast metabolism is the conversion of glucose to ethanol and carbon dioxide under aerobic conditions, a phenomenon known as the Crabtree effect (62, 63). To find out how the altered chromatin structure in *spt10*Δ, *swi6*Δ, *asf1*Δ, and H3K56R cells affects metabolism, we measured growth rates, glucose consumption, and ethanol production in the corresponding strains. The doubling times of wild-type, *spt10*Δ, *swi6*Δ, *asf1*Δ, and H3K56R cells were 1.5, 4.6, 4.4, 1.9, and 2.1 h, respectively (Fig. 4A). As expected, wild-type cells rapidly converted glucose into ethanol and biomass during exponential growth (Fig. 4A to C). *spt10*Δ and *swi6*Δ cells displayed a severe growth defect, utilized glucose slowly, and produced significantly less ethanol (Fig. 4), while the corresponding phenotypes of *asf1*Δ and H3K56R cells were less severe (Fig. 4). Cumulatively, the results show that the altered chromatin structure, particularly in *spt10*Δ and *swi6*Δ cells, results in the highly efficient operation

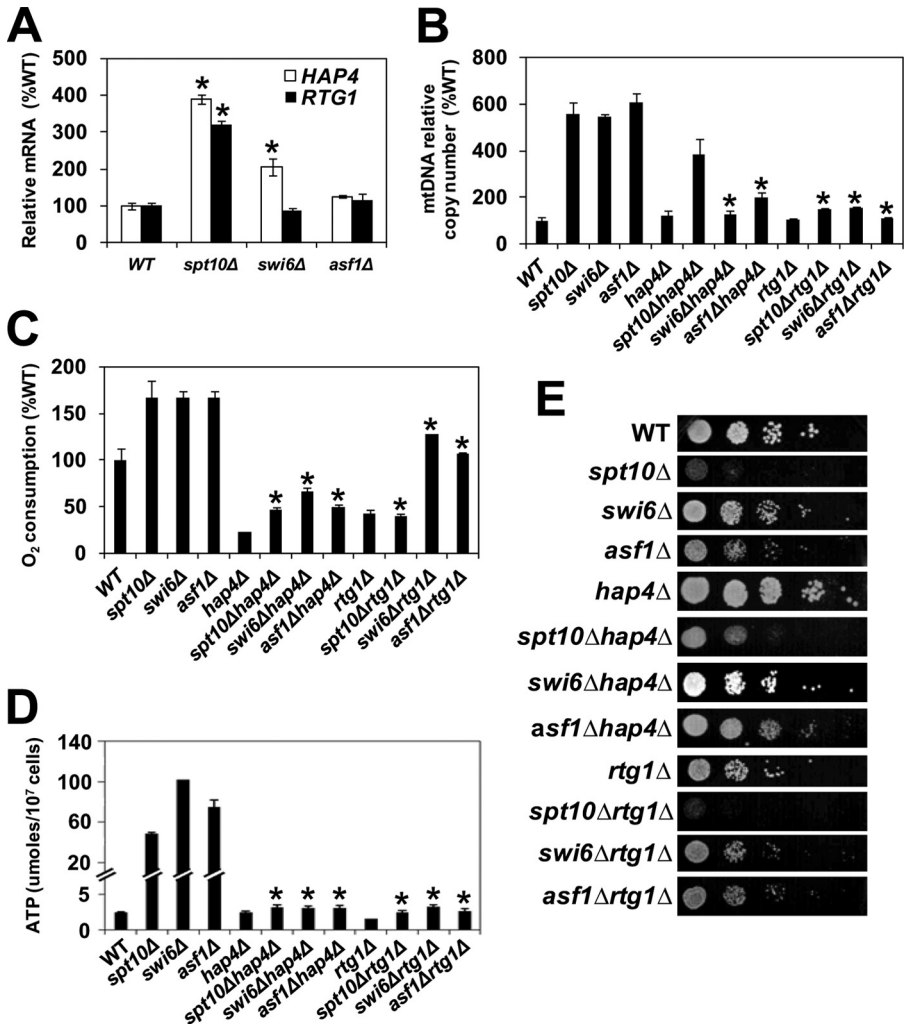

FIG 5 Chromatin defect-induced mitochondrial respiration requires *HAP4* and *RTG1*. (A) *HAP4* and *RTG1* relative mRNA levels in the wild-type (WT) (W303-1a), *spt10*Δ (MZ672), *swi6*Δ (DY5780), and *asf1*Δ (MZ576) strains. The experiment was repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) from those of wild-type cells are indicated by an asterisk. (B to E) Relative mtDNA copy numbers (B), cellular oxygen consumption (C), cellular ATP levels (D), and growth (E) of the wild-type (W303-1a), *spt10*Δ (MZ672), *swi6*Δ (DY5780), *asf1*Δ (MZ576), *hap4*Δ (LG579), *spt10*Δ *hap4*Δ (LG625), *swi6*Δ *hap4*Δ (LG633), *asf1*Δ *hap4*Δ (LG576), *rtg1*Δ (TZ299), *spt10*Δ *rtg1*Δ (LG693), *swi6*Δ *rtg1*Δ (TZ441), and *asf1*Δ *rtg1*Δ (TZ371) strains. For panels B to D, the experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) for the double mutants in comparison to the corresponding single mutants (*spt10*Δ, *swi6*Δ, and *asf1*Δ) are indicated by an asterisk. (E) Tenfold serial dilutions of cells were spotted onto yeast extract-peptone-dextrose plates and grown for 2 days at 30°C. Typical results from three independent experiments are shown.

of mitochondrial respiration (Fig. 1 and 2), slower utilization of glucose (Fig. 4B), and decreased synthesis of ethanol (Fig. 4C and D).

Chromatin defect-induced mitochondrial respiration requires *HAP4* and *RTG1*. Expression of the TCA cycle and OXPHOS genes is regulated by glucose levels independently of PKA and SNF1 by the Hap2/3/4/5p transcription complex, suggesting that the Hap2/3/4/5p complex provides an additional mechanism of transcriptional regulation of mitochondrial respiration (5). The Hap2/3/4/5p complex binds to DNA through the Hap2, -3, and -5 subunits, which are constitutively expressed. The activation domain of the complex is contained within the Hap4p subunit (64). *HAP4* expression increases upon glucose depletion (65), and overexpression of *HAP4* induces respiration, even in a glucose-repressed state (53, 66). Since the induction of Hap2/3/4/5p complex-responsive genes is independent of SNF1 activity, we decided to test the possibility that decreased expression of histone genes induces *HAP4* expression. We found that *HAP4* expression levels are elevated 3.9- and 2.1-fold in *spt10*Δ and *swi6*Δ cells, respectively, in comparison to those in wild-type cells (Fig. 5A). As expected, deletion of *HAP4* significantly reduced mtDNA copy numbers, oxygen consumption, and ATP levels in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells (Fig. 5B to D). Interestingly, we found that the reduced respiration due to the *hap4*Δ mutation in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells improved their slow growth (Fig. 5E). It is possible that the shunting of carbon metabolism from oxidative phosphorylation to glycolysis in *hap4*Δ mutants allows cells to use more pyruvate and other metabolic intermediates for biosynthetic purposes.

Respiration is also regulated by RTG signaling. We found that

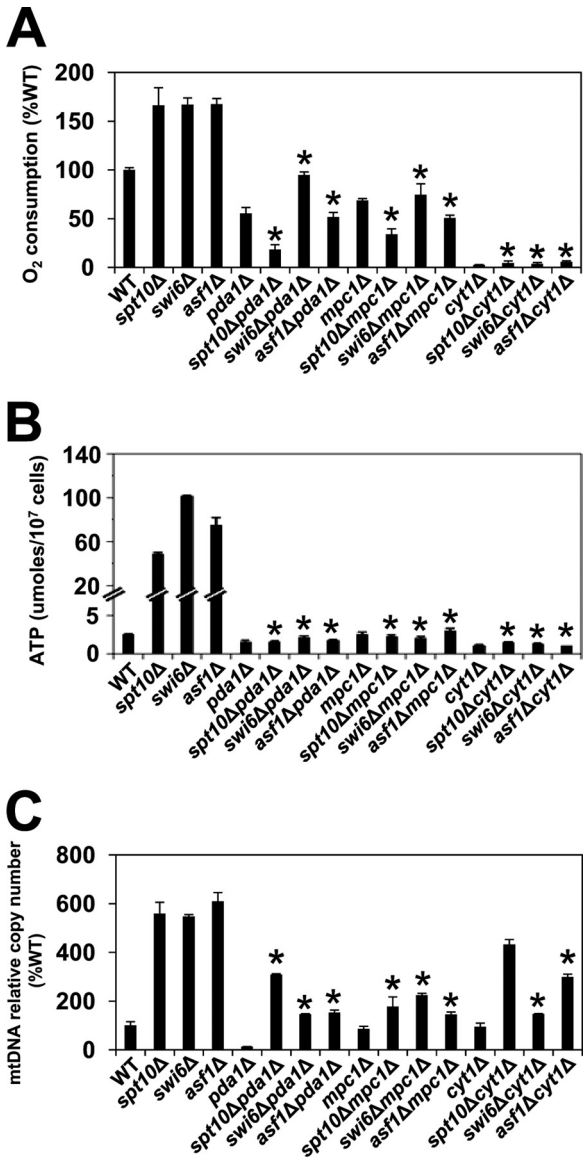

FIG 6 Chromatin defect-induced mitochondrial respiration requires transport and metabolism of pyruvate. (A to C) Cellular oxygen consumption (A), cellular ATP levels (B), and relative mtDNA copy numbers (C) of the wild-type (WT) (W303-1a), *spt10*Δ (MZ672), *swi6*Δ (DY5780), *asf1*Δ (MZ576), *pda1*Δ (TZ354), *spt10*Δ *pda1*Δ (LG699), *swi6*Δ *pda1*Δ (TZ438), *asf1*Δ *pda1*Δ (TZ401), *mpc1*Δ (TZ341), *spt10*Δ *mpc1*Δ (LG696), *swi6*Δ *mpc1*Δ (TZ435), *asf1*Δ *mpc1*Δ (TZ412), *cyt1*Δ (LG533), *spt10*Δ *cyt1*Δ (LG580), *swi6*Δ *cyt1*Δ (LG567), and *asf1*Δ *asf1*Δ (LG570) strains. The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) for the double mutants in comparison to the corresponding single mutants (*spt10*Δ, *swi6*Δ, and *asf1*Δ mutants) are indicated by an asterisk.

CIT2 expression, the hallmark of activation of the RTG pathway, is increased in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells (Fig. 2A), despite *RTG1* expression being increased only in *spt10*Δ cells (Fig. 5A). Therefore, the activation of RTG signaling in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells is likely due to both increased *RTG1* expression (in *spt10*Δ cells) and increased translocation and/or DNA binding of the Rtg1/3p complex (in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells). Similarly to *HAP4*, deletion of *RTG1* significantly reduced mtDNA copy numbers, oxygen consumption, and ATP levels in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells (Fig. 5B to D). Unlike *hap4*Δ cells, *rtg1*Δ cells grew more slowly than wild-type cells, and the *rtg1*Δ mutation did not suppress the slow growth of *spt10*Δ, *swi6*Δ, and *asf1*Δ cells (Fig. 5E). It is likely that the sets of transcriptional targets of the Hap2/3/4/5p and Rtg1/3p complexes are not identical and that the inactivation of the two complexes affects transcription and cellular physiology differently.

Chromatin defect-induced mitochondrial activity requires transport and metabolism of pyruvate. To determine whether pyruvate transport or metabolism affects mitochondrial activity in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells, we impaired the transport and metabolism of pyruvate in mitochondria by deleting the *MPC1* and *PDA1* genes. The pyruvate transporter Mpc1p is localized in the inner mitochondrial membrane, and mitochondria isolated from *mpc1*Δ cells have decreased pyruvate uptake (67, 68). Pda1p is a subunit of the pyruvate dehydrogenase complex that catalyzes the conversion of pyruvate to acetyl-CoA in mitochondria. As expected, introduction of the *mpc1*Δ or *pda1*Δ mutation into *spt10*Δ, *swi6*Δ, and *asf1*Δ cells reduced oxygen consumption and ATP synthesis to levels comparable to or lower than those of wild-type cells (Fig. 6A and B). mtDNA copy numbers were also significantly reduced in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells in combination with the *mpc1*Δ or *pda1*Δ mutation (Fig. 6C). In a parallel experiment, we found that the increased oxygen consumption and ATP levels in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells were reduced to levels lower than those in wild-type cells by a defect in the electron transport chain due to deletion of the cytochrome c_1 subunit *CYT1* (Fig. 6A and B). Interestingly, while the *mpc1*Δ, *pda1*Δ, or *cyt1*Δ mutation reduced oxygen consumption and ATP synthesis to levels comparable to or lower than those of wild-type cells, the mtDNA copy numbers in the double mutants remained higher than those in wild-type cells.

Mitochondrial metabolism is required for expression of genes carried by both nuclear and mitochondrial DNA. Coordination between the mitochondria and nucleus is required for the balanced production of proteins necessary for mitochondrial respiration. Disruptions in mitochondrial metabolism are known to regulate the nuclear genome through the activation of RTG signaling (19, 69). However, the mechanism by which the chromatin structure in the nucleus affects the expression of genes carried by mitochondrial DNA is less obvious. A major mechanism that regulates the activity of the mitochondrial RNA polymerase Rpo41p/Mtf1p *in vitro* is the concentration of ATP (70, 71). To test whether this mechanism regulates the transcription of *COX1*, *COX2*, and *COX3*, three genes carried by mitochondrial DNA, we measured the corresponding transcript levels in *spt10*Δ cells. As expected, the transcription of all three genes was significantly elevated in *spt10*Δ cells (Fig. 7A). Inactivation of *PDA1*, *MPC1*, *HAP4*, and *RTG1* in *spt10*Δ cells results in reduced cellular ATP levels (Fig. 5D and 6B) and reduced transcription of *COX1*, *COX2*, and *COX3* (Fig. 7A). However, despite the decreased transcription of *COX1* in *spt10*Δ *cyt1*Δ cells, the transcription of *COX2* and *COX3* was increased in *spt10*Δ *cyt1*Δ cells in comparison to *spt10*Δ cells (Fig. 7A). Since the *cyt1*Δ mutation eliminates ATP synthesis in mitochondria and significantly reduces the cellular ATP level, these results suggest that the mitochondrial ATP level is not the major or the only mechanism regulating the transcription of *COX1*, *COX2*, and *COX3* in *spt10*Δ cells. To explore other possible mechanisms that regulate the transcription of genes carried by

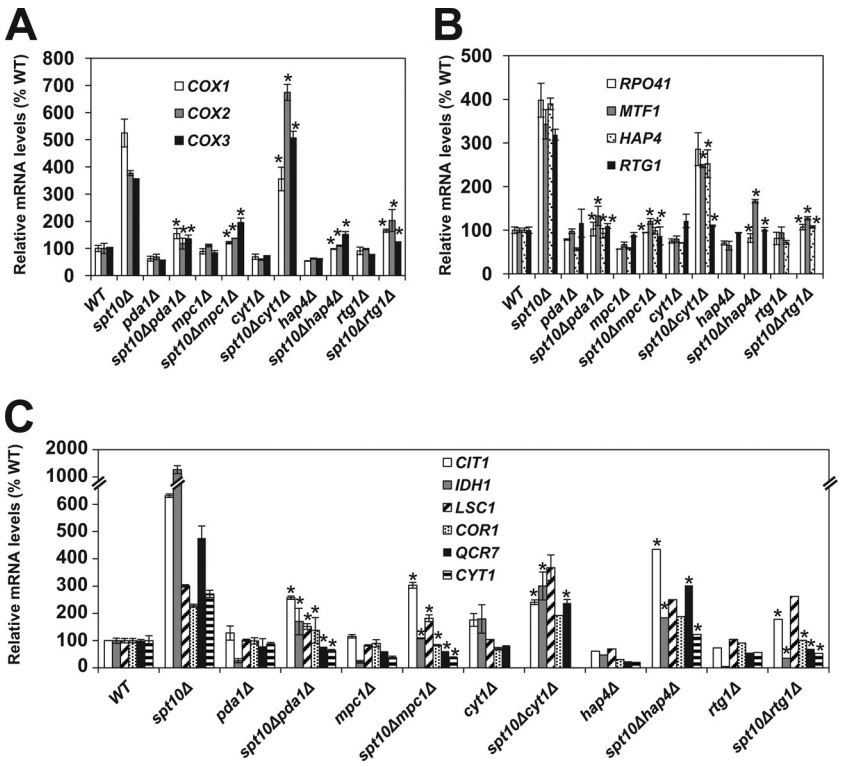

FIG 7 Mitochondrial metabolism is required for expression of genes carried by both nuclear and mitochondrial DNA. Shown are relative mRNA levels of the wild-type (WT) (W303-1a), *spt10*Δ (MZ672), *pda1*Δ (TZ354), *spt10*Δ *pda1*Δ (LG699), *mpc1*Δ (TZ341), *spt10*Δ *mpc1*Δ (LG696), *cyt1*Δ (LG533), *spt10*Δ *cyt1*Δ (LG580), *hap4*Δ (LG579), *spt10*Δ *hap4*Δ (LG625), *rtg1*Δ (TZ299), and *spt10*Δ *rtg1*Δ (LG693) strains. The experiments were repeated three times, and the results are shown as means ± SD. Values that are statistically different ($P < 0.05$) for the double mutants in comparison to the corresponding single mutants (*spt10*Δ, *swi6*Δ, and *asf1*Δ) are indicated by an asterisk.

mitochondrial DNA, we determined the expression levels of *RPO41*, *MTF1*, *HAP4*, and *RTG1* in *spt10*Δ cells (Fig. 7B). *RPO41* and *MTF1* encode the mitochondrial RNA polymerase and its transcriptional factor, respectively (70). Expression of both *RPO41* and *MTF1* was significantly increased in *spt10*Δ cells, and the increased expression depended on *HAP4*, *RTG1*, *PDA1*, and *MPC1* (Fig. 7B). Similarly to the expression of *COX1*, *COX2*, and *COX3*, the expression of *RPO41*, *MTF1*, and *HAP4* was less affected by the *cyt1*Δ mutation (Fig. 7B). These results suggest that the increased expression levels of *COX1*, *COX2*, and *COX3* in mitochondria are driven by higher levels of transcription of *RPO41* and *MTF1* in the nucleus, which depend on *HAP4*, *RTG1*, *PDA1*, and *MPC1* (Fig. 7B). Expression of *RPO41* and *MTF1* is not known to be regulated by either the Hap2/3/4/5p or the Rtg1/3p complex. However, analysis of the database of predicted binding sites of yeast transcription factors (54) shows likely binding sites for the Hap2/3/4/5p complex in the promoter regions of both *RPO41* and *MTF1*. Accordingly, the mechanism of increased *RPO41* and *MTF1* transcription in *spt10*Δ cells likely involves increased *HAP4* transcription (Fig. 5A and 7B). The possibility that *RPO41* and *MTF1* are regulated by the Hap2/3/4/5p complex is supported by the decreased expression of *RPO41* and *MTF1* in *spt10*Δ *hap4*Δ cells (Fig. 7B).

The increased expression of *HAP4* and *RTG1* in *spt10*Δ cells is mutually interdependent: increased expression of *HAP4* requires *RTG1*, and, conversely, increased expression of *RTG1* requires *HAP4*. The requirement for *HAP4* for the expression of *RTG1* is supported by a microarray analysis that showed regulation of *RTG1* by the Hap2/3/4/5p complex (55). However, the mechanism through which the Rtg1/3p complex regulates *HAP4* transcription is likely indirect, as the *HAP4* promoter does not contain an Rtg1/3p binding site. In addition, the increased expression of *HAP4* and *RTG1* in *spt10*Δ cells requires the transport and metabolism of pyruvate in mitochondria (Fig. 7B).

On the basis of these results, we propose a model in which chromatin defects in *spt10*Δ cells lead to increased expression of *HAP4* and *RTG1*, which results in increased expression of nuclear genes encoding TCA cycle and OXPHOS enzymes (Fig. 7C) as well as increased expression of the mitochondrial RNA polymerase *RPO41*/*MTF1* (Fig. 7B). The higher expression levels of *RPO41* and *MTF1* together with elevated mtDNA copy numbers (Fig. 1B) result in increased transcription of genes carried by mitochondrial DNA, *COX1*, *COX2*, and *COX3* (Fig. 7A). The higher expression levels of *HAP4* and *RTG1* in *spt10*Δ cells require a mitochondrial metabolite derived from pyruvate. It is possible that this metabolite is heme, derived from succinyl-CoA, an intermediate of the TCA cycle (72, 73). Heme is required for the function of Hap1p as a transcriptional activator (72, 74, 75), and heme also stimulates the activity of the Hap2/3/4/5p complex (64, 76, 77). It is thus likely that mitochondrial metabolism regulates the transcription of genes carried by both nuclear and mitochondrial DNA through heme synthesis and the activity of Hap1p and Hap2/3/4/5p factors (Fig. 8).

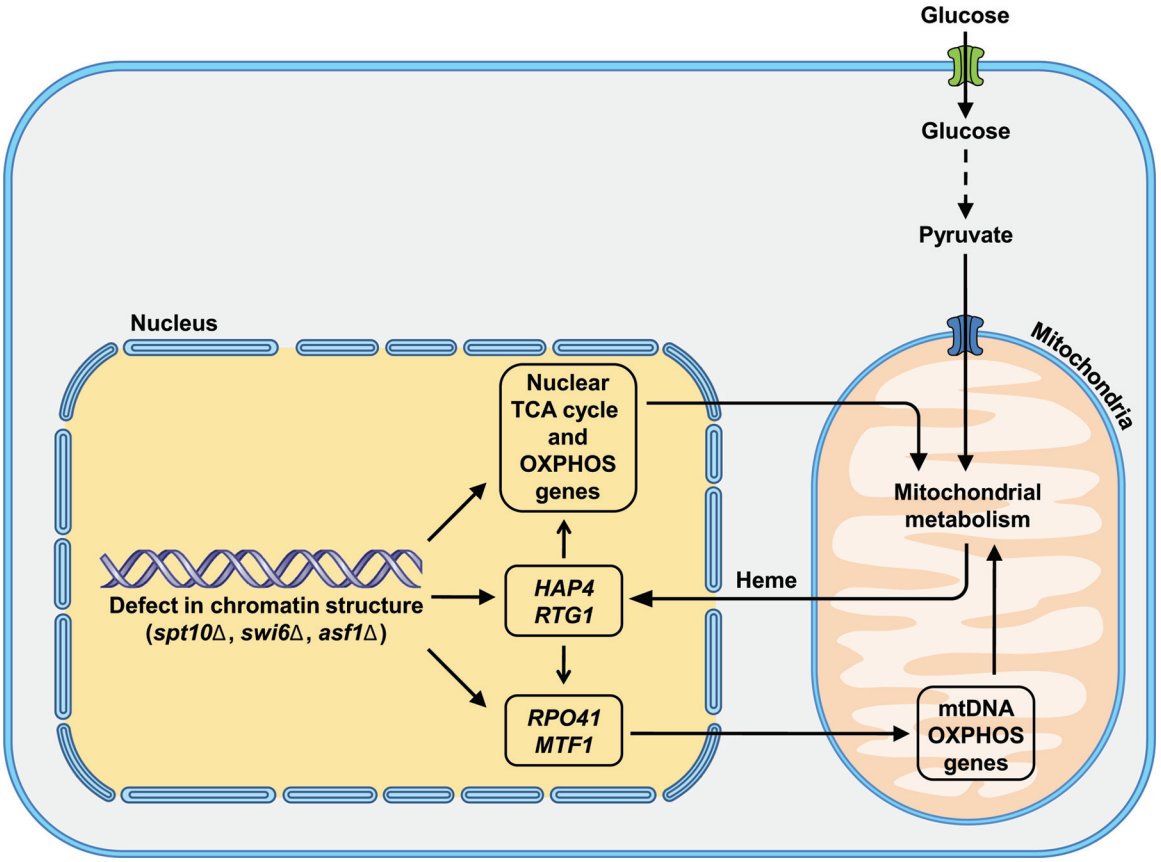

FIG 8 Model for the role of chromatin structure in the regulation of mitochondrial respiration.

DISCUSSION

Despite the central position of mitochondria in cell metabolism and physiology, the signaling mechanisms and the coordinated transcriptional regulation by which mitochondrial abundance and activity are aligned with nutritional and environmental conditions are not well understood. Mitochondrial biogenesis and activity are controlled by the PKA, TOR, Sch9p, Snf1p, and Mec1p/Rad53p signaling pathways (11, 16–19) and require several transcription factors, including Hap1p, Hap2/3/4/5p, Rtg1/3p, and Hcm1p (19–22, 76). Our results show that mitochondrial biogenesis and activity are also regulated by chromatin-mediated pathways.

From the perspective of nucleosomal chromatin architecture, yeast genes can be classified into two broad groups: growth genes and stress genes (78). Growth genes are typically expressed at high levels and feature a region depleted of nucleosomes upstream of the coding region, referred to as the nucleosome-free region. The nucleosome-free region exposes DNA, and transcription factors can bind there in an unobstructed way. The stress genes are generally expressed at lower levels, and their promoters are dominated by delocalized "fuzzy" nucleosomes rather than by the nucleosome-free region. Consequently, stress genes are regulated by factors that affect the structure of chromatin, including histone levels and acetylation. The respiratory genes in *Saccharomyces cerevisiae* belong to the stress category, unlike respiratory genes in yeast species with oxidative metabolism, such as *Candida albicans*, or in higher eukaryotes (79, 80). Our results demonstrate that the expression of genes required for mitochondrial respiration is upregulated by the decreased expression of histone genes (*spt10*Δ, *swi6*Δ, *swi4*Δ, or *mbp1*Δ) or by a defect in chromatin assembly (*asf1*Δ, *rtt109*Δ, or H3K56R). Our results are also in agreement with data from a recent study that showed that histone and nucleosome losses that occur during yeast replicative aging result in transcriptional induction of all yeast genes and particularly stress genes that contain the TATA box (81).

Decreased expression of histone genes or a defect in chromatin assembly results in increased mitochondrial respiration that differs from mitochondrial respiration in wild-type cells grown on raffinose or galactose in two important aspects. The increased mitochondrial activity in *spt10*Δ, *swi6*Δ, or *asf1*Δ cells is associated with the induction of the RTG pathway, as indicated by the increased expression of the RTG pathway target gene *CIT2*. This induction is more prominent in *spt10*Δ cells than in *swi6*Δ or *asf1*Δ cells. Wild-type cells grown on raffinose or galactose do not display a significant induction of the retrograde pathway (Fig. 2B). In addition, the cellular ATP levels in *spt10*Δ, *swi6*Δ, or *asf1*Δ cells are elevated 20- to 50-fold in comparison to those in wild-type cells grown on glucose and are higher than the ATP levels in wild-type cells grown on raffinose or galactose (Fig. 1D). A comparison of oxygen consumption and cellular ATP levels in *spt10*Δ, *swi6*Δ, or *asf1*Δ cells grown on glucose with those in wild-type cells grown on glycerol indicates that the altered chromatin structure in *spt10*Δ, *swi6*Δ, or *asf1*Δ cells changes transcriptional regulation in a way that results in the induction of mitochondrial respiration.

In addition, unlike wild-type cells grown on galactose, raffinose, or glycerol, *spt10*Δ, *swi6*Δ, or *asf1*Δ cells display significantly increased mtDNA copy numbers (Fig. 1B).

The explanation for the increased transcription of nuclear genes required for mitochondrial respiration in cells with altered chromatin structure is fairly straightforward: decreased occupancy or delocalization of nucleosomes in the corresponding promoters likely allows for increased recruitment of Hap2/3/4/5p, Rtg1/3p, and perhaps other transcription factors to the corresponding promoters and increased transcription of genes required for mitochondrial respiration. However, how does a defect in the chromatin structure of nuclear genes affect the expression of genes carried by mitochondrial DNA? *In vitro*, the transcription of genes carried by mitochondrial DNA is regulated by the ATP concentration, and different genes carried by mitochondrial DNA differ in their responsiveness to the ATP level (70, 71). However, our results indicate that the increased expression of genes carried by mitochondrial DNA (*COX1*, *COX2*, and *COX3*) in *spt10*Δ cells requires the nuclear factors Hap4p and Rtg1p and is driven by the increased expression of the *RPO41* and *MTF1* genes, encoding mitochondrial RNA polymerase and its associated factor, respectively.

The Hap2/3/4/5p and Rtg1/3p complexes appear to be the key transcription factors involved in increased mitochondrial respiration in *spt10*Δ, *swi6*Δ, and *asf1*Δ cells. While the transcription of *HAP4* is significantly increased in *spt10*Δ and *swi6*Δ cells, the transcription of *RTG1* is increased only in *spt10*Δ cells. Despite increased mitochondrial respiration in *asf1*Δ cells, the expression of neither *HAP4* nor *RTG1* was elevated (Fig. 5A). This supports the notion that increased transcription of *HAP4* and *RTG1* is not a requirement for the induction of mitochondrial respiration in cells with altered chromatin structure. On the other hand, increased transcription of *HAP4* and *RTG1* in *spt10*Δ cells probably contributes to the robust induction of expression of genes encoding TCA cycle and OXPHOS enzymes (Fig. 2A). The main mechanism responsible for the induction of mitochondrial respiration in cells with altered chromatin structure is likely the increased recruitment of Hap2/3/4/5p, Rtg1/3p, and perhaps other transcription factors to the corresponding promoters, permitted by the increased delocalization of promoter nucleosomes.

Increased expression of both nuclear and mitochondrial DNA-carried genes required for mitochondrial respiration in *spt10*Δ cells requires *HAP4*, *RTG1*, and the transport and metabolism of pyruvate in mitochondria (Fig. 7). Interestingly, the increased expression of *HAP4* and *RTG1* themselves requires a product of mitochondrial carbon metabolism. It is possible that this carbon metabolite is heme. Heme biosynthesis is initiated in mitochondria and requires succinyl-CoA, an intermediate of the TCA cycle (73, 82). Heme is a cofactor of the transcriptional activator Hap1p and stimulates the activity of the Hap2/3/4/5p complex (76, 77). Both Hap1p and Hap2/3/4/5p are important for the expression of genes required for mitochondrial respiration. It is likely that a defect in the transport and metabolism of pyruvate in mitochondria results in the diminished synthesis of succinyl-CoA, 5-aminolevulinic acid (ALA), and, ultimately, heme, affecting the activity of Hap1p and Hap2/3/4/5p. Since the expression of *HAP4* is decreased in *pda1*Δ and *mpc1*Δ cells and the *HAP4* promoter features a binding site for the Hap2/3/4/5p complex (56), it is possible that heme activates the Hap2/3/4/5p complex, which results in increased transcription of *HAP4* and other target genes of the Hap2/3/4/5p complex (Fig. 8). This mechanism could also explain the activation of the Hap2/3/4/5p complex during the diauxic shift. As glucose is depleted and mitochondria begin to utilize ethanol, levels of synthesis of succinyl-CoA, ALA, and heme probably increase, leading to the activation of the Hap2/3/4/5p complex and increased transcription of *HAP4* and genes encoding enzymes of the TCA cycle and OXPHOS. Since many of the Hap2/3/4/5p complex-regulated genes are repressed by glucose independently of the protein kinase A and Snf1 pathways, this mechanism would explain the activation of the Hap2/3/4/5p complex and the increased transcription of the Hap2/3/4/5p complex-regulated TCA cycle and OXPHOS genes upon glucose depletion.

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