Published online 30 April 2009

Nucleic Acids Research, 2009, Vol. 37, No. 12 ***3969–3980***
doi:10.1093/nar/gkp270

A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA

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Received February 4, 2009; Revised April 2, 2009; Accepted April 11, 2009

ABSTRACT

Little is known about what enzyme complexes or mechanisms control global lysine acetylation in the amino-terminal tails of the histones. Here, we show that glucose induces overall acetylation of H3 K9, 18, 27 and H4 K5, 8, 12 in quiescent yeast cells mainly by stimulating two KATs, Gcn5 and Esa1. Genetic and pharmacological perturbation of carbon metabolism, combined with ^{1}H-NMR metabolic profiling, revealed that glucose induction of KAT activity directly depends on increased glucose catabolism. Glucose-inducible Esa1 and Gcn5 activities predominantly reside in the picNuA4 and SAGA complexes, respectively, and act on chromatin by an untargeted mechanism. We conclude that direct metabolic regulation of globally acting KATs can be a potent driving force for reconfiguration of overall histone acetylation in response to a physiological cue.

INTRODUCTION

Histones H2A, H2B, H3 and H4, the protein components of the nucleosome core particle, are subject to numerous chemically distinct post-translational modifications. In terms of function and regulation, the best characterized amongst these is reversible acetylation of lysine residues in the conserved histone amino-terminal tails. Histone acetylation is mediated by lysine acetylases (KATs) and reversed by histone deacetylases (HDACs), and controlled to a large extent by mechanisms that impinge on these enzymes (1,2). This report concerns physiological regulation of histone acetylation in budding yeast in response to glucose, the preferred carbon source of this organism (3).

Our experiments extend previous studies in which it was shown by immunoblotting analysis of total cellular proteins that overall H3/H4 acetylation declines as yeast cells progress into stationary phase (SP) in response to nutrient depletion from their environment (4,5). Conversely, SP cells inoculated into fresh medium give rise to an expanding population with a relatively high level of histone acetylation (data not shown). Although glucose refeeding in SP does not trigger entry into S phase, it does elicit gross morphological changes characteristic of preparation for re-proliferation (6). We therefore reasoned that glucose might also induce overall H3/H4 acetylation. Here, we show that glucose refeeding indeed triggers robust acetylation of nucleosomal H3 (at K9, 14, 18, 27) and H4 (at K5, 8, 12) in SP cells. For simplicity, we refer to these events collectively as 'H3/H4 acetylation'.

Physiological resetting of overall histone acetylation uncoupled from replication is well documented in mammalian cells. For example, H3 K9 and H4 acetylation are induced prior to S phase in mitogenically stimulated B and T cells (7,8), H4 acetylation is induced one day after the onset of embryonic stem cell differentiation (9), H3/H4 acetylation is induced in cells of the hippocampus and cortex during neuronal rewiring (10), and H3 K9 acetylation is induced in the course of epigenetic reprogramming in the germ line (11). Despite the abundant evidence that overall histone acetylation is subject to physiological regulation in non-replicating cells, little is known about the mechanisms of this regulation. We therefore further characterized glucose stimulation overall histone acetylation in SP yeast cells.

What mechanism could account for glucose induction of acetylation in SP cells? A straightforward and compelling model is suggested by two principles in chromatin biology which are widely appreciated and generally accepted. The first is that physiological cues can trigger signal transduction events which cause transcriptional induction of some genes, and repression of others. The second is that induction of transcription is typically accompanied by increased acetylation of chromatin (1,12). In yeast, it is well established that signaling pathways activated by glucose can drive reprogramming of transcription (3), and while our work was ongoing, it was reported that almost 1400 genes are induced when SP cells are fed glucose (13). We further show here that glucose induction of H3/H4 acetylation largely depends on two KATs which play a pivotal role in transcription

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in yeast and higher eukaryotes (14–17). These are Gcn5, which acetylates H3, and Esa1, which acetylates H4 (1).

The scenario suggested by previous studies (and consistent with prevailing views in the field) is that glucose induction of overall acetylation in SP cells is simply the sum of targeted acetylation events associated with pervasive induction of transcription driven by glucose-dependent signaling. Surprisingly, this is not the case. Glucose induction of H3/H4 acetylation in SP yeast cells is principally due to direct metabolic induction of KATs which act globally throughout chromatin.

MATERIALS AND METHODS

Yeast strains, fractionation and culture/refeeding protocols

Strains are listed in Supplementary Table S1. Cells were cultured for 8 days in rich medium with 2% glucose (YPD) before treatments outlined in the text.

Cell analysis and determination of glucose concentration of the culture medium

Automated counting, flow cytometry analysis and viability assays were performed after sonication to disrupt cell aggregates. Extracellular glucose was measured using a blood glucose meter.

Antibodies

H3ac and H4ac antibodies were from Upstate Biotechnology (06-599 and 06-598). Site-specific H4 K5, 8, 12 or 16ac antibodies were from Serotec (AHP414-AHP417). Site-specific H3 K9, 14 and 27ac antibodies were from Upstate (06-942, 07-353 and 07-360). H3 K18ac antibody was from Abcam (ab1191). Bulk H3 and H4 were detected using in-house antibodies [(5), also see Supplementary Figure S8]. The TAP tag was detected using Upstate IgG 07-482, specific for the calmodulin binding peptide (CBP) epitope. Anti-acetyl lysine antibody 9441S was from Cell Signaling. Anti-actin monoclonal antibody MAB1501 was from Chemicon.

Protein isolation and immunoblotting

Total protein was isolated from cells by NaOH/β-mercaptoethanol lysis and trichloroacetic acid (TCA) precipitation (5). Histones were fractionated as described (18). Immunoblotting was performed by standard methods. Sample loadings are all normalized for cell number.

Metabolite profiling by 1H-NMR spectroscopy

Flash-frozen cells were disrupted by bead-beating in 5% TCA and spun to yield a supernatant that was further clarified by centrifugation. This supernatant was adjusted to pH 7, lyophilized, then resuspended in D_2O (Isotec Inc., Miamisburg, Ohio), and DSS-d_6, 2,2-dimethyl-2-sila 3,3,4,4,5,5-hexadeuteropentane sulphonic acid (Chenomx Inc., Edmonton, Alberta) as a chemical shift indicator and concentration standard. All spectra were acquired on a 600 MHz Inova NMR spectrometer using the tnnoesy pulse sequence (circa Vnmr 6.1 software, Varian Inc), and analyzed using the Chenomx NMR Suite Professional software v5.0.

Enzymatic measurement of cellular acetate

Flash-frozen cells were chemically lysed and spun to obtain a supernatant that was assayed for acetate using the Enzytec™ Acetic Acid Kit from Scil Diagnostics GmbH.

Details of all procedures are provided in the Supplemental Material.

RESULTS

Glucose regulation of H3/H4 acetylation in SP cells

We explored the nutrient response of SP cells in 8 day cultures as outlined in Figure 1A. At set times after nutrient addition, the glucose content of the medium and cell viability were determined (Figure 1B), and cell aliquots were processed for automated counting, flow cytometry (to monitor DNA content) and isolation of total protein. Effects of refeeding on H3/H4 metabolism were assessed by immunoblotting. Briefly, 'acetylated H4' (H4ac) was detected with an antibody raised against an H4 tail peptide acetylated at K5, 8, 12 and 16, 'acetylated H3' (H3ac) was detected using an antibody raised against an H3 tail peptide acetylated at K9 and K14, and protein loading was monitored using antibodies against bulk H3/H4 or actin. Changes in acetylation revealed by the H3ac/H4ac antibodies were also readily detected by an anti-acetyl lysine antibody.

We observed robust H4 acetylation following simultaneous refeeding with glucose and amino acids. Acetylation peaks at 4 h (Figure 1C, lanes 9, 12) and is maintained for up to 24 h (Figure 1C and data not shown). After peaking, H4 acetylation slowly declines to below the starting level (Figure 1C, lanes 1, 24). These changes were not accompanied by changes in steady-state bulk H4 expression. Parallel feeding revealed: (i) that increased acetylation in cells fed glucose and amino acids is due to the provision of glucose (Figure 1C, lanes 9–12; average glucose induction corrected for bulk H4 recovery = 4.7, $n = 3$), and (ii) that the persistence of H4ac is positively correlated with the glucose dose (Figure 1D, compare lanes 11, 12 to 17, 18; glucose measurements are in Figure 1B). Probing with site-specific antibodies revealed that fluctuations in acetylation detected by the H4ac antibody (Figure 1C and D) are due to acetylation and deacetylation at K5, K8 and K12, but not K16 (Figure 1E). Figure 1E also shows that H3 K9/K14 acetylation is regulated similarly to H4 K5, 8, 12 acetylation (a more detailed analysis showing glucose induction of H3 K9, K14, K18 and K27 acetylation is presented in Figure 3C). We conclude that glucose triggers transient acetylation at K5, 8 and 12 of H4 and at K9, 14, 18 and 27 of H3 in SP cells. Control and glucose-fed cells do not differ in their viability during the period of histone acetylation (Figure 1B), so this response is not due to entry into a state from which reproliferation is not possible. Fractionation studies revealed that the free histone pool is extremely small in SP cells, and that glucose strongly induces acetylation of chromatin-associated

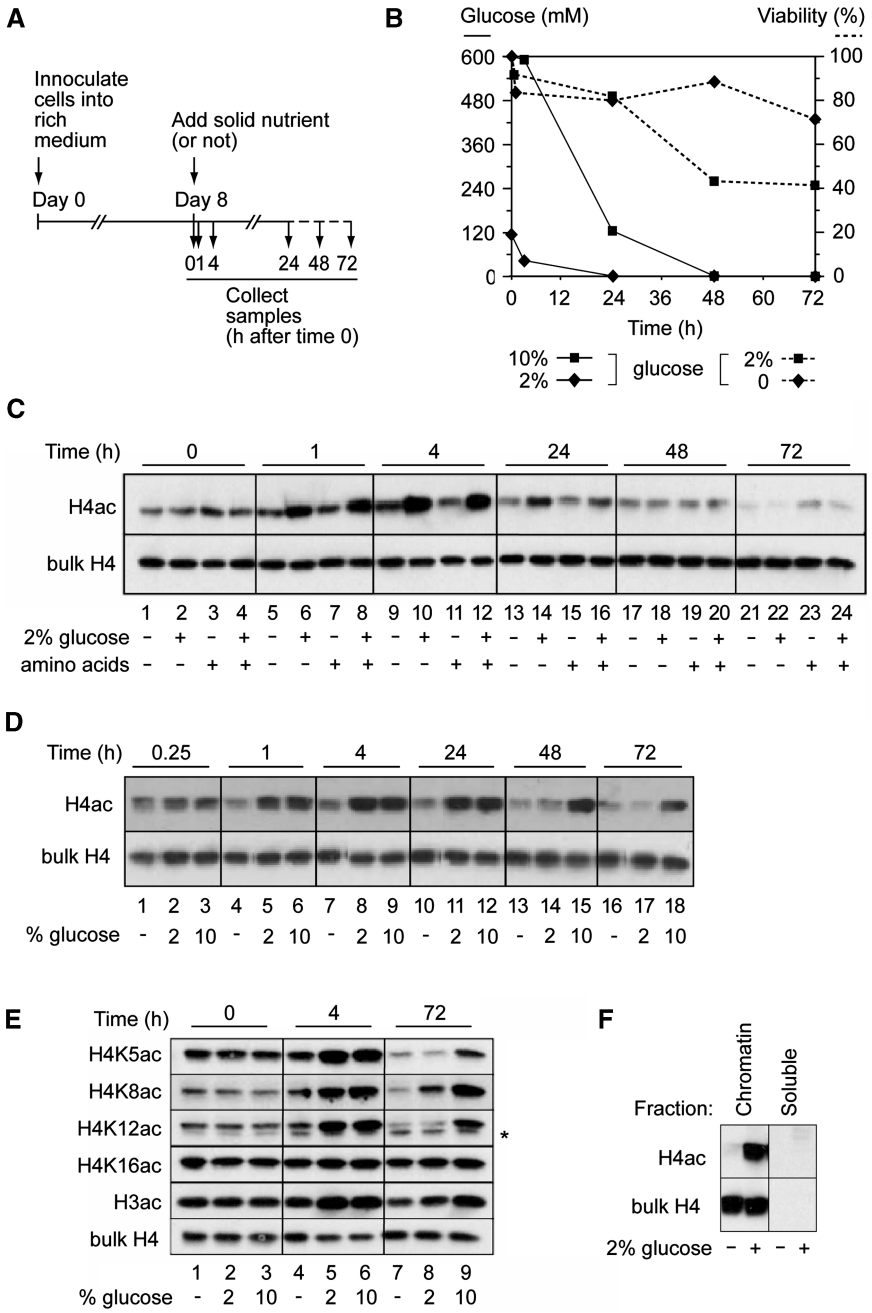

Figure 1. Overview of nutrient refeeding effects on histone acetylation in SP yeast cells. (**A**) Outline of culture/refeeding protocol. Time 0 corresponds to 8 days in culture, before nutrient addition. (**B**) Culture medium glucose concentration (solid lines), and cell viability (dashed lines), plotted as a function of time after refeeding. Viability is normalized to 100% live cell recovery at time 0. (**C**) Immunoblot analysis of H4 acetylation (H4ac antibody) and bulk H4 expression (loading control) at the indicated time points after nutrient refeeding. (**D**) Immunoblot analysis of H4 acetylation and bulk H4 expression at the indicated time points after refeeding with 2 or 10% glucose. (**E**) Immunoblot analysis of histone acetylation using residue- and acetylation-specific anti-H4 antibodies, and an antibody raised against an H3 K9ac K14ac tail peptide. *Indicates an unknown cross-reacting species. (**F**) Immunoblot analysis of acetylated and bulk H4 in the soluble and DNA-associated pools of histones obtained from cells before and after (4 h) glucose refeeding.

H4 (Figure 1F). Therefore, the results obtained by immunoblotting of total protein extracts likely reflect the regulation of histones that reside in nucleosomes, or become incorporated into nucleosomes.

The pattern of induction and repression of H3/H4 acetylation following glucose refeeding, and the residue-specificity of acetylation, resemble the regulation of overall H3/H4 acetylation during G1 to M cell cycle progression (19). However, glucose induction of acetylation is not coupled to nuclear DNA replication (Supplementary Figures S1, S2). Our further work therefore focused on identification of the histone modifying enzymes and complexes that are regulated by glucose, and the mechanism of their regulation.

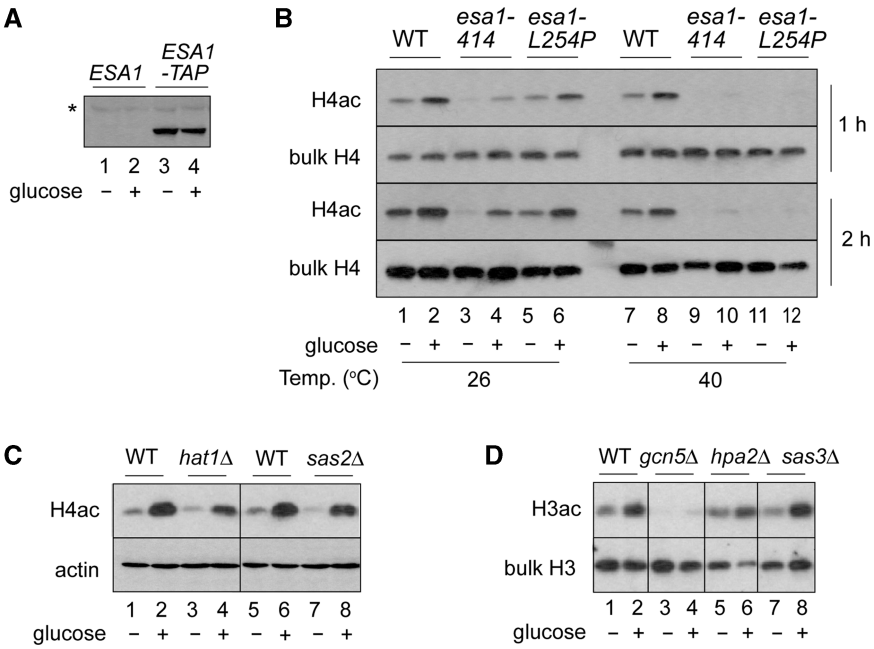

Figure 2. Identification of the KATs required for glucose induction of H3/H4 acetylation in SP. (**A**) Expression of Esa1-TAP in unfed and glucose-fed (1 h) SP cells monitored by anti-CBP immunoblotting. Equivalent loading is confirmed by the intensity of the background band (*). (**B**) Immunoblotting analysis of H4 acetylation in wild-type and two *ESA1* temperature-sensitive strains. Cells were unfed or glucose-fed, at either the permissive (26°C) or restrictive (40°C) temperature. (**C**) Immunoblotting analysis of H4 acetylation in wild-type and *HAT1* and *SAS*2 null mutants. Glucose-fed cells were harvested at 1 h. (**D**) Immunoblotting analysis of H3 acetylation in wild-type and *GCN5*, *HPA2* and *SAS*3 null mutants. Glucose-fed cells were harvested at 1 h. Glucose was added to 2% weight/volume for all experiments.

Histone lysine acetylases Esa1 and Gcn5 mediate glucose induction of H3/H4 acetylation in SP

Theoretically, glucose induction of H3/H4 acetylation in SP could be driven by increased KAT activity, decreased HDAC activity, or both. If this regulation is due to events that impinge on an individual KAT or HDAC, then a mutation that compromises the activity of that enzyme should dampen glucose induction of acetylation. We tested candidate KATs based on this expectation after obtaining evidence that regulation of HDAC activity is unlikely to play a central role in glucose induction of H3/H4 acetylation (Supplementary Figure S3).

We predicted an important role for Esa1 in the regulation of H4ac in SP as this KAT is required for maintenance H4 acetylation in log phase (20) and is expressed in SP (Figure 2A). Because Esa1 is essential for viability, its function was tested by analysis of two temperature sensitive mutants (20) that were allowed to reach SP at the permissive temperature.

Figure 2B shows that Esa1 activity is required for maintenance of basal acetylation in SP. At room temperature esa1-414 protein is partially inactive (21), and *esa1-414* cells display lower than wild type H4 acetylation (lanes 1, 3). Furthermore, H4 acetylation drops sharply in *esa1-L254P* cells when they are shifted to the restrictive temperature in SP (Figure 2B, lanes 5, 11), but does not do so in similarly treated wild-type cells (Figure 2B, lanes 1, 7). Accordingly, we propose that dynamic H4 acetylation in SP directly involves Esa1.

A central role for Esa1 in glucose induction of H4ac in SP was uncovered by refeeding at the permissive and restrictive temperatures. At the permissive temperature, induction of H4 acetylation was slower in $esa1^{ts}$ mutants (particularly *esa1-414*) than in wild-type cells (Figure 2B, lanes 1–4, compare 1 and 2 h). When cells grown for 8 day at the permissive temperature were incubated for 1 h at the restrictive temperature and then fed at the restrictive temperature, there was an even more pronounced effect specifically in $esa1^{ts}$ mutants: H4 acetylation was almost completely blocked (Figure 2B, lanes 7–12). On the other hand, null mutants of *HAT1* and *SAS2*, which encode the other two major H4 KATs in yeast, induce H4ac normally (Figure 2C). We conclude that Esa1 activity is critical for robust glucose induction of H4 acetylation in SP. Esa1 does not contribute to H3 acetylation in log phase cells (22), and is not important for glucose induction of H3ac in SP (Supplementary Figure S4).

Of the known H3 KATs, Gcn5 was expected to play an important role in the regulation of H3ac in SP because it has long been known to contribute to the control of overall acetylation of H3 K9 in log phase (22–24). Indeed, H3 acetylation in 8 day *gcn5Δ* cells is much lower than in wild type cells, and glucose induction of H3ac is severely compromised in this mutant (Figure 2D, lanes 1–4). On the other hand, *GCN5* deletion has little effect on induction of H4ac (Supplementary Figure S5), and the regulation of H3 acetylation is not perturbed by deletion of two other H3 KATs (Hpa2 and Sas3; Figure 2D, lanes 5–8). We conclude that Gcn5 functions in the establishment and/or maintenance of global H3 acetylation in SP, and has an important role in induction of H3ac upon glucose refeeding.

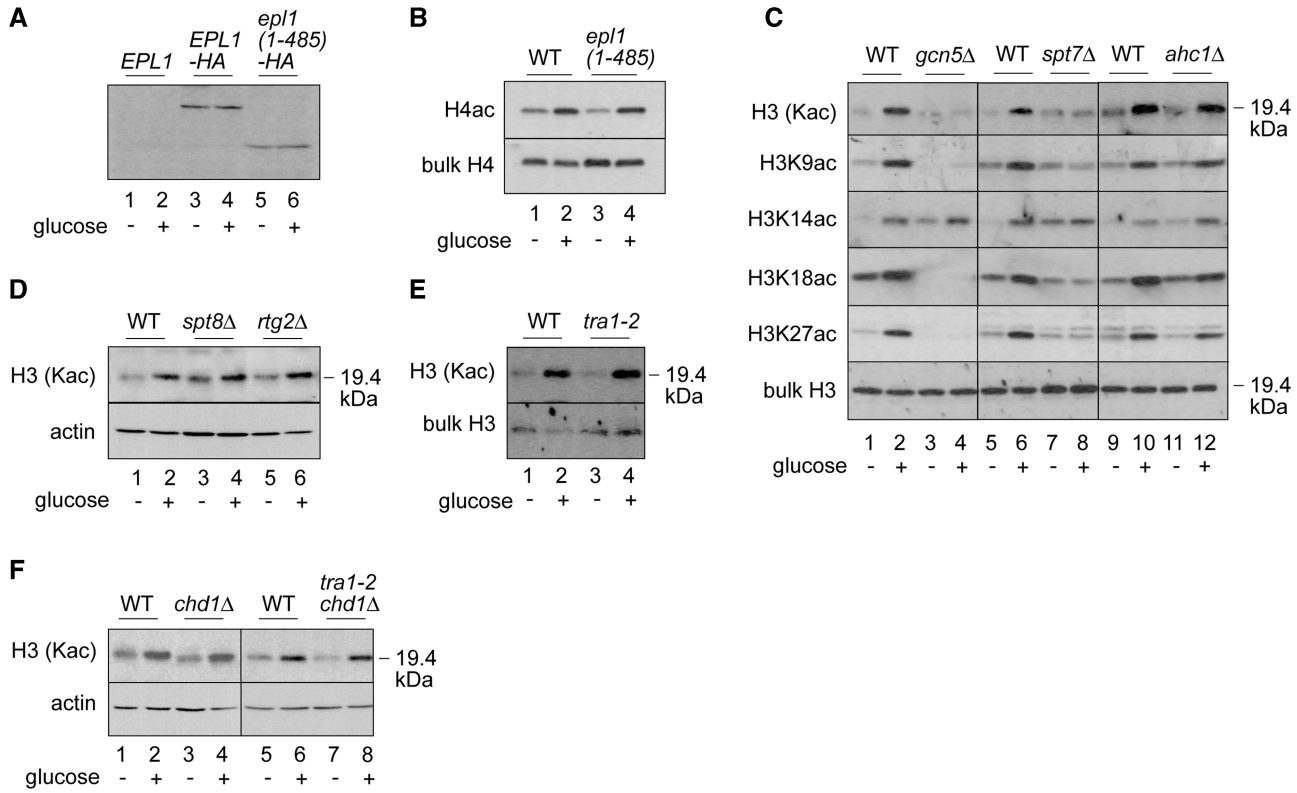

Figure 3. Identification of H3 and H4 KAT complexes required for glucose induction of H3/H4 acetylation in SP. (**A**) Expression of HA-tagged Epl1 and epl1(1–485) monitored by anti-HA immunoblotting. In (A–D), glucose-fed cells were harvested at 1 h. (**B**) Immunoblotting analysis of H4 acetylation in wild-type and *epl1(*1–485*)* cells. (**C**) Immunoblotting analysis of H3 acetylation in wild-type cells compared to null mutants lacking the genes encoding Gcn5, Spt7 (specific for SAGA/SLIK) and Ahc1 (a specific subunit of ADA). (**D**) Immunoblotting analysis of H3 acetylation in wild-type and null mutant strains lacking the genes encoding Spt8 (a specific subunit of SAGA) and Rtg2 (a specific subunit of SLIK). (**E**) Immunoblotting analysis of H3 acetylation in wild-type and a *TRA1* temperature sensitive strain. Cells were incubated for 30 min at the restrictive temperature (40°C), then harvested after another 1 h at the same temperature in the presence or absence of added glucose. (**F**) Immunoblotting analysis of H3 acetylation in wild-type and mutant strains lacking *CHD1* in a *TRA1* or *tra1-2* background. The *chd1Δ* single mutant was fed at room temperature and harvested at 1 h. The double mutant was fed after 30 min at the restrictive temperature (40°C) and harvested 1 h later. In (C–F), 'H3 Kac' denotes acetylated H3 detected using an anti-acetyl lysine antibody.

One could argue, because *esa1* and *gcn5* mutants have a lower level of acetylation in SP than wild-type cells and fail to induce acetylation to wild-type levels when fed glucose (Figure 2B and D), that induction of acetylation in SP depends on Esa1/Gcn5 solely for establishment of a threshold level of acetylation that is permissive for glucose induction by other KATs. We do not favour this scenario because glucose induction of acetylation is normal in a non-KAT mutant that has low acetylation in SP (*rpb1-1*, Supplementary Figure S1F).

Our results do not exclude the possibility that glucose induction of H3/H4 acetylation in SP cells partly involves KATs in addition to Gcn5 and Esa1. Given the evident importance of Gcn5 and Esa1 in this regulation, however (Figure 2B and D), our further work focused on these enzymes.

Glucose induction of histone acetylation by Esa1 and Gcn5 in SP is predominantly due to the untargeted activities of picNuA4 and SAGA

Overall, histone acetylation is a combined readout of targeted (de)acetylation events that are directly coupled to transcriptional reprogramming, and untargeted (de)acetylation events that modulate global acetylation in the absence of recruited KAT/HDAC activities. The KATs responsible for H3/H4 acetylation in response to glucose, Esa1 and Gcn5, both function in targeted and untargeted acetylation, and both exist in multiple complexes that acetylate chromatin (2,25). We used a genetic approach to assess the relative contribution of targeted and untargeted acetylation to induction of overall acetylation, and to identify the KAT complexes responsible for histone acetylation upon glucose refeeding.

Esa1 occurs in one complex (NuA4) which functions in targeted acetylation and another (picNuA4), which is specialized for untargeted acetylation (26). We differentiated the activities of NuA4 and picNuA4 by monitoring the glucose response of a viable mutant that lacks NuA4 activity because its Epl1 subunit is expressed in a C-terminally truncated form (26). epl1(1–485) is present in SP at levels comparable to Epl1 (Figure 3A) and epl1(1–485) cells support robust glucose induction of H4ac (Figure 3B). Therefore, although Esa1 is required for H4 acetylation triggered by glucose (Figure 2B), NuA4 is not. Accordingly, we propose that glucose induction of H4 acetylation in SP is mainly due to untargeted Esa1 activity.

The Esa1-dependent picNuA4 complex likely performs untargeted acetylation of nucleosomal H4 in glucose-fed cells, since it acetylates nucleosomal histones more readily than it does free histones, and it lacks the Tra1 targeting protein (26). Also of note, the cellular yield of picNuA4 increases with culture density (26), and its Esa1, Epl1 and Yng2 subunits are expressed in SP (Figures 2A and 3A, data not shown).

Although Gcn5 can acetylate nucleosomes on its own (27), in yeast this enzyme mostly exists in complex with other proteins (25). Therefore, we predicted that a Gcn5 complex performs glucose-induced H3 acetylation. Our first step in testing this prediction was to comprehensively analyze the site-specificity of Gcn5-dependent H3 tail acetylation in glucose-fed cells. For this experiment, blots were probed with antibodies which exclusively recognize H3 K9ac, H3K14ac, H3K18ac and H3K27ac, as well as an antibody directed against acetyl-lysine (Kac). This experiment revealed that acetylation of H3 K9, 14, 18 and 27 is induced by glucose (Figure 3C, lanes 1, 2). As expected from published studies of the KAT-dependency of H3 tail acetylation in log phase cells (14,22,23), acetylation of H3 K9, K18 and K27, but not K14, strongly depends on Gcn5 (Figure 3C, lanes 3, 4).

Three complexes which contain Gcn5 and can acetylate nucleosomal substrates have been well characterized. These are SAGA, SLIK and ADA. We tested if proteins required for the stability of such complexes are required for H3 acetylation in response to glucose. SAGA and SLIK are not detectable in cells lacking Spt7, *rtg2Δ* cells lack SLIK but not SAGA (28), and the ADA complex is absent in cells lacking Ahc1 (29). We find that glucose induction of Gcn5-dependent H3 acetylation is completely blocked in *spt7Δ* cells, but unaffected in *AHC1* and *RTG2* null mutants (Figure 3C and D). This profile suggests that SAGA is sufficient for glucose induction of H3 K9, K18, K27 acetylation, and that neither ADA nor SLIK on its own is 'necessary and sufficient' for the response. Deletion of *SPT8*, which interferes with many known functions of SAGA (28), does not block glucose induction of H3 acetylation (Figure 3D). Induction of acetylation therefore is either separable from Spt8-dependent functions of SAGA, or can also be mediated by SLIK. A straightforward and parsimonious interpretation of these results is that SAGA is sufficient for glucose-induction of H3 acetylation by Gcn5. An alternative possibility is that an unknown SAGA-like complex mediates untargeted H3 acetylation in glucose-fed cells. We do not favor this possibility because intensive characterization of Gcn5-dependent KATs by multiple independent groups has failed to reveal Spt7-dependent Gcn5 complexes in addition to SAGA/SLIK (25).

We tested if Gcn5 targeting is important for glucose induction of H3 acetylation using strains harboring mutations in known targeting subunits of SAGA/SLIK. These are Tra1, which mediates association of SAGA/SLIK with trans-activators, and the chromodomain protein Chd1 which binds to K4-methylated H3 in nucleosomes [H3 K4me is commonly found in promoter nucleosomes; (28)]. *tra1-2* cells (30) acetylate H3 normally in response to glucose at the restrictive temperature (Figure 3E), as do *chd1Δ* cells and a *tra1-2 chd1Δ* double mutant at the restrictive temperature (Figure 3F). It follows that neither Tra1 nor Chd1 plays a substantial role in overall acetylation of H3 after glucose refeeding in SP. Accordingly we propose that SAGA-dependent H3 acetylation in glucose-fed cells occurs by an untargeted mechanism. Furthermore, since inactivation of Tra1 prior to refeeding does not dampen glucose induction of H3 acetylation (Figure 3E), we consider it unlikely that H3 acetylation is due to passive spreading of targeted H3 KATs from induced genes (24).

We do not exclude glucose induction of targeted Esa1 or Gcn5 activity in SP cells. It is clear from our results, however, that induction of targeted activity makes only a minor contribution to glucose induction of overall Esa1/Gcn5-dependent acetylation in the amino-terminal tails of H3 and H4.

A metabolic mechanism for glucose induction of global histone acetylation

Two distinct regulatory mechanisms that could contribute to glucose induction of untargeted KATs have been under investigation. Since much cellular regulation depends on signal transduction cascades, we have been testing if signaling previously implicated in gene induction by glucose contributes to the control of untargeted KAT activity. This work is ongoing (so far we have shown that individually compromising the protein kinase A and target of rapamycin signal transduction pathways has little effect on glucose induction of overall histone acetylation in SP; Figures S6, S7 in Supplementary Material). Based on the idea that increased synthesis of acetyl-CoA, an essential co-substrate of KATs, could stimulate acetylation, we have also tested if glucose metabolism is necessary for upregulation of overall histone acetylation by glucose. This idea builds on the following published results. First, artificial depletion of nuclear acetyl-CoA in log phase cells causes overall histone acetylation to drop precipitously (31). Second, acetyl-CoA essentially disappears from cells in early SP (32). And finally, cells that have depleted glucose from the medium (1 day culture) can synthesize acetate and (by inference) ATP when fed glucose (33). As noted by Takahashi *et al.* (31), Acs1 and Acs2 receive substrates from glycolytic metabolism. Since glycolytic activity can fluctuate during the life history of a cell, physiological variations in histone acetylation might be regulated by changes in the availability of acetyl-CoA, which result directly from changes in glycolytic rate. Accordingly, we envisioned that acetate and/or ATP resulting from glucose metabolism would be used by acetyl-CoA synthases to increase the availability of acetyl-CoA for histone acetylation by untargeted KATs [Figure 4A, adapted from ref. (34)].

Our first step in testing this hypothesis was to determine if glucose-fed SP cells produce the precursors for acetyl-CoA synthesis. Acid soluble intracellular metabolites were obtained from clarified cell lysates. Targeted metabolic profiling of these samples was performed by ^{1}H NMR spectroscopy (35). In this quantitative method, NMR spectra of purified compounds at known concentrations

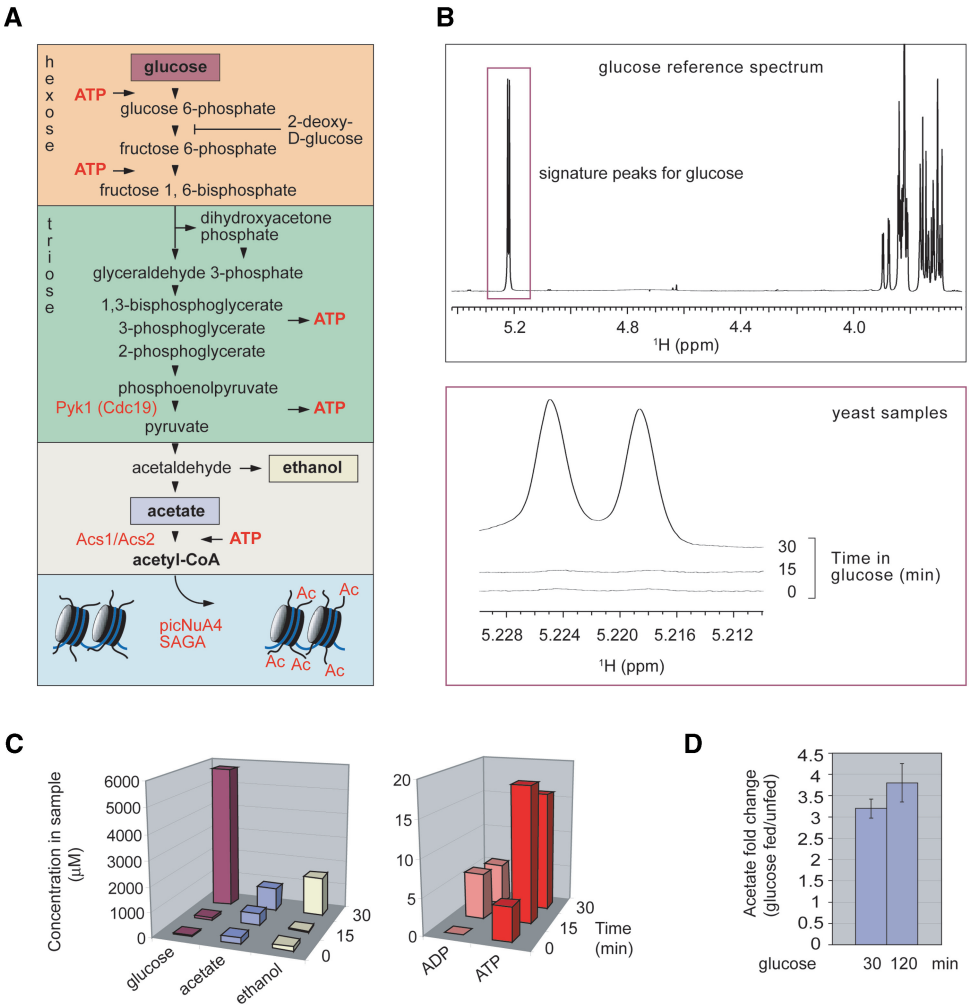

Figure 4. Metabolite profiles in unfed and glucose-fed SP cells. (**A**) Relationship of glycolysis to acetyl-CoA production for use by nuclear KATs. (**B**) ^{1}H-NMR spectra of a glucose reference sample (top panel) and samples of metabolites isolated from SP cells (bottom panel; only glucose peaks are shown). (**C**) Targeted quantitative profiling of cellular metabolite levels by ^{1}H-NMR, before and after glucose refeeding. Identical volumes were analyzed from samples prepared from the same number of cells and resuspended in the same final volume. The results are the average of two independent experiments. (**D**) Cellular level of acetate in glucose-fed cells relative to the level in unfed cells (average of three independent experiments ± SD). Acetate was measured by an enzymatic assay.

are compared to spectra collected from cell samples. Representative spectra for glucose in one refeeding experiment are shown in Figure 4B. As expected the concentration of glucose is very low in unfed cells, and rises sharply after refeeding (Figure 4B; the upper panel includes the reference signature peaks for glucose; the lower panel shows glucose in cells). These changes occur in concert with glucose depletion from the medium (Figure 1B). Increases in cellular ADP, ATP, acetate and ethanol concentrations accompany glucose uptake (Figure 4C). An enzymatic assay confirmed acetate production in glucose-fed SP cells (Figure 4D). We conclude that glucose feeding in SP results in new synthesis of ATP and acetate, the metabolites required for acetyl-CoA production.

The acetyl-CoA used by nuclear KATs (and some other enzymes) is synthesized by acetyl-CoA synthase Acs1 and/or Acs2 (31). If glucose induction of acetylation in SP depends on increased synthesis of acetyl-CoA for use by KATs, then Acs1 and/or Acs2 should be expressed in SP, and necessary for glucose induction of acetylation. Figure 5A shows that Acs1 and Acs2 are both present in SP cells. Acs1 expression in SP is likely due, at least in part, to derepression of *ACS1* transcription under conditions of glucose limitation (36). Note that although glucose refeeding might inhibit *ACS1* transcription in SP, Acs1 protein expression remains high during the period when histones are acetylated (Figure 5A), and enzyme activity declines only slowly under conditions that repress *ACS1* transcription (37). Therefore, acetyl-CoA synthesis in SP cells treated with glucose could result from the activity of both Acs1 and Acs2.

We tested the dependence of acetylation on acetyl-CoA synthase activity using strains that harbor a deletion allele of *ACS1*, a temperature sensitive allele of *ACS2* (*acs2-Ts1*), or both (31). Figure 5B shows that H3 K9 and H4 acetylation decline in SP when Acs1 is absent and acs2-Ts1 is thermally inactivated; therefore maintenance of acetylation in SP requires acetyl-CoA synthesis

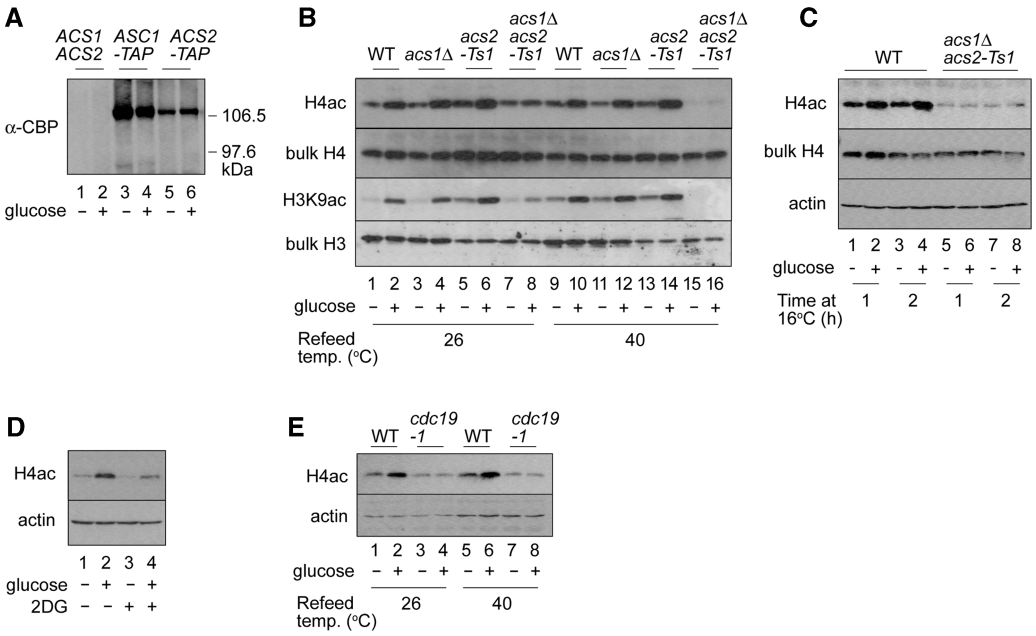

Figure 5. Metabolic regulation of global histone acetylation by glucose. (**A**) Expression of TAP-tagged Acs1 and Acs2 in unfed and fed cells monitored by anti-CBP immunoblotting. Glucose-fed cells were harvested at 1 h. (**B**) Immunoblotting analysis of H3 K9 and H4 acetylation in wild-type and *ACS1*/*ACS2* mutant strains. Cells were unfed or glucose-fed (1 h), at either the permissive (26°C) or restrictive (40°C) temperature. (**C**) Immunoblotting analysis of H4 acetylation in wild-type and *acs1Δ acs2-Ts1* mutant strains shifted from room temperature to 16°C at the time of glucose refeeding. (**D**) Immunoblotting analysis of H4 acetylation in wild-type cells fed 2% glucose, 2% 2-deoxy-D-glucose (2DG), or a mixture of both sugars at 1% each. (**E**) Immunoblotting analysis of H4 acetylation in wild-type and a *PYK1* (*CDC19*) temperature sensitive mutant strain. Cells were unfed or glucose-fed (1 h), at either the permissive (26°C) or restrictive (40°C) temperature.

(by either enzyme). At the permissive temperature the *acs1Δ* and *acs2-Ts1* mutations in combination have no effect on maintenance acetylation, but glucose induction of H4ac is consistently dampened (Figure 5B, lanes 7, 8). If acs2-Ts1 protein is partly defective for acetyl-CoA production at room temperature, then the blunted response in the double mutant might reflect the dependence of glucose induction of acetylation on increased metabolic flux through the ACS enzymes. It follows that the difference in induction of histone acetylation between mutant and wild-type cells could be accentuated at lower temperatures, where reaction rates are slowed. Consistent with this prediction, at 16°C induction of H4ac by glucose is robust in wild-type cells but is not evident in *acs1Δ acs2-Ts1* cells even at 2 h after refeeding (Figure 5C; note that both strains are viable at 16°C). We conclude that ACS activity is essential for glucose induction of acetylation. From a broader perspective, we hypothesize that increased glycolysis provides the metabolites necessary for increased flux through acetyl-CoA synthases Acs1 and Acs2, which in turn expands an acetyl-CoA pool that is used by untargeted KATs for histone acetylation.

A strong prediction of this hypothesis is that induction of histone acetylation requires execution of all the steps of glycolysis. Consistent with this prediction, the glucose analogue 2-deoxy-D-Glucose (2DG), which is not metabolized after being phosphorylated in the first step of glycolysis (Figure 4A), does not induce histone acetylation (Figure 5D). Feeding a 1:1 mixture of glucose and 2DG dampens the response in comparison to feeding of glucose alone (Figure 5D), probably because 2DG phosphorylation consumes ATP and blocks ATP production by glycolysis, thereby reducing ATP availability for acetyl-CoA synthesis.

To further test if glucose catabolism is important for glucose-stimulated histone acetylation, we analyzed glucose regulation of H4ac in a strain defective in pyruvate kinase 1 (Pyk1, encoded by *CDC19*), which catalyzes the final step of glycolysis (Figure 4A, phosphoenolpyruvate → pyruvate). Note that Pyk1 inactivation does not deplete pre-existing ATP stores because ATP consumption during the hexose phase of glycolysis is balanced by its production in the subsequent steps leading to phosphoenolpyruvate synthesis (Figure 4A). As shown in Figure 5E (lanes 1, 3, 5, 7), unfed wild-type and *PYK1* mutant cells (38) have the same level of acetylation at the permissive and restrictive temperatures. The mutant, however, does not support induction of H4ac at either temperature (Figure 5E, lanes 1, 2 compared to 3, 4; 5, 6 compared to 7, 8). We conclude that flux through Pyk1 allows for both net ATP generation by glycolysis and production of pyruvate which is used to form acetate that is required for acetyl-CoA synthesis. Glucose induction of histone acetylation in SP is thus critically dependent on its metabolism to produce the acetyl-CoA used by untargeted KATs.

DISCUSSION

A major ongoing effort in biology is focused on determining if there are causal links between physiological fluctuations in metabolism and the activity of other biochemical

modules of cell function (39). Such links between carbon metabolism and NAD^+-dependent HDACs are now well established (40). Here, we provide the first evidence that physiological regulation of acetylation can also depend on direct metabolic control of KAT activity.

Glucose induction of untargeted KAT activity

Glucose induction of overall H3 K9, 18, 27 and H4 K5, 8, 12 acetylation in SP cells depends mainly on the activity of Gcn5 and Esa1, respectively. How does glucose upregulate Gcn5-/Esa1-dependent histone acetylation in SP cells? As part of our effort to answer this question, we assessed the relative contribution of targeted and untargeted KAT activity to the stimulation of acetylation. Given that hundreds of mRNAs are induced when SP cells are refed glucose (13), targeted KAT activity associated with induction of glucose-regulated genes was expected to account for most of the increase in acetylation triggered by glucose. Surprisingly, this is not the case: Esa1-dependent acetylation of H4 can largely be attributed to picNuA4, which is specialized for untargeted acetylation (26), and Gcn5-dependent H3 acetylation by SAGA (or a closely related complex) does not require SAGA subunits that are known or likely to target it to genes in the course of transcriptional activation (28,30).

A role for SAGA in untargeted acetylation might seem surprising in view of its well-established function in locus-specific histone modification. It is noteworthy, however, that all Gcn5 complexes including SAGA readily acetylate chromatin by an untargeted mechanism in vitro (21,41). Therefore, our evidence for untargeted acetylation by SAGA *in vivo* is consistent with its demonstrated substrate specificity.

Generally speaking our finding that the acetylation state of H3 and H4 in SP cells is largely controlled by untargeted KATs is consistent with published evidence that untargeted KATs have pervasive effects on chromatin acetylation in log phase (42–46).

Untargeted KAT activity is controlled by glycolytic metabolism

Our further analysis of glucose regulation of Gcn5 and Esa1 activity revealed a previously unknown physiological mechanism by which histone acetylation is modulated in vivo. That is, induction of KAT activity by glucose refeeding in SP depends on increased availability of acetyl-CoA. We base this conclusion on several key observations. The steady state concentration of metabolites required for acetyl-CoA synthesis—acetate and ATP—increases in SP cells within 15 min of glucose feeding. These metabolites are likely used to produce the acetyl-CoA required for histone acetylation, as induction of acetylation depends on enzymes that require acetyl-CoA (the KATs Esa1 and Gcn5) and the enzymes that generate acetyl-CoA (Acs1 or Acs2). Because net production of ATP by glycolysis, and glucose induction of acetate, both require the last step of glycolysis (phosphoenolpyruvate → pyruvate), we propose that glucose-fed SP cells must execute the entire glycolytic program in order to produce acetyl-CoA for use by KATs. This proposal is supported by the finding that inhibition of Pyk1, which catalyzes the final reaction of glycolysis, strongly dampens glucose induction of H3/H4 acetylation. Mitochondria could provide acetate or acetyl-CoA for use by nucleocytosolic acetyl-CoA synthases and nuclear KATs (respectively). However such pathways (if they exist) do not make a major contribution to maintenance histone acetylation in log phase (31), and therefore probably have only a minor role in glucose induction of H3/H4 acetylation in SP.

Since pulse-feeding of glucose causes a transient increase in acetylation, and the duration of peak acetylation depends on the amount of glucose provided to the cells (Figure 1D), glucose may be a rheostat-like controller of KAT activity. In this model, histones are deacetylated at late time points after glucose repletion because the balance between KAT and HDAC activity gradually shifts in favor of HDACs as the KAT-inducing signal from glucose decays.

Glucose-induction of overall H3/H4 acetylation depends mainly on the untargeted activities of Gcn5 and Esa1 (in SAGA and picNuA4, respectively), and glucose catabolism is required for glucose-induction of overall H3/H4 acetylation; it follows that glucose catabolism controls untargeted KAT activity. This finding has several important implications for our understanding of chromatin regulation during entry into and exit from quiescence. In view of the evidence that acetyl-CoA depletion is an early metabolic event in the transition into SP (32), it helps to explain why overall H4 acetylation declines during SP entry (4,5) despite induction of picNuA4 at this time (26). Specifically we propose that protein induction of picNuA4 is compatible with overall H4 deacetylation because cells entering SP do not make enough acetyl-CoA to support increased picNuA4 activity. By extension, it is plausible that metabolic regulation of KAT activity is a mechanism for modulating the acetylation state of chromatin even in cells in which nuclear KATs are otherwise fully activated.

Glucose triggers systems-wide physiological reprogramming in SP cells: many characteristics of quiescence are lost, hundreds of mRNAs are induced, and events that promote budding are initiated (6,13,47,48). Induction of global histone acetylation accompanies these changes (this study), and like them, is a hallmark of re-entry into a proliferative state. Generally speaking this regulation would be beneficial from a fitness perspective in yeast because it intrinsically couples the process of overall chromatin 'activation' to competence for metabolic activity that is associated with a high rate of proliferation. Targeted KATs might also be directly regulated by glycolytic metabolism, although in SP cells this regulation does not make a substantial contribution to changes in acetylation detected by immunoblotting.

In order to characterize the contribution of untargeted acetylation to glucose regulation of specific chromatin-dependent biochemical activities, it would be optimal to obtain mutants in which untargeted activity is compromised. Using such mutants in glucose refeeding experiments, for example, one could determine how the absence of untargeted KAT activity affects the kinetics of gene

induction. Although we are attempting to identify untargeted KAT mutants, none has yet been obtained.

In the meantime, we speculate that direct glucose induction of untargeted acetylation in SP cells contributes to transcriptional activation. We favor this possibility based on recent work in yeast. Using an activator bypass approach, Imoberdorf *et al*. (42) showed that high levels of untargeted H3 acetylation allow robust transcription in the context of weak recruitment of the basal transcription machinery. Their work also suggests that the strength of interaction between an activator and its target in the transcriptional machinery might determine the extent to which induction of natural promoters depends on untargeted acetylation. We predict that glycolytic induction of untargeted acetylation in SP cells helps to overcome the gene induction barrier presented by low global acetylation in G0 (4,5). When SP cells are refed all the nutrients required for proliferation (glucose, amino acids, vitamins, etc.), the full spectrum of genes that must be induced for a balanced physiological response to the growth stimulus will benefit from chromatin opening triggered by glucose.

Beyond yeast

Untargeted KAT activity almost certainly contributes to chromatin acetylation in higher organisms (15). The HBO1 and PCAF/STAGA/TFTC complexes of human are respectively equivalent to picNuA4 and SAGA of yeast (25). HBO1, like picNuA4, is specialized for untargeted acetylation of nucleosomal H4 (49), and the human SAGA-related enzymes are capable of untargeted acetylation of nucleosomal H3 *in vitro* (50–52)

What we have learned in yeast suggests that direct metabolic regulation of untargeted KATs probably manifests itself in cells which depend heavily on glycolysis for their metabolic needs. Such cells include T cells and cancer cells. T cells, like yeast, can be triggered to re-enter proliferation from a non-dividing state in which metabolism is relatively low. Intriguingly, this T cell 'activation' requires induction of glycolytic metabolism and (at least *in vitro*) is associated with replication-independent induction of overall H4 tail acetylation (7,53). We speculate induction of histone acetylation in T cells partly involves glycolytic upregulation of untargeted KAT activity.

Many cancer cell types are specialized for energy generation by glycolysis (54). Cancer cells likely experience substantial fluctuations in glucose availability during vascular remodeling within solid tumors and during egress from poorly vascularized tumors (55). Perhaps fluctuations in glycolysis accompany such changes in access to glucose and directly modulate untargeted KAT activity, thereby affecting transcriptional reprogramming that promotes cancer cell survival. What makes this scenario attractive is the evidence that the glycolytic phenotype of cancer cells, and tumor cell growth *in vivo*, depend on expression of pyruvate kinase isoform PKM2 (56). PKM2 is the human equivalent of yeast Pyk1, the glycolytic enzyme required for glucose induction of global acetylation in G0.

SUPPLEMENTARY DATA

Supplementary data are available at NAR Online.

ACKNOWLEDGEMENTS

Signy Chow and Karen Robinson are thanked for the experiment in Figure S8A. We thank Dave Stuart for access to a Coulter counter, and Dean Zaragoza and Olivier Julien for computing and technical assistance. Lorraine Pillus, Margaret Werner-Washburne, Jacques Côté, Jeff Boeke and Jerry Workman kindly provided strains. Dave Stuart, Margaret Werner-Washburne, Jacques Côté, Alain Verreault, Jerry Workman and Bernie Lemire are thanked for helpful discussions.

FUNDING

Alberta Heritage Foundation for Medical Research (to M.C.S., S.N.R.); Canadian Institutes of Health Research (to B.D.S., M.CS.); Canadian Institutes of Health Research to B. Lemire (co-supervisor of S.N.R.). Funding for open access charge: Canadian Institutes of Health Research.

Conflict of interest statement. None declared.

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