Carbohydrates Induce Mono-ubiquitination of H2B in Yeast

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Histone modifications have emerged to be a major regulatory mechanism for gene expression (1–4). However, it is not clear how histone modifications are physiologically regulated. Here, we show that mono-ubiquitinated H2B at lysine 123 (uH2B) in the yeast (Saccharomyces cerevisiae) is present in exponential phase and absent in stationary phase. A wide array of carbohydrates or sugars, including glucose, fructose, mannose, and sucrose, are capable of inducing uH2B in stationary phase yeast. In contrast, non-metabolic glucose analogs are defective in inducing uH2B. Furthermore, uH2B induction is inhibited by iodoacetate, an inhibitor of glyceroldehyde-3-phosphate dehydrogenase in glycolysis. Moreover, uH2B induction is markedly impaired in yeast mutants, in which glycolytic genes are deleted. These data indicate that glycolysis is required for the carbohydrate-induced mono-ubiquitination of H2B at lysine 123. Therefore, our study reveals a novel paradigm of metabolic regulation of histone modifications.

Chromatin undergoes diverse post-translational modifications such as methylation, acetylation, phosphorylation, and ubiquitination, which play an important role in gene expression (1–4). Histone H2A is the first protein found to be post-translationally modified by covalent ligation to ubiquitin (5, 6). Ubiquitinated histones are the most abundant ubiquitin conjugates in higher eukaryotes (7). In the budding yeast (Saccharomyces cerevisiae), histone H2B is mono-ubiquitinated at lysine 123 (uH2B) (8), which is required for H3 methylation at lysines 4 and 79, a phenomenon termed trans-regulation of histone modification (9–11). uH2B is mediated by Bre-1, a ubiquitin E3 ligase, and regulated by Rad6, Lge1, Rtf1, and Ubp5 (8, 12–16). uH2B plays a role in gene silencing in telomeres (9, 17) and in controlling cell size control (12). Despite the potentially important function of uH2B, it is not known how the histone modification is physiologically regulated. Here, we found that carbohydrates were potent inducers of mono-ubiquitination of H2B. We further showed that mono-ubiquitination of H2B required glycolysis, the central metabolic pathway of carbohydrates.

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EXPERIMENTAL PROCEDURES

Yeast Strains and DNA Constructs—Yeast strain Y96 (MATa ura3-52 lys2-801 ade2-101 his3-11,15 leu2-3,112 trp1) was derived from YPH500 (Stratagene) as described before (18). Y208 was the same as Y96 except HTA1::hkb1 (K123R), Y119 (hkb2); URA3::htb1::KANMX, Y127 (cys1::HIS5), and Y105 (gpd1::HIS5), and Y104 (his3::LEU2 pht1::A H3::HIS5) were derived from Y97 and constructed by Cre-mediated gene knockout method (19). Y218 (PPK1 Pfk2 HTA1::HTB1 (CEN URA3)) was derived from BY4733 (Research Genetics). Y206 was Y119 with FLAG-BRE1 (CEN TRP). Y97 was Y96 with CRE1 (CEN TRP1) (19). Y98 (hkb22), Y101 (snf2a::HIS5), Y102 (gpd1::HIS5), Y103 (gpd1::HIS5), and Y104 (his3::LEU2 pht1::A H3::HIS5) were derived from Y97 and constructed by Cre-mediated gene knockout method (19).

BRE1 with its endogenous promoter region was cloned by PCR from yeast genomic DNA and cloned to SmaI site of pRS414. Subsequently, N-terminal FLAG-BRE1 was generated by site-directed mutagenesis (Stratagene) and sequence verified. All the N-terminal FLAG-tagged H2B constructs were generated either by site-directed mutagenesis (Stratagene) or generously provided by M. A. Ooley.

Western Blot Analysis and Glucose Assay—Whole-cell extracts from normal culture or cells were prepared, analyzed on 12 or 7.5% SDS-polyacrylamide gels, and Western-blotted with anti-FLAG antibodies or anti-His antibodies. The proteins were visualized by chemiluminescence.

RESULTS AND DISCUSSION

We made a serendipitous observation that uH2B was not detected in yeast Y96 with FLAG-tagged H2B as the only copy of H2B in water (Fig. 1A, lane 4). We further found that uH2B was also present in stationary phase Y96 that was cultured in YPD (1% yeast extract, 2% peptone, and 2% glucose) for 7 days (Fig. 1A, lane 3). In striking contrast, significant amount of uH2B was not present in exponential phase Y96 (e.g. A600 = 1) in YPD (Fig. 1A, compare lanes 1, 3, and 4). These initial observations indicated that the induction of uH2B was subjected to environmental and/or nutritional regulation.

Stationary phase yeast is in a non-proliferating or G0 state, resulting from nutrient depletion (20). Because uH2B was not detected in stationary phase yeast, we then sought to explore whether addition of nutrients to stationary phase yeast could induce uH2B. To do so, we collected stationary phase Y96, washed it with water, and resuspended it with 5 ml of the following solutions in separation or in combination (A600 = 1): 2% glucose, nitrogen base, ammonium sulfate, and amino acid mixture. After incubating the yeast at 30 °C for 1 h, we prepared whole cell extracts for assaying the presence of uH2B. We found that glucose alone was capable of inducing uH2B (Fig. 1A, lane 11). In contrast, amino acids, nitrogen base, and ammonium sulfate were defective in inducing uH2B (Fig. 1A, lanes 7 and 12). In addition, uH2B could be readily induced by a range of glucose concentrations (0.05 to 2% tested) within a few minutes of incubation in Y96 (data not shown). The data demonstrate that glucose is an inducer of uH2B.

There are four stages of yeast growth on the glucose-based medium YPD: exponential phase during which glucose is present, diauxic shift when glucose is depleted, post-diauxic shift when yeast growth depends on respiration, and stationary
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Fig. 1. Glucose induces ubiquitination of H2B at Lys123. A, Y96 whole cell extracts (WCEs) prepared from equal number of cells from different conditions were analyzed for mono-ubiquitination by Western blot using monoclonal antibody against the FLAG epitope (M2). uH2B phase when nutrient is depleted (20). To examine the presence of uH2B in the four stages of yeast growth, we inoculated Y96 in YPD and measured glucose levels in the medium, cell density, and uH2B levels at various time points. We found that uH2B was significantly present in exponential phase ($\lambda_{600}$ was less than 12) (Fig. 1B, lanes 3–5; Fig. 1C). However, only less than 4% of the original bulk uH2B was present in post-diauxic shift ($\lambda_{600}$ was from about 17 to 41) (Fig. 1B, lanes 6 and 7; Fig. 1C). Moreover, uH2B was not detectable in stationary phase (Fig. 1B, lanes 8 and 9; Fig. 1C). Therefore, the presence of uH2B closely correlated with the presence of glucose (Fig. 1C), further suggesting that glucose is an inducer of uH2B.

Genetic studies indicate that mono-ubiquitination of H2B is catalyzed in vivo by Bre-1, a ubiquitin E3 ligase (12, 13). Because uH2B was not detected in stationary phase, we sought to determine whether Bre1 was expressed in stationary phase. To assess Bre1 expression, we first tagged chromosomal BRE1 with green fluorescence protein to be catalytically inactive (data not shown). Therefore, we generated a bre1Δ mutant (Y119, derived from BY4733 strain) and introduced the bre1Δ mutant (Y119, derived from BY4733 strain) and introduced the bre1Δ mutant with pRS414-(2x) FLAG-Bre1 (CEN TRP) under the control of BRE1 endogenous promoter (Y206) and cultured it in YPD until stationary phase was reached. pRS414-(2x) FLAG-Bre1 directed expression of FLAG-Bre1, in which tandem FLAG epitopes were tagged after its start methionine. We directed expression of FLAG-FLAG-Bre1, in which tandem FLAG epitopes were tagged after its start methionine. We found that FLAG-FLAG-Bre1 protein was present throughout the four growth stages, whereas the presence of uH2B was mainly in exponential phase (Fig. 1D) as described earlier. These data suggest that the Bre1 enzymatic activity on H2B in is the slower migrating, mono-ubiquitinated species of histone H2B. Lanes 1 and 2 are the WCEs of isogenic strains Y96 (WT, FLAG-H2B) and Y208 (FLAG-H2B-K123R) ($\lambda_{600}$ = 1, exponential phase). Lane 3 is the WCE of 7-day-old Y96 (stationary phase). Lane 4 is the WCE of Y96 that was cultured in YPD (exponential phase) and subsequently incubated in water for several hours. Lanes 5–12 are the WCEs of 7-day-old Y96 culture (stationary phase) that was washed with water, resuspended with indicated nutrients, and incubated at 30 °C for 1 h. The concentration of nitrogen base (NB), ammonia sulfate (AS), amino acid mixture (AA), and Glu (2% glucose) was identical to those in yeast synthetic complete medium. B, yeast WCEs from various growth stages (lanes 3–9, normalized by cell numbers) from one of the triplicate experiments. C, uH2B mostly was present in exponential phase, significantly decreased in post-diauxic shift, and was not detected in stationary phase. Data for glucose levels and optical density are the mean values ± S.E. (n = 3). D, Bre1 and uH2B levels were examined at 6, 9, 24, and 72 h from normalized number of cells after Y206 was inoculated in SC-Trp2% glucose media ($\lambda_{600}$ = 1) as described. The asterisk denotes an unknown protein that cross-reacts with the FLAG antibody.

Fig. 2. uH2B can be induced in snf1, snf2, gpr1, and hxk2 glb1 isogenic strains. Y96 (WT), Y208 (bre1::K123R), Y97 (WT), Y98 (hxk2Δ), Y99 (snf1Δ::HIS5), Y101 (snf2Δ::HIS5), Y102 (gpr1Δ::HIS5), Y103 (gpr1Δ::HIS5), and Y104 (hxk2Δ::loxP-glh1::HIS5) were cultured in YPD (A$_{600}$ = 2). uH2B was analyzed as described in the legend to Fig. 1.
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FIG. 3. Glycolysis is required for carbohydrate-induced ubiquitination of H2B. A, ubiquitination of H2B is induced by a variety of carbohydrates except non-metabolic glucose analogs. Stationary phase Y96 was collected, washed with water, and resuspended in 2% indicated carbohydrates (A$_{600} = 2$) and incubated at 30 °C for 6 h. B, induction of uH2B is inhibited by IAA. Stationary phase Y96 was diluted with fresh YPD (2% glucose) containing various amount of IAA (A$_{600} = 2$) and further incubated at 30 °C for 30 min. C, Stationary phase Y96 was diluted with fresh YPD (2% glucose) (A$_{600} = 2$) containing various amount of rotenone and further incubated at 30 °C for 1 h. D, stationary phase isogenic strains Y218 (WT), Y219 (pfk1), and Y223 (pfk1 pfk2) (lanes 3, 5, and 7) were collected, resuspended in YPD, and incubated at 30 °C for 3 h (lanes 4, 6, and 8). E, stationary phase isogenic strains Y230 (WT), Y237 (cyt1), and Y238 (cox9) were collected and resuspended in YPD and incubated at 30 °C for 3 h. uH2B was analyzed as in Fig. 1.

vivo is down-regulated in post-diauxic shift and stationary phase. Alternatively, expression and/or activity of positive regulators for mono-ubiquitination of H2B such as Rad6, Lge1, or Rgt1 (8, 12, 14, 15) or negative regulators such as ubiquitin protease Upb8 (16) may be subjected to the carbohydrate regulation.

Glucose is a major carbon and energy source for yeast. There are a number of known glucose sensors monitoring the presence of glucose, which include hexokinase II, Snf3, Snf1, Rgt2, Gpr1, and glucokinase I (21–26). Therefore, we sought to explore whether individual glucose sensors play an essential role in inducing uH2B. To test this, we generated isogenic strains of Y96, namely, hxk2Δ, snf1Δ, snf3Δ, gpr1Δ, and hxk2Δ glk1Δ mutants, in which the entire open reading frames of these genes were deleted. We cultured them in YPD until A$_{600}$ reached about 2, and prepared whole cell extracts for analyzing the presence of uH2B as described. We found that uH2B was present in all the mutant strains (Fig. 2). These data demonstrate that these sensors do not play an essential role in inducing uH2B.

In addition to glucose, yeast can also utilize a variety of carbohydrates as its carbon and energy sources. Therefore, we sought to test whether other carbohydrates could also induce uH2B. To do so, we collected stationary phase Y96, washed it with water, resuspended it with 2% various carbohydrates (A$_{600} = 2$), and incubated these cultures at 30 °C for 6 h. We found that mannose, fructose, and sucrose were also very potent in inducing uH2B when added to stationary phase yeast (Fig. 3A, lanes 5–8). Raffinose and maltose could also induce uH2B (Fig. 3A, lanes 12 and 13). These data indicate that uH2B can be induced by a wide array of carbohydrates.

In contrast, l-glucose, 3-O-methyl-d-glucopyranose, and 2-deoxyglucose, non-metabolic glucose analogs, were defective in inducing uH2B (Fig. 3A, lanes 9–11). l-Glucose is not transported by the yeast (21). 3-O-Methyl-d-glucopyranose is transported; however, it is not phosphorylated at the C-6 position (21). 2-Deoxyglucose is transported and phosphorylated at the C-6 position but is not further phosphorylated at the C-1 position (21), which is a key step for the entry of glycolysis. Therefore, our data suggest that glucose-mediated mono-ubiquitination of H2B requires glucose metabolism.

These observations led us to explore whether glycolysis, the central carbohydrate metabolic pathway (27), plays a role in inducing uH2B. We first examined whether iodoacetate (IAA), 1 an inhibitor of glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase step of glycolysis (28), could inhibit the induction of uH2B. We collected stationary phase Y96 and resuspended it with fresh YPD containing various amount of IAA (A$_{600} = 2$) and further incubated it at 30 °C for 30 min. We found that glucose-induced uH2B levels were markedly reduced in the presence of IAA in a dose-dependent manner (Fig. 3B). In contrast, rotenone, an inhibitor of respiratory chain (29), had no effect on inducing uH2B (Fig. 3C). These data suggest that glycolysis, but not respiration, is required for carbohydrate-induced ubiquitination of H2B.

To further examine the role of glycolysis on carbohydrate-induced uH2B, we examined the presence of uH2B in glycolytic mutants, in which genes for the entry of glycolysis are deleted. The first irreversible step for glycolysis is the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, catalyzed by 6-phosphofructokinase activity encoded by PFK1 or PFK2 (30). Deletion of both PFK1 and PFK2 genes abolishes glycolysis (31). To test the effect of glycolysis on the induction of uH2B, we cultured isogenic strains Y218 (WT), Y219 (pfk1), and Y223 (pfk1 pfk2) in SC-U/GE (1% glycerol and 3% ethanol) for 3 days. uH2B was not present in the WT, pfk1, and pfk1 pfk2 strains before YPD was added (Fig. 3D, lanes 3, 5, and 7). After resuspending the WT, pfk1, and pfk1 pfk2 strains in YPD (A$_{600} = 2$) and incubating them at 30 °C for 3 h, we found that uH2B was induced in the WT strain as expected (Fig. 3D, lane 8). However, the induction of uH2B was impaired in the pfk1 mutant (Fig. 3D, compare lanes 4 and 6). Furthermore, the induction of uH2B was abolished in the pfk1 pfk2 mutant (Fig. 3D, compare lanes 4, 6, and 8). In contrast, deletion of CYT1 or COX9 (Y237 or Y238), which encodes essential respiration protein cytochrome c$_1$ or cytochrome c oxidase subunit VIIA, respectively, had no effect on uH2B (Fig. 3E). These data further validate our model that glycolysis is required for the carbohydrate-induced mono-ubiquitination of H2B.

Mono-ubiquitination of H2B at Lys$^{123}$ is implicated in yeast telomeric silencing (9). Consistent with the gene-silencing role, our comparative genome-wide expression data showed that about 70 metabolic genes, which are normally repressed in exponential phase and up-regulated in post-diauxic shift and stationary phase, were up-regulated in a h2b-K123R strain when it was in exponential phase in YPD. 2 Taken together, our data suggest that uH2B might function as a histone marker for the carbohydrate metabolism or carbon source in yeast.

In summary, we have found that carbohydrates are potent

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1 The abbreviations used are: IAA, iodoacetate; WCE, whole-cell extract.

2 L. Dong and C. W. Xu, unpublished data.
Inducers for mono-ubiquitination of histone H2B in yeast. We have further discovered that the induction of mono-ubiquitinated H2B requires glycolysis, the central carbohydrate metabolic pathway. To our knowledge, this study is the first demonstration of novel metabolic regulation of histone modifications by sugars or carbohydrates.

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