Acetyl-CoA Carboxylase Regulates Global Histone Acetylation**

Luciano Galdieri and Ales Vancura
From the Department of Biological Sciences, St. John’s University, Queens, New York 11439

Histone acetylation depends on intermediary metabolism for supplying acetyl-CoA in the nucleocytoplasmic compartment. However, because nucleocytoplasmic acetyl-CoA is also used for de novo synthesis of fatty acids, histone acetylation and synthesis of fatty acids compete for the same acetyl-CoA pool. The first and rate-limiting reaction in de novo synthesis of fatty acids is carboxylation of acetyl-CoA to form malonyl-CoA, catalyzed by acetyl-CoA carboxylase. In yeast *Saccharomyces cerevisiae*, acetyl-CoA carboxylase is encoded by the ACC1 gene. In this study, we show that attenuated expression of ACC1 results in increased acetylation of bulk histones, globally increased acetylation of chromatin histones, and altered transcriptional regulation. Together, our data indicate that Acc1p activity regulates the availability of acetyl-CoA for histone acetyltransferases, thus representing a link between intermediary metabolism and epigenetic mechanisms of transcriptional regulation.

The eukaryotic genome is highly organized and compacted into chromatin within the nucleus. The packaging of DNA into chromatin poses a barrier for certain fundamental cellular processes that require access to DNA, and cells rely on complexes that post-translationally modify histones to make the chromatin more accessible (1). Histone acetylation is a dynamic modification that occurs on all four core histones; it affects chromatin structure and regulates diverse cellular functions, such as gene expression, DNA replication and repair, and cellular proliferation (2–4). The enzymes responsible for histone acetylation are the histone acetyltransferase (HAT)2 complexes that catalyze the transfer of acetyl groups from acetyl-CoA to lysine residues, whereas histone deacetylases (HDACs) remove acetyl groups from histones. Perturbation of the balanced action of HATs and HDACs alters the structure of chromatin and the expression pattern of genes (5, 6).

Histone acetylation depends on intermediary metabolism for supplying acetyl-CoA in the nucleocytoplasmic compartment. The connection between carbon metabolism and histone acetylation in yeast cells was highlighted by demonstrating that global histone acetylation depends on acetyl-CoA synthetase-mediated synthesis of acetyl-CoA in the nucleocytoplasmic compartment (7). When the synthesis of acetyl-CoA is compromised, rapid histone deacetylation ensues (7). Decreasing concentration of glucose in the medium as cells enter the stationary phase is accompanied by decreased histone acetylation (8, 9), whereas addition of glucose induces histone acetylation by picNuA4 and SAGA HAT complexes (10). In mammalian cells, the nucleocytoplasmic enzyme ATP-citrate lyase (ACL) was shown to be the major source of acetyl-CoA for histone acetylation (11). ACL generates acetyl-CoA in the nucleocytoplasmic compartment from glucose-derived citrate, and glucose availability affects histone acetylation in an ACL-dependent manner. Thus, in both yeast and mammalian cells, the nucleocytoplasmic acetyl-CoA is the link between cellular energy and carbon metabolism and histone acetylation and chromatin regulation (12–15).

The preferred sources of carbon and energy for the yeast *Saccharomyces cerevisiae* are fermentable sugars, such as glucose. 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 into ethanol in the cytosol; only a small fraction of acetaldehyde is converted into acetate (16–18). This acetate is subsequently converted to acetyl-CoA by nucleocytosolic acetyl-CoA synthetase 2 (Acs2p) (7, 19). Because glucose represses the tricarboxylic cycle and respiration in *S. cerevisiae*, only a very small fraction of glycolytically produced pyruvate is translocated into mitochondria and converted to acetyl-CoA by the pyruvate dehydrogenase complex (16–18). The mitochondrial pool of acetyl-CoA in glucose-grown cells is very small, and because it is biochemically isolated, it cannot be used for histone acetylation (7).

© 2012 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.
thesis of fatty acids is carboxylation of acetyl-CoA to form malonyl-CoA, catalyzed by acetyl-CoA carboxylase (Acc1p) (20).

In this study, we show that lowering the flux of acetyl-CoA through the fatty acid synthesis pathway by reducing ACC1 expression results in globally increased histone acetylation and altered transcriptional regulation. Our study demonstrates that homeostasis of nucleocytosolic acetyl-CoA plays an important role in the regulation of histone acetylation and gene expression.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Media**—All yeast strains are listed in Table 1. The strains used in this study are isogenic to the W303 strain background. Standard genetic techniques were used to manipulate yeast strains, and to introduce mutations from non-W303 strains into the W303 background (21). Cells were grown in rich medium (YPD; 1% yeast extract, 2% Bacto™ Peptone, 2% glucose) or under selection in synthetic complete medium containing 2% glucose and, when appropriate, lacking specific nutrients to select for a strain with a particular genotype. Meiosis was induced in diploid cells by incubation in 1% potassium acetate.

**Western Blotting**—Yeast cells were inoculated to an $A_{600} = 0.1$ and grown in YPD medium with or without doxycycline. Four $A_{600}$ units were harvested and immediately boiled in SDS sample buffer. Denatured proteins were separated on a 15% denaturing polyacrylamide gel. Western blotting with anti-histone H3 polyclonal antibody (ab1791; Abcam) at a dilution of 1:1000, anti-acetyl histone H3 (Lys-14) polyclonal antibody (ab1791; Abcam) at a dilution of 1:1000, anti-acetyl histone H3 (Lys-14) polyclonal antibody (ab1791; Abcam) at a dilution of 1:1000, anti-acetyl histone H3 polyclonal antibody (ab1791; Abcam) at a dilution of 1:1000 was carried out as described previously (22). Western blots were quantified using NIH Image software (Image), National Institutes of Health, image.nih.gov.

**Real Time RT-PCR**—Total RNA was isolated from cultures grown in 50 ml of YPD medium to an $A_{600} = 1.0$ by the hot acid phenol method, as described previously (23), treated with RNase-free DNase (Qiagen), and purified with the RNeasy mini kit (Qiagen). RNA (100 ng per reaction) was analyzed by real time reverse transcriptase-PCR (25 µl of reaction mixture) using the iScript one-step RT-PCR SYBR Green Supermix kit (Bio-Rad). The following primers were used with real time RT-PCR: ACT1 (5′-TATGTGTAAGCCCGGTTCG-3′ and 5′-GACATAACGTGTTCAATTTG-3′); ACC1 (5′-TAGAT-CCGTCAAAATTGGGC-3′ and 5′-TACGAGATTATCTC-GCGGTGGC-3′); INO1 (5′-ACGAGCTGTCACAAAGTACAG-3′ and 5′-TGAACAGCTAGTCCTTCAGAATGG-3′); UBC8 (5′-TTGGAGGTTACATGTAGAGTTGCC-3′ and 5′-CCCGATGCGATATCAATGTTAG-3′); FIT2 (5′-CTACAG-TTATGACTGCCGGTCCTG-3′ and 5′-GGTGTGCGTAAACCTTATTG-3′); PFK26 (5′-ACTTCCTGAAACATT-CCTGTGC-3′ and 5′-TCGGGGAAATAGGATCTCATG-3′); and GIT1 (5′-CTTCCAATATGTCCTTCAGCA-3′ and 5′-GGTGTGGTCAGAAGACCTTC-3′).

**Acc1p Activity Assay**—Acc1p was purified from whole cell extracts by avidin affinity chromatography as described previously (24) with minor modifications. All steps were performed at 4 °C. Briefly, wild-type and $tetO_{ACC1}$ cells were inoculated to an $A_{600}$ of 0.1 in YPD medium containing 0 or 0.05 µg/ml doxycycline and were grown at 30°C for 5 h. An equal amount of cells ($10^{10}$) for each strain was harvested by centrifugation at $3000 \times g$ for 5 min, converted to spheroplasts with zymolase, and resuspended in lysis buffer (50 mM Tris-HCl, 100 mM NaF, 1 mM EDTA, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and complete protease inhibitors (Roche Applied Science)). Glass beads (0.5 mm) were added, and cells were disrupted by vigorous mixing 10 times in 15-s bursts. The homogenate was centrifuged at 16,000 $\times g$ for 20 min. Supernatant was loaded onto an anti-acc1p antibody column (2 ml; Pierce). Proteins that did not bind were eluted with 20 ml of PBS buffer, and bound proteins were eluted with 7 ml of PBS buffer containing 2 mM D-biotin. Acc1p activity was determined as described previ-
ously (25) at 30 °C, and the activity was calculated as microunits/10^7 cells (1 enzyme unit corresponds to the carboxylation of 1 μmol of acetyl-CoA per min).

**Chromatin Immunoprecipitation and Quantitative Real Time PCR Analysis**—In vivo chromatin cross-linking and immunoprecipitation were performed essentially as described (22, 26, 27). Immunoprecipitation was performed with the following antibodies: for anti-histone H3 (Abcam ab1791), anti-acetyl-histone H3 (Lys-14) (Upstate Cell Signaling Solutions 07-353), and for anti-hyperacetylated histone H4 (Penta) (Upstate Cell Signaling Solutions 06-946). The primers used for real time PCR are as follows: POL1 (5’-TCCGTGACAAAGAGGCAATAGAAG-3’ and 5’-TAAAACACCGTGACTCCCGTTTCTG-3’); PYK1 (5’-CCAGTTTATCATGTGGTCCCTTTCCCT-3’ and 5’-CCAGAAACACGTTTAAATGAGGTG-3’); ACT1 (5’-CTCTTGATTCCTCCTCCCCCTTC-3’ and 5’-ATGGTGCAAGCGGTAGAACATAC-3’); INO1 (5’-ACGACTGTGCTTCAAGAACTACAG-3’ and 5’-TGAAACACGTAGTCCTGAACAGTGG-3’); UBC8 (5’-TTGGAGGTACATGTAGATTGC-3’ and 5’-CCGGATGCTATACATGGTAG-3’); HSP12 (5’-AACACTCTGACGGAGAAGTGATAC-3’ and 5’-GAATCTCTTTTCACTGCTGAG-3’); INT6 (5’-GGAAGCGAATTCAAGGGTG-3’ and 5’-TTGGATCAGTGTGCAAGTCGC-3’); and INT8 (5’-CAGTTTATCATGTGGTCCCTTTCCCT-3’ and 5’-TACTAACCACATCATGCAATCCTTGG-3’).

**Histone Preparation and Analysis**—Histones were extracted and precipitated as described previously (28–30) with several minor modifications. Briefly, yeast cells were inoculated to an A_600 = 0.1 and grown in YPD medium with 0.05 μg/ml doxycycline for 5 h. One hundred and sixty A_600 nm units of cells were harvested and disrupted by vigorous mixing with glass beads (0.5 mm) 20 times in 15-s bursts in the presence of 0.2 M H_2 SO_4, complete protease inhibitors (Roche Applied Science), and HDAC inhibitors (100 μM trichostatin A, 50 mM nicotinamide, and 50 mM sodium butyrate) followed by precipitation of histones with 20% trichloroacetic acid. Purification of histones was performed with the histone purification mini kit (Active Motif), according to the manufacturer’s instructions. Partially purified histones were analyzed in a 20 × 20-cm 18% acrylamide gel containing 1 M acetic acid, 8 M urea, 0.5% Triton X-100, and 45 mM NH_4 OH as described previously (31, 32). Proteins were visualized using silver staining (Pierce), according to the manufacturer’s instructions.

**Immunoprecipitation**—Immunoprecipitation of acetylated proteins was performed as described previously (33, 34) with several minor modifications. Briefly, yeast cells were inoculated to an A_600 = 0.1 and grown in YPD medium with 0.05 μg/ml doxycycline. One hundred and sixty A_600 nm units of cells were harvested, flash-frozen with dry-ice and ethanol, and kept at −80 °C. Cells were disrupted by vigorous mixing with glass beads (0.5 mm) 20 times in 15-s bursts with lysis buffer (100 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1 mM DTT, and complete protease inhibitors (Roche Applied Science)) in the presence of HDAC inhibitors (100 μM trichostatin A, 50 mM nicotinamide, and 50 mM sodium butyrate). A whole cell lysate aliquot was saved at −80 °C as immunoprecipitation input control. After preclearing the whole cell lysate with 50 μl of protein A/G Plus-agarose (Santa Cruz Biotechnology), acetylated proteins were immunoprecipitated by incubating with 60 μl of protein A/G Plus-agarose preadsorbed with 12 μl of mouse monoclonal anti-pan-acetyl-lysine antibody (9681S; Cell Signaling) or normal mouse IgG (sc-2025; Santa Cruz Biotechnology) for 3 h at 4 °C. Immunoprecipitates were washed four times at 4 °C for 10 min with lysis buffer and boiled with 20 μl of 1× SDS buffer. To detect immunoprecipitation of Sip2p, Cdc11p, and Shs1p, a rabbit polyclonal anti-TAP antibody (cab1001; Pierce) at a dilution of 1:1000 was used. To detect immunoprecipitation of Pck1p, rabbit polyclonal anti-Myc antibody (sc-789; Santa Cruz Biotechnology) at a dilution of 1:100 was used.

**RESULTS**

**Reduced ACC1 Expression Leads to Increased Acetylation of Histones H3 and H4**—Previous work showed that cytosolic acetyl-CoA derived from the reaction catalyzed by acetyl-CoA synthetase 2 (Acs2p) regulates histone acetylation in yeast (7). Cytosolic acetyl-CoA is also used by ACC to yield malonyl-CoA, a precursor for de novo synthesis of fatty acids (20). We speculated that it would be possible to increase the concentration of acetyl-CoA in the cytosol by reducing the conversion of acetyl-CoA to malonyl-CoA and thus increase the substrate level of acetyl-CoA that is accessible to HATs and available for histone acetylation.

The cytosolic ACC in *S. cerevisiae* is encoded by the *ACC1* gene. To modulate *ACC1* expression, we used the regulatable *tetO*-promoter fused to the *ACC1* coding region (tetO-ACC1) (24). Expression of genes under the *tetO*-promoter is repressed by addition of doxycycline in a concentration-dependent manner (35), whereas doxycycline has no effect on gene expression in the wild-type cells (36). Because genes under the control of the *tetO*-promoter are expressed in the absence of doxycycline, we compared the histone acetylation levels of the wild-type cells and cells harboring the *tetO*-ACC1 construct. Comparable histone H3 and H4 acetylation levels were found for both yeast strains (Fig. 1A), suggesting that placing the *ACC1* gene under the control of the *tetO*-promoter did not alter the acetyl-CoA levels to the extent that would affect histone acetylation. To eliminate the possibility that doxycycline affects cell physiology, altering the histone acetylation levels, we investigated the effect of doxycycline on histone acetylation. The results showed that doxycycline at 0.05 μg/ml did not alter histone acetylation in the wild-type cells (Fig. 1B); however, at a concentration of 0.1 μg/ml, doxycycline somewhat decreased acetylation of histone H3. For this reason, all experiments reported in this study include data obtained with doxycycline at 0.05 μg/ml.

To verify that doxycycline attenuates the *ACC1* expression in the *tetO*-ACC1 strain, we determined the *ACC1* mRNA levels. In the absence of doxycycline, *ACC1* was expressed at a 2-fold higher level in the *tetO*-ACC1 strain than in the wild-type strain. However, addition of doxycycline to 0.05 and 0.1 μg/ml reduced the *ACC1* expression in the *tetO*-ACC1 strain to 3 and 2%, respectively. As expected, doxycycline had no effect on the expression of *ACC1* in the wild-type strain (Fig. 1C).
Acc1 Regulates Histone Acetylation

To confirm the relationship between gene expression and the Acc1p enzymatic activity, total Acc1p activity was determined in purified fractions prepared from wild-type and tetO7-ACC1 cells grown in the presence of 0 and 0.05 μg/ml doxycycline. As expected, tetO7-ACC1 cells treated with 0.05 μg/ml doxycycline showed only 7% of the total Acc1p activity relative to wild-type cells grown in the absence of doxycycline (Fig. 1D). This result is in agreement with the ACC1 expression data (Fig. 1C). Interestingly, tetO7-ACC1 cells grown in the absence of doxycycline displayed only 50% of the total Acc1p activity relative to wild-type cells grown in the absence of doxycycline (Fig. 1D), even though the tetO7-ACC1 cells showed increased ACC1 expression relative to the wild-type cells. Because Acc1p forms aggregates and overexpression of Acc1p results in formation of para-crystalline structures in vivo (20, 37, 38), increased expression of Acc1p from the tetO7 promoter in the absence of doxycycline probably affects the stoichiometry of the Acc1p complexes, which results in decreased Acc1p enzymatic activity (see “Discussion”).

The efficient repression of ACC1 expression and the enzymatic activity by doxycycline in tetO7-ACC1 cells allowed us to test whether the decreased Acc1p activity would result in increased histone acetylation. Western blot analysis showed that the tetO7-ACC1 cells grown in the presence of doxycycline displayed increased acetylation of both histones H3 and H4, although the total levels of histone H3 remained constant throughout the experiment (Fig. 1E). We interpret these results to indicate that the decreased ACC1 expression and activity results in decreased Acc1p-mediated conversion of acetyl-CoA to malonyl-CoA. The elevated concentration of acetyl-CoA in the cytosol then results in increased activity of HATs, thus increasing histone acetylation levels.

To confirm increased histone acetylation in tetO7-ACC1 cells by a technique that does not depend on the specificity of the antibodies that recognize different acetylation states of histones, total histones were extracted with sulfuric acid, partially purified, and analyzed by Triton/acetic acid-urea-PAGE. This method allows separation of proteins based on the number of protonated groups under acidic conditions (32), therefore separating individual histones with different acetylation levels. Although wild-type and tetO7-ACC1 cells did not significantly differ in the amount of unacetylated and monoacetylated H4, tetO7-ACC1 cells contained increased amounts of diacetylated and triacetylated H4 (Fig. 1F). The band corresponding to the tetraacetylated H4 comigrated with a non-histone protein present in our partially purified histone preparations and could not be visualized. However, the result is in agreement with our Western blot analysis and shows increased histone acetylation in tetO7-ACC1 cells.

Histone H3 were plotted. The ratios represent means ± S.D. from three independent experiments. F, reduced expression of ACC1 in the presence of doxycycline increases acetylation of histone H4 in tetO7-ACC1 cells compared with wild-type cells. The wild-type and tetO7-ACC1 cells were inoculated to an A600 = 0.1 and grown in YPD medium at 30 °C for 600 h, 0.05 μg/ml doxycycline. Samples were analyzed by Western blotting with antibodies against histone H3 acetylated at lysine 14 (acH3), hyperacetylated histone H4 (acH4), and total histone H3. The figure represents typical results from three independent experiments.

FIGURE 1. Reduced expression of ACC1 results in increased histone acetylation. A, strain harboring the tetO7-ACC1 construct does not have increased acetylation of histones H3 and H4 compared with the wild-type cells. The wild-type and tetO7-ACC1 cells were grown in YPD medium without doxycycline to A600 = 1.0. Samples were analyzed by Western blotting with antibodies against histone H3 acetylated at lysine 14 (acH3), hyperacetylated histone H4 (acH4), and total histone H3. The figure represents typical results from three independent experiments. The intensity of each acH3 and acH4 band was quantified by densitometry, normalized with H3 as a loading control of each lane, and shown as percentage of wild-type acetylation.

B, presence of 0, 0.05, and 0.1 μg/ml doxycycline increases acetylation of histones H3 and H4. The figure represents typical results from three independent experiments. The intensity of each acH3 and acH4 band was quantified by densitometry, normalized with H3 as a loading control of each lane, and shown as percentage of wild-type acetylation.

C, presence of 0 and 0.05 μg/ml doxycycline increases acetylation of histone H3. The figure represents typical results from three independent experiments.

D, presence of 0, 0.05, and 0.1 μg/ml doxycycline increases acetylation of histones H3 and H4. The figure represents typical results from three independent experiments.
Reduced ACC1 Expression Results in Increased Untargeted Acetylation of Chromatin Histones—To test whether decreased ACC1 expression increases histone acetylation globally or only at specific loci, we evaluated by chromatin immunoprecipitation (ChIP) the occupancy of histone H3 acetylated at lysine 14 (acH3K14) as well as hyperacetylated histone H4 (acH4K5,8,12,16) at the promoter regions of PYK1, ACT1, UBC8, INO1, HSP12, and intergenic regions in chromosome VI (INT6; coordinates 261,412 to 261,538) and chromosome VIII (INT8; coordinates 535,029 to 535,163). These intergenic regions were selected because they are at least 3 kb distant from the nearest coding region. We also determined the histone acetylation levels at the promoter and a 14-kb coding region of MDN1. We used anti-H3 antibody that recognizes the C-terminal region of H3 that is not post-translationally modified. The ChIP signal obtained with this antibody thus represents total H3 occupancy and can be used to calculate the histone acetylation levels per nucleosome content (39–42).

When ACC1 expression in the tetO7-ACC1 cells was reduced with 0.05 μg/ml doxycycline, histone H3 was 1.3 to 2.8 times more acetylated, and histone H4 was 1.3 to 2.5 times more acetylated in different promoters and intergenic regions than histones H3 and H4 in the corresponding loci of the wild-type cells (Fig. 2). The highest values for acetylation of both histones H3 and H4 were found in the promoter region of the INO1 gene. To account for differences in nucleosome density at different genomic loci, we corrected the acH3 and acH4 occupancies for histone H3 content and generated values that represent acetylation per nucleosome. The acetylation of histones H3 and H4 per nucleosome in promoters and intergenic regions was 1.2 to 2.2 and 1.1 to 2.1 times higher in tetO7-ACC1 than in the wild-type cells, respectively (Fig. 2).

To test whether the increased acetylation of histones found in promoter regions is also found in the coding regions, we evaluated by ChIP the occupancy of acetylated histones in the very long coding region of MDN1. Because the DNA fragments obtained through sonication in the ChIP protocol are randomly generated, the long coding region of MDN1 (15 kb) allowed us to design three sets of primers (promoter, middle of coding region, and the 3' end of coding region) far from each other to avoid overlap of the DNA fragments. We found that total levels of acH3 and acH4 in the coding region of MDN1 of tetO7-ACC1 cells were increased 1.3–1.9 and 1.7–1.9 times, respectively, when compared with wild-type cells (Fig. 3, A and C). Correcting the acetylation levels of histones H3 and H4 per nucleosome content showed an increase of 1.4–1.7 and 1.7–2.2 times in the tetO7-ACC1 cells, respectively, when compared with the wild-type cells (Fig. 3, B and D). The increased acetylation of both histones H3 and H4 at all tested loci suggests that the decreased ACC1 expression increases acetylation of chromatin histones in a global untargeted manner and is in agreement with increased acetylation of bulk histones, shown by the Western blot analysis (Fig. 1E).
Acc1 Regulates Histone Acetylation

Increased Histone Acetylation in tetO2-ACC1 Cells Leads to Increased Expression of Genes That Are Regulated by Histone Acetylation—Changes in histone acetylation alter transcriptional regulation of many genes (1, 3, 4, 7). As we found that decreased ACC1 expression in tetO2-ACC1 cells results in globally increased histone acetylation, we decided to evaluate the expression of several genes known to be regulated by histone acetylation. INO1, UBCH8, FIT2, PFK26, and GTT1 genes were found to be up-regulated either in rpd3Δ (43), hda1Δ (44), or in both strains. Rpd3p is a subunit of the class I Sin3/Rpd3 HDAC, although Hda1p is a subunit of the class II HDA1 HDAC complex. Mutations in HDACs were shown to increase the level of histone acetylation and to alter the gene expression pattern (43, 44). All five tested genes showed increased expression in tetO2-ACC1 than in the wild-type cells in the presence of doxycycline (Fig. 4A). The INO1 gene displayed the most pronounced increase, in accordance with the increased acetylations of histones H3 and H4 in the INO1 promoter (Fig. 2). INO1 encodes inositol-1-phosphate synthase shown to be regulated by Gcn5p, a catalytic subunit of the SAGA histone acetyltransferase complex and Rpd3 complex (45–47).

Reduced Expression of ACC1 Suppresses Growth Defect and Histone Hypoacetylation Phenotype of acc2ts Mutant—Because yeast mitochondrial and nucleocytosolic pools of acetyl-CoA are distinct and isolated (7), and direct biochemical assays for measuring each pool separately are not available, we sought genetic proof that attenuated ACC1 expression leads to increased concentration of acetyl-CoA in the nucleocytosolic compartment. On normal glucose-containing media, acetyl-CoA is produced from acetate and CoA by nucleocytosolic acetyl-CoA synthetase 2 (AcS2p). AcS2p is required for growth on glucose, and the acc2ts mutant displays severe global histone hypoacetylation and transcriptional defects at restrictive temperature. The crucial role of nucleocytosolic acetyl-CoA is attested by the fact that inactivation of the acc2ts allele affects expression of more genes than inactivation of any HAT (7). acc2ts cells grow at wild-type rate at 30 °C but are not able to grow at the restrictive temperature of 37 °C. Introducing the tetO2-ACC1 allele into acc2ts cells did not alter the growth rate at 30 °C but it clearly suppressed the growth defect at 37 °C, especially in the presence of doxycycline (Fig. 5A). Because acetyl-CoA is a precursor for malonyl-CoA in the cytosol, it is likely that the impairment of nucleocytosolic acetyl-CoA synthesis in the acc2ts mutant affects both histone acetylation and fatty acid synthesis. The fatty acid synthesis is additionally limited by decreased ACC1 expression in the tetO2-ACC1 cells. Unlike acc2ts, the tetO2-ACC1(acc2ts) cells are able to grow at 37 °C; it thus appears that the reduced level of acetyl-CoA in acc2ts cells inhibits growth more by affecting histone acetylation than by affecting fatty acid synthesis.

To determine whether the histone hypoacetylation phenotype of acc2ts cells can be partially suppressed by the decreased ACC1 expression due to the tetO2-ACC1 allele, we assayed the acetylation of bulk histones. Western blot analysis showed increased acetylation of histones H3 and H4 in acc2ts tetO2-ACC1 cells in comparison with acc2ts cells (Fig. 5B). These results corroborate the conclusion that the nucleocytosolic concentration of acetyl-CoA that is accessible by HATs and thus affects histone acetylation is regulated not only by AcS2p but also by Acc1p.

Reduced Expression of ACC1 Does Not Suppress Growth Defect and Hypoacetylation Phenotype of NuA4 Mutants—Reduced expression of ACC1 partially suppresses histone hypoacetylation in the acc2ts mutant. To test whether the attenuated expression of ACC1 can suppress mutations in a HAT complex, we introduced the tetO2-ACC1 allele in cells carrying mutations in the NuA4 complex. Esal1p is the catalytic subunit of two separate complexes as follows: NuA4, recruited to promoters with targeted activity, and piccolo NuA4, a smaller
complex with global functions (4). Because ESA1 is an essential gene, we used temperature-sensitive esa1-414 allele (from now referred to as esa1ts) that contains a frameshift mutation that results in early truncation of the protein, resulting in decreased HAT activity in vivo and in vitro (48).

Spotting assay of esa1ts cells showed no growth defect at 30 °C, but growth was inhibited at the restrictive temperature of 37 °C (Fig. 6A). Doxycycline did not alter the growth rate of the esa1ts mutant. Introducing the tetO7-ACC1 allele into esa1ts cells did not alter the growth rate at 30 °C and was unable to suppress the growth defect of esa1ts at 37 °C, even in the presence of doxycycline (Fig. 6A). Western blot analysis of cells grown at 30 °C did not show altered histone H3 acetylation in esa1ts cells when compared with the wild-type cells. However, as expected, esa1ts cells displayed decreased acetylation of histone H4, because NuA4 and piccolo NuA4 complexes are affected in NuA4 and piccolo NuA4 mutant cells in such a way that the acetyl-CoA level does not increase sufficiently to affect histone acetylation in cells containing the tetO7-ACC1 allele.

Reduced ACC1 Expression Results in Increased Acetylation of Nonchromatin Proteins—Histones are not the only proteins that are acetylated; many acetylated cytoplasmic proteins were identified in yeast (33, 34, 50). To test whether decreased ACC1 expression increases acetylation of nonchromatin proteins, we assayed acetylation of Pck1p, Sip2p, Cdc11p, and Shs1p by immunoprecipitation with anti-pan-acetyl-lysine antibody (33). These proteins were shown previously to be acetylated in vivo by NuA4 (33, 34, 50). Pck1p is a cytosolic phosphoenolpyruvate carboxykinase that catalyzes the rate-limiting reaction in gluconeogenesis. Acetylation of Pck1p is
important for the enzymatic activity and ability to grow on non-fermentable carbon sources (33). Sip2p is a regulatory subunit of the Snf1 complex. Acetylation of Sip2p enhances interaction with Snf1p, which results in inhibition of Snf1 activity (50).

Cdc11p and Shs1p are septins required for cytokinesis. It appears that acetylation of Cdc11p and Shs1p plays a role in regulation of septin dynamics (34). Our results show that acetylation of Pck1p, Sip2p, Cdc11p, and Shs1p is increased in tetO<sup>7</sup>-ACC1 cells in comparison with wild-type cells (Fig. 7) and suggest that in addition to histones reduced ACC1 expression results in increased acetylation of cytoplasmic proteins.

**DISCUSSION**

The major objective of this study was to test the hypothesis that impaired <i>de novo</i> fatty acid synthesis caused by reduced ACC1 expression would increase histone acetylation by increasing the nucleoeytosolic pool of acetyl-CoA (Fig. 8). This hypothesis was motivated by previous work that showed that the nucleoeytosolic pool of acetyl-CoA regulates histone acetylation (7, 11). In mammalian cells, down-regulation of ACL, which generates the majority of cytosolic acetyl-CoA, resulted in histone hypoacetylation (11). Yeast cells do not have ACL, and cytosolic acetyl-CoA is generated from acetate by acetyl-CoA synthetase, encoded by the ACS1 and ACS2 genes. In yeast, mutation in ACS2 impaired global histone acetylation and transcriptional regulation (7). Regardless of the different pathways used for synthesis of nucleoeytosolic acetyl-CoA, the major pathway that utilizes cytosolic acetyl-CoA in both yeast and metazoans is fatty acid synthesis. The first and rate-limiting reaction in the fatty acid biosynthetic pathway is carboxylation of cytosolic acetyl-CoA to malonyl-CoA, catalyzed by acetyl-CoA carboxylase, in yeast cells encoded by the <i>ACC1</i> gene (20). We speculated that reduced expression of <i>ACC1</i> would result in reduced conversion of acetyl-CoA to malonyl-CoA and hence increased cytosolic pool of acetyl-CoA and increased histone acetylation. In this study, we show that reduced expression of <i>ACC1</i> results in globally increased acetylation of chromatin histones (Figs. 1E, 2, and 3) and altered pattern of gene expression (Fig. 4).

Perhaps the first indication that fatty acid synthesis is linked to transcriptional regulation was provided by a genetic screen for suppressors of inositol auxotrophy in the <i>snf1Δ</i> mutant (24). This screen identified mutations in <i>ACC1</i> and <i>FAS1</i>, which encode a subunit of fatty-acid synthase. Snf1p is a protein kinase involved in regulation of utilization of carbon sources (51, 52). Snf1p also phosphorylates and inactivates Acc1p (53). Shirra et al. (24) showed that the inositol auxotrophy in <i>snf1Δ</i> cells is at least partly due to the elevated Acc1p activity and that
reduction in Acc1p activity restores INO1 expression. On the basis of these results, the authors suggested that metabolites that are generated during fatty acid biosynthesis influence gene expression (24).

Our results show that decreased ACC1 expression in tetO7-ACC1 cells resulted in increased histone acetylation per nucleosome by up to 2.8 times of the wild-type levels in several promoters, intergenic regions (Fig. 2), and a coding region (Fig. 3). This relatively moderate increase in histone acetylation is in accordance with the notion that histone deacetylation is more efficient than histone acetylation (54). The wide distribution (55) and faster kinetics of HDAC complexes (54) may partially counteract the effect of increased levels of acetyl-CoA on histone acetylation. Decreased Acc1p activity in tetO7-ACC1 cells increases the nucleocytosolic acetyl-CoA levels, changing the equilibrium between the activities of HATs and HDACs, leading to hyperacetylation of histones H3 and H4 in an untargeted fashion (Figs. 2 and 3).

The expression of ACC1 in tetO7-ACC1 cells grown without doxycycline is about 2-fold higher in comparison with the wild-type cells (Fig. 1C); however, the enzymatic activity of Acc1p in tetO7-ACC1 cells is reduced to about 50% of the wild-type level (Fig. 1D). It appears that this 50% decrease in Acc1p activity is not sufficient to alter the acetyl-CoA levels to the extent that would affect the acetylation of bulk histones when measured by Western blotting (Fig. 1A); however, it results in a higher expression of INO1 (Fig. 4), a gene whose expression is known to be regulated by acetylation (45–47). In addition, the tetO7-ACC1 allele also partially suppresses the temperature sensitivity of acs2ts strain even in a medium without doxycycline (Fig. 5A). Increased abundance of ACC1 mRNA in tetO7-ACC1 cells grown without doxycycline thus does not correlate with reduced Acc1p enzymatic activity. The explanation for this discrepancy probably lies in the complex mode of Acc1p regulation. In both yeast and mammalian cells, ACC is regulated transcriptionally by protein phosphorylation and by oligomerization (20, 56, 57). In mammalian cells, ACC polymerization and activity are regulated by interaction with regulatory proteins Mig12 and Spot14 and by citrate (56–58). Yeast Acc1p forms aggregates in vivo in a citrate-independent fashion, and overexpression of Acc1p results in formation of para-crystalline structures in vivo (20, 37, 38). Thus, the increased expression level of Acc1p from the tetO7 promoter in the absence of...
doxycycline probably affects the stoichiometry of the Acc1p complexes, which results in decreased Acc1p enzymatic activity.

The transcription of ACC1 is regulated by the transcription factors Ino2p and Opi1p that also regulate expression of enzymes involved in phospholipid metabolism (59, 60). Previous studies also suggested that the expression of the ACC1 gene inversely correlates with the enzymatic activity of Acc1p (24). ACC1 gene expression is increased in an acc1 mutant with decreased enzymatic activity of Acc1p, although it is decreased in snf1Δ cells (24). Because Snf1p phosphorylates and inactivates Acc1p (53), it is possible that decreased Acc1p phosphorylation and thus increased Acc1p enzymatic activity in snf1Δ cells results in decreased ACC1 expression. It is tempting to speculate that the feedback regulation of ACC1 expression by Acc1p activity is mediated by histone acetylation, which is regulated by the level of acetyl-CoA. Increased Acc1p enzymatic activity would lead to a lower level of acetyl-CoA and hence decreased histone acetylation and decreased ACC1 gene expression. Conversely, decreased Acc1p enzymatic activity would lead to an increased level of acetyl-CoA and therefore increased histone acetylation and increased ACC1 gene expression. This hypothesis is in accordance with decreased ACC1 expression found in gen5 cells (61) and cells expressing a non-acetylatable version of histone H4 (H4K5,8,12,16R; see Ref. 62).

Our results suggest that in yeast cells, the concentration of acetyl-CoA that is available for HAT complexes in the nucleus is limiting and is at least one of the factors that set the global level of histone acetylation. This conclusion is in agreement with the finding that inactivation of acs2Δ results in rapid histone deacetylation (7). Most kinetically characterized HATs have high catalytic efficiency ($k_{cat}/K_m \sim 10^9–10^{10} \text{ M}^{-1} \text{s}^{-1}$) and affinity ($K_m$ or $K_d \sim 1–10 \text{ M}$) for acetyl-CoA (63, 64). Because most HATs, including NuA4 and SAGA complexes, have similar affinity for both acetyl-CoA and CoA, and CoA is a competitive inhibitor, the acetyl-CoA/CoA ratio may also be a significant regulatory factor. The notion that the concentration of acetyl-CoA that is available for histone acetylation is limiting and regulates global histone acetylation is supported by the rapid histone deacetylation upon inactivation of acs2Δ (7). In this context, it would be interesting to consider possible changes in the histone acetylation pattern induced by drugs that target ACC and other enzymes in the fatty acid biosynthetic pathway and that are being developed as a treatment for obesity, metabolic syndrome, diabetes, and cancer (65–67). It is not unreasonable to speculate that these drugs, by virtue of inhibiting ACC or fatty acid synthase, could elevate the acetyl-CoA level in the nucleocytosolic compartment and consequently increase histone acetylation and alter transcriptional regulation.

In addition to histones, many acetylated nonchromatin proteins were identified in yeast (33), and recent proteomic studies revealed that human proteome contains at least $\sim 2500$ acetylated proteins (68–70). In comparison, similar analyses of human and mouse proteins identified $\sim 2200$ phosphoproteins (70–72). Thus, it appears that protein acetylation is widespread as phosphorylation (70). In human cells, the acetylated proteins are involved in the regulation of diverse cellular processes, including chromatin remodeling, cell cycle, RNA metabolism, cytoskeleton dynamics, membrane trafficking, and key metabolic pathways, such as glycolysis, gluconeogenesis, and citric acid cycle (68, 69). In yeast cells, NuA4 was found to acetylate proteins involved in several processes, including metabolism, transcription, cell cycle progression, RNA processing, stress response, and cytokinesis (33, 34). Many of the acetylated proteins are located in the nucleus or cytosol, and their acetylation is very likely regulated by the concentration of acetyl-CoA in the nucleocytosolic compartment. Indeed, the cytoplasmic proteins Sip2p, Cdc11p, Shs1p, and Pck1p were more acetylated in tetO–ACC1 cells than in the wild-type cells (Fig. 7). The regulatory role of nucleocytosolic acetyl-CoA thus likely extends beyond histone acetylation and chromatin-dependent processes (Fig. 8).

Yeast cells, like metazoan cells, use a multitude of signaling pathways to respond to nutritional and other changes in the environment (73). Many of these pathways target transcriptional factors and other components of the transcriptional machinery, thus establishing the connection between the metabolic state of the cell and transcription. However, the connection between intermediary metabolism and epigenetic mechanisms of transcriptional regulation has been appreciated only recently (7, 10–12). Our data indicate that Acc1p activity regulates the availability of acetyl-CoA for HATs and provide an additional link between intermediary metabolism and histone acetylation and transcriptional regulation (Fig. 8).

Acknowledgments—We thank Drs. Arndt, Baetz, Boeke, Chuang, Kohlwein, Pillus, Shirra, Tsukiyama, and Zhu for strains and plasmids and members of the Vancura laboratory and Dr. Vancurova for helpful comments.

REFERENCES

1. Kouzarides, T. (2007) Chromatin modifications and their function. Cell 128, 693–705
2. Kurdistani, S. K., Tavazoie, S., and Grunstein, M. (2004) Mapping global histone acetylation patterns to gene expression. Cell 117, 721–733
3. Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., Bell, G. W., Walker, K., Rolfe, P. A., Herbsleb, E., Zeitlinger, J., Levitter, F., Gifford, D. K., and Young, R. A. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. Cell 122, 517–527
4. Millar, C. B., and Grunstein, M. (2006) Genome-wide patterns of histone modifications in yeast. Nat. Rev. Mol. Cell Biol. 7, 657–666
5. Shukla, V., Vaisiere, T., and Herceg, Z. (2008) Histone acetylation and chromatin signature in stem cell identity and cancer. Mutation Res. 637, 1–15
6. Lafon, A., Chang, C. S., Scott, E. M., Jacobson, S. J., and Pillus, L. (2007) MYST opportunities for growth control. Yeast genes illuminate human cancer gene functions. Oncogene 26, 5373–5384
7. Takahashi, H., McCaffery, J. M., Irizarry, R. A., and Boeke, J. D. (2006) Nucleocytosolic acetyl-coenzyme A synthetase is required for histone acetylation and global transcription. Mol. Cell 23, 207–217
8. Ramaswamy, V., Williams, J. S., Robinson, K. M., Sopko, R. L., and Schultz, M. C. (2003) Global control of histone modification by the anaphase-promoting complex. Mol. Cell Biol. 23, 9136–9149
9. Sandmeier, J. J., French, S., Osheim, Y., Cheung, W. L., Gallo, C. M., Beyer, M. C. (2003) Global control of histone modification by the anaphase-promoting complex. Mol. Cell Biol. 23, 9136–9149
10. Friis, R. M., Wu, B. P., Reinke, S. N., Hockman, D. J., Sykes, B. D., and Schultz, M. C. (2009) A glyceralic burst drives glucose induction of global
histone acetylation by picNuA4 and SAGA. *Nucleic Acids Res.* **37**, 3969–3980.

11. Wellen, K. E., Hatzivassiliou, G., Sachdeva, U. M., Bui, T. V., Cross, J. R., and Thompson, C. B. (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* **324**, 1076–1080.

12. Rathmell, J. C., and Newgard, C. B. (2009) Biochemistry. A glucose-to-genic link. *Science* **324**, 1021–1022.

13. Cai, L., Sutter, B. M., Li, B., and Tu, B. P. (2011) Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. *Mol. Cell* **42**, 426–437.

14. Guarente, L. (2011) The logic linking protein acetylation and metabolism. *Cell Metab.* **14**, 151–153.

15. Wellen, K. E., and Thompson, C. B. (2012) A two-way street. Reciprocal regulation of metabolism and signaling. *Nat. Rev. Mol. Cell Biol.* **13**, 270–276.

16. Gombert, A. K., Moreira dos Santos, M., Christensen, B., and Nielsen, J. (2001) Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* under different conditions of glucose repression. *J. Bacteriol.* **183**, 1341–1451.

17. Smedsgaard, J., and Nielsen, J. (2005) Metabolite profiling of fungi and yeast. From phenotype to metabolome by MS and informatics. *J. Exp. Bot.* **56**, 273–286.

18. Heyland, J., Fu, J., and Blank, L. M. (2009) Correlation between TCA cycle flux and glucose uptake rate during respiro-fermentative growth of *Saccharomyces cerevisiae*. *Microbiology* **155**, 3827–3837.

19. Starai, V. J., and Escalante-Semerena, J. C. (2004) Acetyl-coenzyme A synthetase (AMP-forming). *Cell. Mol. Life Sci.* **61**, 2020–2030.

20. Tehlivets, O., Scheuringer, K., and Kohlwein, S. D. (2007) Fatty acid synthesis and elongation in yeast. *Biochim. Biophys. Acta* **1771**, 255–270.

21. Sherman, F. (1991) Getting started with yeast. *Methods Enzymol.* **194**, 3–21.

22. Demczuk, A., Guha, N., Nguyen, P. H., Desai, P., Chang, J., Guzinska, K., Rollins, J., Ghosh, C. C., Goodwin, L., and Vancura, A. (2008) *Saccharomyces cerevisiae* phospholipase C regulates transcription of Msn2p-dependent stress-responsive genes. *Eukaryot. Cell* **7**, 967–979.

23. Iyer, V., and Struhl, K. (1996) Absolute mRNA levels and transcriptional regulation, purification, and analysis of histones and other H2AX-related proteins. *Mol. Cell. Biol.* **16**, 2199–2205.

24. Shirra, M. K., Patton-Vogt, J., Ulrich, A., Liuta-Tehlivets, O., Kohlwein, S. D., Henry, S. A., and Arndt, K. M. (2001) Inhibition of acetyl-coenzyme A carboxylase is required for SAGA NuA4 histone H4 acetylation activity is required for mitotic and meiotic chromosome condensation. *Cell. Mol. Life Sci.* **58**, 5208–5212.

25. Matsuhashi, M. (1969) Acetyl-CoA carboxylase from yeast. *Methods Enzymol.* **14**, 3–8.

26. Geng, F., Cao, Y., and Laurent, B. C. (2001) Essential roles of Snf5p in Snf-Swi chromatin remodeling in *vivo*. *Mol. Cell. Biol.* **21**, 4311–4320.

27. Guha, N., Desai, P., and Vancura, A. (2007) Plc1p is required for SAGA two-dimensional gel analysis of histones and other H2AX-related proteins. *Mol. Cell. Biol.* **18**, 2419–2428.

28. Allis, C. D., Glover, C. V., and Gorovsky, M. A. (1979) Micronuclei of *Tetrahymena* contain two types of histone H3. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4857–4861.

29. Pilch, D. R., Redon, C., Sedelnikova, O. A., and Bonner, W. M. (2004) Two-dimensional gel analysis of histones and other H2AX-related methods. *Methods Enzymol.* **375**, 76–88.

30. Richon, V. M., Zhou, X., Secrist, J. P., Gordon-Cardo, C., Kelly, W. K., Drobnyjak, M., and Marks, P. A. (2004) Histone deacetylase inhibitors: assays to assess effectiveness in *vivo* and in *vivo*. *Methods Enzymol.* **376**, 199–205.

31. Kuo, M. H., Zhou, J., Jambeck, M. E., and Allis, C. D. (1998) Histone deacetylase activity of yeast Gcn5p is required for the activation of target genes in *vivo*. *Genes Dev.* **12**, 627–639.

32. Shechter, D., Dormann, H. L., Allis, C. D., and Hake, S. B. (2007) Extraction, purification, and analysis of histones. *Nat. Protoc.* **2**, 1445–1457.

33. Lin, Y. Y., Lu, J. Y., Zhang, J., Walter, W., Dang, W., Wan, J., Tao, S. C., Qian, J., Zhao, Y., Boeke, J. D., Berger, S. L., and Zhu, H. (2009) Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating glucose homeostasis. *Cell* **136**, 1073–1084.

34. Mitchell, L., Lau, A., Lambert, J. P., Zhou, H., Fong, Y., Couture, I. F., Figuys, D., and Baetz, K. (2011) Regulation of septin dynamics by the *Saccharomyces cerevisiae* lysine acetyltransferase NuA4. *PLoS One* **6**, e25336.

35. Smedsgaard, J., and Nielsen, J. (2005) Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* in yeast. From phenotype to metabolome by MS and informatics. *J. Exp. Bot.* **56**, 273–286.

36. Guha, N., Desai, P., and Vancura, A. (2007) Plc1p is required for SAGA NuA4 histone H4 acetylation activity. *J. Biol. Chem.* **282**, 23643–23653.

37. Schneiter, R., Hitomi, M., Iwao, A., Ehrhardt, A., and Bonner, W. M. (2004) Histone deacetylase inhibitors: assays to assess effectiveness in *vivo* and in *vivo*. *Methods Enzymol.* **376**, 199–205.

38. Ganshori, L. J., Dollard, C., Tan, P., and Winston, F. (1995) The *Saccharomyces cerevisiae* SPT7 gene encodes a very acidic protein important for transcription in *vivo*. *Genetics* **139**, 523–536.

39. Roberts, S. M., and Winston, F. (1996) SPT20/ADA5 encodes a novel protein functionally related to the TATA-binding protein and important for transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**, 3206–3213.

40. Budak, K. A., Lopes, J. M., and Henrique, J. (2001) A pleiotropic phospho-lipid biosynthetic regulatory mutation in *Saccharomyces cerevisiae* is allelic to sin1(Δ) (sm4, rpd1). *Genetics* **163**, 475–483.

41. Clarke, A. S., Lovern, J. L., Jacobson, S. I., and Pillus, L. (1999) Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol. Cell. Biol.* **19**, 2515–2526.

42. Choy, J. S., Tobe, B. T., Huh, J. H., and Kron, S. J. (2001) Yng2p-dependent NuA4 histone H4 acetylation activity is required for mitotic and meiotic progression. *J. Biol. Chem.* **276**, 43653–43662.

43. Lu, J. Y., Lin, Y. Y., Shen, J. C., Wu, J. T., Lee, F. J., Chen, Y., Lin, M. L., Chang, F. T., Tai, T. Y., Berger, S. L., Zhao, Y., Tsai, K. S., Zhu, H., Chuang, L. M., and Boeke, J. D. (2011) Acetylation of yeast AMPK controls intrinsic aging independently of caloric restriction. *Cell* **146**, 969–979.

44. Hardie, D. G., Carling, D., and Carlson, M. (1998) The AMP-activated/ SNF1 protein kinase subfamily. Membrane receptors of the eukaryotic cell? *Ann. Rev. Biochem.* **67**, 821–855.

45. Hedbäck, K., and Carlson, M. (2008) SNF1/AMPK pathways in yeast. *Front. Biosci.* **13**, 2408–2420.

46. Woods, A., Munday, M. R., Scott, J., Yang, X., Carlson, M., and Carling, D. (1994) Yeast Snf1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase in *vivo*. *J. Biol. Chem.* **269**, 19509–19515.

47. Katan-Khayovich, Y., and Struhl, K. (2002) Dynamics of global histone
Acetylation and deacetylation in vivo. Rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes Dev.* **16**, 743–752

55. Kurdistani, S. K., Robyr, D., Tavazoie, S., and Grunstein, M. (2002) Genome-wide binding map of the histone deacetylase Rpd3 in yeast. *Nat. Genet.* **31**, 248–254

56. Colbert, C. L., Kim, C. W., Moon, Y. A., Henry L., Palnitkar M., McKean W. B., Fitzgerald K., Deisenhofer J., Horton J. D., and Kwon H. I. (2010) Crystal structure of Spot 14, a modulator of fatty acid synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18820–18825

57. Choudbary, C., Kumar, C., Gnadt, F., Nielsen, M. L., Rehman, M., Waltber, T. C., Olsen, J. V., and Mann, M. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **325**, 834–840

58. Choudhari, C., Pillus, L. (2009) Collaboration between the essential Esa1 acetyltransferase and the Rpd3 deacetylase is mediated by H4K12 histone acetylation in *Saccharomyces cerevisiae*. *Genetics* **183**, 149–160

59. Tong, L., and Harwood, H. J. (2006) Acetyl-coenzyme A carboxylases. Versatile targets for drug discovery. *J. Cell Biochem.* **99**, 1476–1488

60. Kim, C. W., Moon, Y. A., Park, S. W., Cheng, D., Kwon, H. J., and Horton, J. D. (2010) Induced polymerization of mammalian acetyl-CoA carboxylase by MIG12 provides a tertiary level of regulation of fatty acid synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 9626–9631

61. Gregolin, C., Ryder, E., Kleinschmidt, A. K., Warner, R. C., and Lane, M. D. (1966) Molecular characteristics of liver acetyl-CoA carboxylase. *Proc. Natl. Acad. Sci. U.S.A.* **56**, 148–155

62. Zadra, G., Priolo, C., Patnauf, A., and Loda, M. (2010) New strategies in prostate cancer. Targeting lipogenic pathways and the energy sensor AMPK. *Clin. Cancer Res.* **16**, 3322–3328

63. Chirala, S. S., Zhong, Q., Huang, W., and al-Feel, W. (1994) Analysis of FAS3/ACC regulatory region of *Saccharomyces cerevisiae*. Identification of a functional UASINO and sequences responsible for fatty acid-mediated repression. *Nucleic Acids Res.* **22**, 412–418

64. Schneper, L., Düvel, K., and Broach, J. R. (2004) Sense and sensibility. Nutritional response and signal integration in yeast. *Curr. Opin. Microbiol.* **7**, 624–630

65. Chang, C. S., and Pillus, L. (2009) Collaboration between the essential Esa1 acetyltransferase and the Rpd3 deacetylase is mediated by H4K12 histone acetylation in *Saccharomyces cerevisiae*. *Genetics* **183**, 149–160

66. Lindstrom, K. C., Vary, J. C., Parthun, M. R., Delrow, J., and Tsukiyama, T. (2006) Isw1 functions in parallel with the NuA4 and Swr1 complexes in stress-induced gene repression. *Mol. Cell. Biol.* **26**, 6117–6129

67. Zhao, S., Xu, W., Jiang, W., Wu, W., Lin, Y., Zhang, T., Yao, J., Zhou, L., Zeng, Y., Li, H., Li, Y., Shi, J., An, W., Hancock, S. M., He, F., Qin, L., Chin, J., Yang, P., Chen, X., Lei, Q., Xiong, Y., and Guan, K. L. (2010) Regulation of cellular metabolism by protein lysine acetylation. *Science* **327**, 1000–1004

68. Schneper, L., Düvel, K., and Broach, J. R. (2004) Sense and sensibility. Nutritional response and signal integration in yeast. *Curr. Opin. Microbiol.* **7**, 624–630

69. Kim, G. W., and Yang, X. J. (2011) Comprehensive lysine acetylomes emerging from bacteria to humans. *Trends Biochem. Sci.* **36**, 211–220

70. Olsen, J. V., Blagoev, B., Gnadt, F., Macek, B., Kumar, C., Mortensen, P., and Mann, M. (2006) Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell* **127**, 635–648

71. Villén, J., Beausoleil, S. A., Gerber, S. A., and Gygi, S. P. (2007) Large scale phosphorylation analysis of mouse liver. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1488–1493

72. Albaugh, B. N., Arnold, K. M., and Denu, J. M. (2011) KAT(ching) metabolism by the tail. Insight into the links between lysine acetyltransferases and metabolism. *ChemBioChem.* **12**, 290–298

73. Rak, J. L., and Abu-Elheiga L. A. (2009) Fatty acid metabolism. Target for metabolic syndrome. *J. Lipid Res.* **50**, S138–S143

74. Tong, L., and Harwood, H. J. (2006) Acetyl-coenzyme A carboxylases. Versatile targets for drug discovery. *J. Cell Biochem.* **99**, 1476–1488

75. Lindstrom, K. C., Vary, J. C., Parthun, M. R., Delrow, J., and Tsukiyama, T. (2006) Isw1 functions in parallel with the NuA4 and Swr1 complexes in stress-induced gene repression. *Mol. Cell. Biol.* **26**, 6117–6129