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ATP-citrate lyase links cellular metabolism to histone acetylation

Kathryn E. Wellen^{1,*}, Georgia Hatzivassiliou^{1,*}, Uma M. Sachdeva¹, Thi V. Bui¹, Justin R. Cross¹, and Craig B. Thompson¹

¹Department of Cancer Biology, Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA 19104

Abstract

Histone acetylation in single cell eukaryotes relies on acetyl-CoA synthetase enzymes that utilize acetate to produce acetyl-CoA. Metazoans, however, use glucose as their main carbon source and have exposure to only low concentrations of extracellular acetate. We show that histone acetylation in mammalian cells is dependent on ATP-citrate lyase (ACL), the enzyme that converts glucose-derived citrate into acetyl-CoA. We find that ACL is required for increases in histone acetylation in response to growth factor stimulation and during differentiation, and that glucose availability can impact histone acetylation in an ACL-dependent manner. Together, these findings suggest that ACL activity is required to link growth-factor-induced increases in nutrient metabolism to the regulation of histone acetylation and gene expression.

The accessibility of DNA in eukaryotic cells is determined by its organization in a DNA-protein complex known as chromatin. Chromatin structure is regulated in part through dynamic modifications of the constituent proteins, primarily histones. Histone acetylation has critical roles in regulating global chromatin architecture and gene transcription (1-3). Acetylation of histones can provide binding sites for proteins containing bromodomains, alter chromatin subnuclear localization and structure, and neutralize histone positive charge, which may loosen interactions between histones and DNA (2,4-6). Histone acetylation can be dynamically regulated by several classes of histone deacetylases (HDACs) and families of histone acetyltransferases (HATs), which act both on targeted regions of chromatin to regulate specific gene transcription as well as in a more global manner (1,3,7).

Studies of the NAD⁺-dependent sirtuins (class III HDACs), which target both histone and non-histone proteins, have demonstrated that deacetylation is responsive to metabolic cues (8-12). Sirtuins are dependent on NAD⁺ hydrolysis for their deacetylase activity and their activity is sensitive to changes in the intracellular NAD⁺/NADH ratio. HATs have not been shown to be regulated by the bioenergetic status of the cell, but production of acetyl-CoA by the enzyme acetyl-CoA synthetase (Acs2p), which generates acetyl-CoA from acetate, is linked to the regulation of histone acetylation in the yeast *S. cerevisiae* (13). This enzyme is itself regulated in a nutrient-responsive manner, and is activated by sirtuin-dependent deacetylation (14). Though mammalian cells contain a homologue to Acs2p, AceCS1, which synthesizes acetyl-CoA from acetate and is also regulated by sirtuins (15), most mammalian cells do not use acetate as a major bioenergetic substrate. Rather, the major carbon source in mammalian cells is glucose. Acetyl-CoA can be produced from glucose by the enzyme

To whom correspondence should be addressed: Craig B. Thompson, M.D. Director, Abramson Cancer Center University of Pennsylvania 1600 Penn Tower 3400 Spruce Street Philadelphia, PA 19104 Phone: 215-662-3929 craig@mail.med.upenn.edu.
²present address: Genentech Inc., South San Francisco, CA 94080

*These authors contributed equally to this work.

Production of glucose-derived acetyl-CoA by ATP-citrate lyase is a key determinant of net histone acetylation levels.

ATP-citrate lyase (ACL), which generates acetyl-CoA from mitochondria-derived citrate. ACL-dependent production of acetyl-CoA for lipogenesis is important for the proliferation of glycolytically-converted tumor cells (16).

In yeast, Acs2p localizes to both the cytoplasm and the nucleus, suggesting that acetyl-CoA is produced in both compartments in this organism (13). Using deconvolution microscopy to image EGFP-tagged ACL, we detected significant levels of EGFP-ACL in the nucleus in addition to the cytoplasm in two different mammalian cell lines (Figs. 1A,S1A). Similar results were obtained with myc-tagged ACL in murine FL5.12 hematopoietic cells (Fig. S1B). Subcellular fractionation of HCT116 human colon carcinoma cells confirmed the presence of endogenous ACL in the nucleus as well as the cytoplasm (Fig. 1B). AceCS1 was largely cytoplasmic in HCT116 cells, with a small but significant amount of protein detected in the nuclear fractions (Fig. 1B). Since both citrate and acetate are small molecules able to freely diffuse through the nuclear pore complex (17), these data suggest that acetyl-CoA production may occur in both the cytoplasmic and nuclear compartments in mammalian cells.

To investigate whether ACL or AceCS1 is important for the maintenance of global histone acetylation in mammalian cells, we analyzed global histone acetylation after siRNA-mediated silencing of ACL, AceCS1, or both. Silencing of ACL significantly decreased the amount of histone acetylation for all core histones assessed (Fig. 2A). In contrast, suppression of AceCS1 had less of an effect on net histone acetylation and the reduction failed to reach statistical significance (Fig. 2A). In the absence of physiologic ACL levels, incubation of cells with supraphysiologic levels of acetate, the substrate used by AceCS1, rescued histone acetylation in a dose-dependent and AceCS1-dependent manner (Fig. 2, A and B). Thus, ACL is a major source of acetyl-CoA for global histone acetylation under normal growth conditions in HCT116 cells, but AceCS1 also participates and can compensate for loss of ACL function when its substrate is available.

We next sought to determine whether ACL-dependent production of acetyl-CoA selectively affects acetylation of histones or has equivalent effects on the acetylation of other cellular proteins. Upon DNA damage, p53 K³⁸² acetylation is catalyzed by p300 and may increase the ability of p53 to bind DNA and perform its functions as a transactivator (18). We assessed the efficiency of p53 acetylation in response to treatment with the DNA-damaging agent doxorubicin and found that acetylation of p53 K³⁸² was unaffected by silencing of ACL (Fig. 2C). Thus, high ACL activity is not a general requirement for cellular acetylation events, and acetylation of histones might be specifically linked to the pool of citrate-derived acetyl-CoA produced by ACL.

Net histone acetylation represents a balance between rates of acetylation and deacetylation catalyzed by HATs and HDACs, respectively. We therefore tested whether the decrease in global histone acetylation observed upon silencing of ACL could be rescued by inhibition of HDACs. Treatment of cells with the HDAC inhibitor trichostatin A (TSA) suppressed the effects of ACL deficiency on histone acetylation (Fig. 2D). Thus, in the absence of substantial histone deacetylation, ACL activity has less impact on the net acetylation of the core histones.

To better evaluate the scale of the histone acetylation defects observed upon ACL suppression, we compared the relative decrease in histone acetylation upon siRNA silencing of ACL to that upon silencing of GCN5, a HAT with an important role in the maintenance of global histone acetylation (19,20). Under these conditions, suppression of either ACL or GCN5 led to a statistically significant reduction in the acetylation of histones H2B, H3, and H4 (Fig. 2E, S2). Suppression of both ACL and GCN5 did not lead to additive inhibition of

the acetylation of the histones examined, suggesting that the two enzymes may function within the same pathway to regulate chromatin structure. Differences between the effects of silencing ACL and GCN5 may reflect the involvement of other HATs and production of acetyl-CoA by AceCS1. Acetylation of tubulin was unaffected by silencing of either GCN5 or ACL, again emphasizing that production of acetyl-CoA by ACL is not a general requirement for cellular protein acetylation (Fig. 2E). Suppression of AceCS1 or both ACL and AceCS1 also failed to reduce tubulin acetylation (Fig. 4A), which could reflect a low rate of turnover for tubulin acetylation, sufficient residual activity of either ACL or AceCS1, compensatory regulation of HDACs, or availability of acetyl-CoA from another source. Further investigation will be required to ascertain the relative contributions of different acetyl-CoA sources to acetylation of non-histone cellular proteins.

Regulation of histone acetylation levels may depend on both the cell type and state of cellular differentiation or activation (21-26). For example, we observed that apoptosis-deficient cells subjected to extended growth factor withdrawal exhibited low levels of histone acetylation that progressively increased upon growth factor add-back, correlating with increasing protein levels of ACL (Fig S3A). We thus examined the effect of ACL silencing on two cellular processes associated with changes in global histone acetylation: mitogenic stimulation of quiescent cells and adipocyte differentiation.

Acetylation of histones is regulated in a cell-cycle dependent manner and can contribute to normal cell cycle progression (27-29). Serum starvation followed by reintroduction can synchronize cells within a population by inducing them to collectively exit and re-enter the cell cycle (30). This mitogenic stimulation is accompanied by a net increase in histone acetylation, as cells enter and progress through S phase (24). We assessed the ability of cells to induce histone acetylation in response to mitogenic stimulation in immortalized MEFs. In control cells, acetylation of histone H3 increased following serum stimulation, and silencing of ACL inhibited this response (Fig. 3A). Levels of acetylated tubulin were unaffected by ACL suppression. To assess whether this ACL-dependent increase in histone acetylation could be regulated by glucose availability, serum stimulation was repeated in the presence or absence of glucose. Indeed, serum-induced changes in histone acetylation were dependent on glucose and failed to occur in the absence of ACL (Fig. 3, B and C). Under glucose deprivation conditions, cells can utilize fatty acid oxidation to support their bioenergetic needs. However, fatty acid oxidation results in the production of mitochondrial but not nucleocytoplasmic acetyl-CoA. Consistent with this, supplementation of glucose-starved cells with fatty acids failed to rescue histone acetylation, supporting the model that only nucleocytoplasmic acetyl-CoA is available to participate in histone acetylation (Fig S3B). Reduced histone acetylation after silencing of ACL was also observed during proliferation of MEFs in culture (Fig. S3C), suggesting that ACL is also involved in maintaining histone acetylation during proliferative expansion, even when sufficient acetyl-CoA is present to support cell growth.

Histone acetylation increases have also been observed during differentiation of murine 3T3-L1 preadipocytes into adipocytes (25). Silencing of ACL reduced histone acetylation in adipocytes, while suppression of AceCS1 had little effect (Fig. 4A). In addition, the increase in histone acetylation observed during adipocyte differentiation was almost entirely suppressed upon ACL silencing and could be rescued by acetate in a dose-dependent manner, suggesting that AceCS1 can compensate in this model depending on acetate availability (Fig. 4B).

We next sought to determine whether ACL silencing specifically affected differentiation-induced histone acetylation or whether it more broadly impaired the ability of the cell to implement the differentiation program. Using Oil Red O staining to assess differentiation-

induced lipid accumulation, we observed that cells depleted of ACL contained visibly less lipid than control cells, whereas silencing of AceCS1 had minimal effects on lipid accumulation (Fig. 4C). Lipid accumulation in ACL-deficient cells, however, could be partially rescued by incubation of cells with acetate (Fig. S4A), suggesting that the reduced lipid accumulation was primarily due to low availability of acetyl-CoA rather than impaired differentiation and that, in the absence of supraphysiological acetate, acetyl-CoA is derived mainly from citrate in adipocytes.

These observations raised the possibility that ACL-dependent changes in histone acetylation are required for adipocytes to take up and metabolize the amounts of glucose needed to engage in fat storage. Transcriptional profiling in yeast has previously indicated that a reduction in global histone acetylation as a result of GCN5 deficiency results in altered expression of multiple carbohydrate metabolism genes (31). Therefore, we examined whether the expression of genes involved in glucose uptake and metabolism was affected in cells in which ACL is silenced. Strikingly, we found that expression of the insulin-responsive glucose transporter, Glut4, as well as three key regulators of glycolysis, hexokinase 2 (HK2), phosphofructokinase-1 (PFK-1), and lactate dehydrogenase A (LDH-A), were all significantly suppressed upon ACL silencing and rescued by acetate (Fig. 4D). These transcriptional effects correlated well with cellular glycolytic activity; ACL silencing resulted in a 32% decrease in glucose consumption ($p < 0.001$), which was reversed by acetate supplementation. In addition, expression of genes involved in conversion of glucose into nonessential amino acids and fatty acids, including soluble glutamate: oxaloacetate transaminase (Got1) and carbohydrate responsive transcription factor ChREBP, were similarly regulated (Fig. 4D). These effects on glucose metabolism genes appeared to be selective, as markers of adipocyte differentiation such as aP2, as well as the HAT GCN5, were not regulated upon either ACL suppression or acetate supplementation (Fig. 4D).

Glut4 expression was selected for further examination, since it is a well-characterized determinant of adipocyte glucose consumption. The reduction in Glut4 expression was specific for ACL silencing and was not observed upon silencing of AceCS1 (Fig. 4E). Notably, other differentiation markers including aP2, adiponectin, and fatty acid synthase were expressed at equal or higher levels upon silencing of either ACL or AceCS1, indicating that the adipogenic program is successfully initiated without ACL or AceCS1 (Fig. 4E, S4B). Upon silencing of ACL, Glut4 expression could be rescued in a dose-dependent manner by acetate supplementation (Fig. 4F), in concert with total histone acetylation levels (Fig. 4B). Furthermore, chromatin immunoprecipitation experiments revealed that acetylation of histones H3 and H4 at the Glut4 promoter was specifically reduced upon ACL silencing and could be rescued by acetate (Fig. 4G).

We next tested whether the regulation of histone acetylation and Glut4 expression by ACL in differentiating adipocytes is nutrient-dependent by exposing the cells to various concentrations of glucose during differentiation. Standard medium for adipocyte differentiation contains 25 mM glucose, but 3T3-L1 cells can also differentiate at lower concentrations of glucose, although they accumulate lower amounts of lipid (32). We differentiated adipocytes in 1, 4 and 25 mM glucose and observed that they accumulated increasing amounts of lipid when exposed to higher levels of glucose, correlating with increasing Glut4 expression (Fig. S4, C and D). Cells exposed to either 4 or 25 mM glucose exhibited similar increases in expression of the differentiation marker aP2, whereas 1 mM glucose resulted in lower levels of aP2 gene expression (Fig S4, C and D).

Histone acetylation was regulated during differentiation in a nutrient-dependent manner, increasing according to both glucose and acetate availability (Fig. S4E). Glucose-dependent regulation of histone acetylation was dependent on ACL, while supraphysiologic

concentrations of acetate increased histone acetylation in the presence or absence of ACL (Fig. 4H). Similarly, the expression of Glut4 and the glycolytic genes HK2, PFK-1, and LDH-A were regulated according to glucose availability, in an ACL-dependent manner (Fig. 4I). These results demonstrate that, during adipocyte differentiation, global histone acetylation is determined by glucose availability through an ACL-dependent pathway and that supraphysiologic levels of acetate can also contribute through AceCS1. Our data also suggest that nutrient-responsive histone acetylation may selectively impact the expression of genes required to reprogram intracellular metabolism to utilize glucose for ATP production and macromolecular synthesis.

In summary, we have demonstrated that ACL plays a critical role in determining the total amount of histone acetylation in multiple mammalian cell types. ACL-dependent production of acetyl-CoA contributes to increased histone acetylation during cellular response to growth factor stimulation and during adipocyte differentiation, both energy-intensive processes in which nuclear activity needs to be coordinated with cellular metabolic state. ACL-dependent acetylation can also contribute to the selective regulation of genes involved in glucose metabolism. Our data indicate that ACL activity is required to link growth factor-induced nutrient uptake and metabolism to the regulation of histone acetylation. Thus, it appears that histone acetylation can be dynamically regulated by physiologic changes in concentrations of acetyl-CoA produced by ACL. Our results also suggest that the current model for metabolic regulation of histone acetylation should be expanded to include not only redox regulation of deacetylases, but also regulation of HATs by physiologic changes in the generation of acetyl-CoA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. ACL localizes to the nucleus and cytoplasm

(A) Subcellular localization of EGFP-tagged ACL in LN229 cells imaged by deconvolution microscopy. EGFP-ACL, visible in both nucleus and cytoplasm, is contrasted to DsRed mitochondrial marker, which is excluded from the nucleus. DAPI staining of nuclear DNA is restricted to the nucleus. (B) Western blot analysis of cytoplasmic and nuclear protein extracts from HCT116 cells. Quantitation represents mean $\pm$ SD from 4 [ACL, AceCS1] or 8 [PARP, tubulin] nuclear and cytoplasmic samples. ACL and AceCS1 are both significantly (***, $p < 0.0001$) enriched in the nuclear fraction as compared to tubulin.

Fig. 2. ACL is the major source of acetyl-CoA for histone acetylation in mammalian cells

In all panels, HCT116 cells were used and transfected with CTRL, ACL and/or AceCS1 siRNA as indicated for 72 hours. (A) Western blot analysis of total and acid extracts from HCT116 cells treated +/- 5mM acetate for 24 hours prior to lysis. Acid-extracted histones were visualized by Coomassie staining. Quantitation represents percent reduction of acetylated/total histone levels in siACL- and/or siAceCS1-transfected cells compared to siCTRL-transfected cells in 3 independent experiments (mean +/- SEM, *, $p < 0.05$; **, $p < 0.01$) (B) HCT116 cells were incubated with 0, 1, or 5 mM acetate for 24 hours prior to lysis. Quantitation of acetylated/total H3 levels represents triplicate samples run in parallel, mean +/- SD (*, $p < 0.05$; ***, $p < 0.0005$). (C) Western blot analysis of p53 acetylation in

HCT116 cells treated with 0.5 μ M doxorubicin for 24 hours. Phosphorylation of H2AX confirmed that the DNA damage response was activated by doxorubicin. (D) Western blot analysis of H3 and tubulin acetylation in HCT116 cells treated with 500 nM trichostatin A (TSA) for 24 hours. (E) Western blot analysis of histone acetylation in total extracts of HCT116 cells transfected for 72 hours with CTRL, ACL, and/or GCN5 siRNAs. Results are representative of 4 independent experiments. For quantitation, see Figure S2.

Fig. 3. Role of ACL and glucose availability in the acetylation of histones during cellular response to serum stimulation

Immortalized MEFs transfected with CTRL or ACL siRNAs as indicated were synchronized by serum deprivation overnight followed by serum stimulation. (A) Western blot analysis of total protein extracts from siCTRL-or siACL-treated cells at indicated time points after re-addition of serum to the culture (left panel). Ratio of acetylated histone H3 to total H3 was quantitated at each time point and fitted to a linear regression (right panel). Comparable results were obtained in each of 3 independent experiments. (B) Western blot analysis of total protein extracts from control cells at indicated times following serum re-introduction in the presence (+ = 25 mM) or absence (- = 0 mM) of glucose. (C) Western blot analysis of total protein extracts from cells transfected with siCTRL or siACL as described in Materials and Methods (33) and cultured for 48h in the presence or absence of glucose.

Fig. 4. Regulation of acetylation of histones during adipocyte differentiation by ACL and nutrient availability

3T3-L1 preadipocytes were transfected with control, ACL, and/or AceCS1 siRNAs and 2 days later stimulated to differentiate into adipocytes. (A) Total lysates and acid extracts from 3T3-L1 cells 4 days after induction of differentiation, analyzed by Western blot. (B) Acid extracts from 0 and 4 days after induction of differentiation in the presence of 0, 1, or 5 mM sodium acetate were analyzed by Western blot. (C) Cells were fixed and Oil Red O lipid staining was performed 5 days after induction of differentiation. Similar results were obtained in each of 3 independent experiments. (D) RNA was isolated 4 days after induction of differentiation in the presence or absence of 5 mM sodium acetate. Gene expression was analyzed by quantitative RT-PCR and normalized to 18S rRNA (mean $\pm$ S.D. of triplicate samples). (E) Gene expression 4 days after induction of differentiation was quantified by Taqman quantitative RT-PCR and normalized to 18S rRNA. Data represent triplicate wells from 2 independent experiments, mean $\pm$ S.D. (F) RNA was isolated 4 days after induction of differentiation in the presence of 0, 1, or 5 mM sodium acetate. Glut4 expression was analyzed by Taqman RT-PCR and normalized to 18S rRNA (mean $\pm$ S.D. of triplicate samples). (G) Chromatin immunoprecipitation was performed using antibodies to Ac-H3 and Ac-H4. Immunoprecipitated Glut4 promoter sequence was analyzed by quantitative PCR. H4 genomic sequence was used as an endogenous control. The amount of immunoprecipitated H4 DNA was unchanged upon ACL silencing. Data represent mean $\pm$ S.D. of triplicate samples from each of 2 independent experiments (H) Western blots of cells differentiated 0 or 4 days in 4 or 25 mM glucose, +/- 5 mM sodium acetate. (I) Gene expression in cells 4 days after induction of differentiation in 1, 4, or 25 mM glucose was assessed by Taqman quantitative RT-PCR and normalized to 18S rRNA. Data are the averages of 3 independent experiments (mean $\pm$ SEM). All statistical analyses are

comparisons of 2 data sets and were performed using t-tests (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$). C= siCTRL; A= siACL.