Histone 2B (H2B) Expression Is Confined to a Proper NAD\(^{+}\)/NADH Redox Status*

Received for publication, June 4, 2008, and in revised form, August 4, 2008. Published, JBC Papers in Press, August 5, 2008, DOI 10.1074/jbc.M804307200

Ru-Ping Dai1,2, Fa-Xing Yu1, Shuang-Ru Goh3, Hsiao-Wee Chng4, Ya-Li Tan1, Jian-Lin Fu1, Lei Zheng3,5, and Yan Luo1,4

From the 1Institute of Molecular and Cell Biology, Proteos, Singapore 138673, Singapore and the 4Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21230

S-phase transcription of the histone 2B (H2B) gene is dependent on Octamer-binding factor 1 (Oct-1) and Oct-1 Co-Activator in S-phase (OCA-S), a protein complex comprising glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase (p38/GAPDH and p36/LDH) along with other components. H2B transcription in vitro is modulated by NAD(H). This potentially links the cellular redox status to histone expression. Here, we show that H2B transcription requires a proper NAD\(^{+}\)/NADH redox status in vitro and in vivo. Therefore, perturbing a properly balanced redox upsets H2B transcription. A redox-modulated direct p38/GAPDH-Oct-1 interaction nucleates the occupancy of the H2B promoter by the OCA-S complex, in which p36/LDH plays a critical role in the hierarchical organization of the complex. As for p38/GAPDH, p36/LDH is essential for the OCA-S function in vivo, and OCA-S-associated p36/LDH possesses an LDH enzyme activity that impacts H2B transcription. These studies suggest that the cellular redox status (metabolic states) can directly feedback to gene switching in higher eukaryotes as is commonly observed in prokaryotes.

S-phase progression requires cyclin E/cdk2 signaling, which orchestrates coupled DNA replication and histone expression. This is mediated by N帕T, i.e. nuclear protein, the ataxia-telangiectasia locus, a cyclin E/cdk2 substrate (1–3). Transcription of histone genes is mediated by subtype-specific promoter elements and associated transcription factors and/or co-activators (4–6), and the overall histone expression levels are regulated post-transcriptionally as well (5). In addition, the histone expression is highly coordinated (4) and is tightly coupled to S-phase progression (7).

The transcription of the histone 2B (H2B) gene depends on Octamer-binding factor 1 (Oct-1) and Oct-1 Co-Activator in S-phase (OCA-S), a co-activator complex comprising the glycolytic enzymes p38/GAPDH and p36/LDH among other subunits (4, 5, 8–11). Oct-1 binds the essential octamer site in the H2B promoter throughout the interphase; however, OCA-S occupies the H2B promoter only in the S-phase (10). A direct Oct-1-p38/GAPDH interaction and H2B transcription are modulated by NAD(H) in vitro (10). This implicates a redox-modulated H2B expression in vivo and potentially links the redox status (metabolic states) of the cell to gene switching. Quite common in prokaryotes, such links have been rarely reported in higher eukaryotes (12).

Here, we provide evidence that a proper NAD\(^{+}\)/NADH redox status (ratio) is important for optimal H2B expression both in vitro and in vivo. The direct Oct-1-p38/GAPDH interaction plays a role nucleating the H2B promoter occupancy by OCA-S, which dictates the redox-modulated H2B transcription. p36/LDH is also essential for H2B expression in vivo and plays a critical role in the hierarchical organization of OCA-S. Finally, the OCA-S-associated p36/LDH possesses an intrinsic catalytic activity that exerts an impact upon H2B transcription. Our studies suggest that the nuclear moonlighting transcription functions of metabolic enzymes can be modulated by the cellular redox status, which directly links gene switching to the cellular metabolic states in higher eukaryotes.

EXPERIMENTAL PROCEDURES

Antibodies, Published Materials, and Methods—Antibodies against the OCA-S components were raised with in-house rabbits. The rabbit antibodies against Oct-1, Nnmnt-1, or Namp were from Biovision, Santa Cruz Biotechnologies, and Abcam, respectively. The mouse anti-BrdU monoclonal antibody was from BD Biosciences. Protocols for RNA interference (RNAi) using small interfering (si)RNAs (13), chromatin immunoprecipitation (ChIP), and reverse transcription (RT)-PCR (10), were outlined in corresponding references. J.-Y. Zhao (University of Rochester) provided the H2B promoter-luciferase (fire-
fly) construct (2). The in vitro transcription templates and procedures were as described (14).

The Sequence Information for siRNAs and RT-PCR Primer Pairs—The Duplexes of siRNAs targeting Nmnt-1, Nampt, or p36/LDH and a control siRNA (Random) are: GGCGUCAA-GACACCAUCAA (Nmnt-1 no. 1); ACACCGGACCAAC-AUUCAC (Nmnt-1 no. 2); GGAAGGUAUAUAGGGA (Nampt no. 1); UACCUUACAGGAACUUGUA (Nampt no. 2); GUGCCUAUGAAGUCAUCAC (p36/LDH no. 1); GCUUUAUACGGCGCU (p36/LDH no. 2) and UCAGUGUCAUAGUGUGACAG (Random). The RT-PCR primer pairs are: GCCGACAGGATGCAGAAGGAGATCA and AAGC-ATTTGCCGTTGAAGATGA (β-actin); CAGTGTCAATGC-CAAGGCAAGCGGA and CTGTTTACTTAG CGCTGGTG-TACTTGGTGTA (H2B).

Real-time PCR Assays—Sybr Green Core Reagents (Applied Biosystems) were used in the real-time PCR assays to quantify histone mRNA levels normalized to β-actin mRNA levels.

B Cell Nuclear Extract (NE)-based Transcription Mixture—A B cell NE (in buffer BC-100, 100 mM KCl) was passed through a P11 phosphocellulose column equilibrated in BC-100, and the column washed with BC-100. General transcription factor IIA, purified from the flow-through fraction, was reconstituted with BC-300, BC-500, and BC-1,000 (mM KCl) fractions (10). This procedure removed the pre-existing redox components, resulting in the B cell NE-based transcription mixture containing an intact set of transcription factors that support IgH and H2B transcription and allowed the modulation of the H2B transcription by (low level) exogenous NAD(H) in vitro (Ref. 10 and “Results”).

Immunodepletion—Equilibrated in BC-500, the B cell NE-based transcription mixture was loaded onto a protein A-Sepharose column (mock) or a column coupled with the anti-p38/GAPDH, anti-p36/LDH, or anti-p60/Sti1 antibodies, equilibrated also in BC-500. After re-loading five times, the flow-through was dialyzed to BC-100 prior to assays.

Imobilized H2B Promoter Pull-down Assays—A DNA fragment corresponding to −90 to −17 of the H2B promoter (15) was biotin-labeled and immobilized to streptavidin-agarose beads (100 ng of DNA for 25-μl beads). The beads were made in 50% slurries. Then, 10 μl of the B cell NE-based transcription mixture and 2 μg of poly(dl-dc)-poly(dl-dc), and NAD(H), when appropriate, were mixed in 25 μl and incubated with the immobilized DNA slurries. The beads were then extensively washed, and the bound proteins further analyzed by immunoblot and enzyme assays.

GAPDH and LDH Enzyme Assays—1 μl of the B cell NE-based transcription mixture, or 1 milliliter of GAPDH or LDH, was mixed with streptavidin-agarose beads (25 μl in 50% slurries), as was the H2B promoter-bound OCA-S in the beads (see above). For the LDH enzyme reaction, slurries were mixed with 25 μl of transcription buffer with 2 mM each of NAD+, NADH, and sodium pyruvate, and for the GAPDH enzyme reaction, 2 mM each of NAD+, NADH, glyceraldehyde 3-phosphate and KH₂AsO₄. The generated or consumed NADH levels were recorded on a fluorometer (340 nm excitation, 460 nm emission).

Evaluations of Free Cellular NAD⁺/NADH Ratios (Redox Status) and Total NAD⁺, NADH, or ATP Levels—Free NAD⁺/NADH ratios were scored on the premise that the coenzymes are in equilibrium with real-time pyruvate and lactate levels (16–17). Thus, cells were lysed with 1 m perchloric acid to stop all metabolism and the lysate neutralized for enzymatic assays. A reaction for determining the pyruvate level contained 100 mM imidazole (pH 7.0), 40 μM NADH, 4 units/ml LDH, and a lysis titration. A reaction for determining the lactate level contained a lysis titration, 450 mM glycine (pH 9.5), 200 mM hydrazine sulfate, 2.5 mM EDTA, 120 μM NAD⁺, and 8 units/ml LDH. All reactions were carried out at 25 °C for 30 min. All generated or consumed NADH levels were recorded on a fluorometer (340-nm excitation, 460-nm emission). The total cellular NAD⁺ or NADH levels were determined by established assays (18). Cellular ATP levels were scored by the ATPlite™ Luminescence Assay System from PerkinElmer.

BrdU-FACS Analyses—Cells grown in 6-well plates were treated with BrdU (10 μM) for 45 min before harvesting, fixed with 70% cold ethanol, treated with 3 N HCl, washed, and incubated with the mouse anti-BrdU monoclonal antibody for 30 min. After washing, cells were incubated with the Alexa Fluor® 488 anti-mouse IgG (Invitrogen) for 30 min. Finally, cells were treated with propidium iodide and RNase A for 30 min and subjected to FACS analyses.

Luciferase Assays in Xenopus Oocytes and HeLa Cells—Increasing NAD⁺, p38/GAPDH (or bovine serum albumin), and an H2B promoter-luciferase (firefly) reporter (2) were co-injected into oocyte nuclei (10 nl per nucleus, 1.4 ng of protein). At 40 h, the oocytes were lysed to measure the luciferase activities. To assess the effects of redox perturbations on H2B promoter in vivo, HeLa cells were transfected with the H2B promoter-luciferase reporter, treated with NaN₃ overnight, and then harvested to measure the luciferase activities. In both assays, the internal reference was a Renilla luciferase gene driven by the SV40 promoter. The reference activities were largely constant, which further supports H2B promoter specificity of redox effects (see “Results”).

RESULTS

Biphasic Response of H2B Transcription to Varied NAD⁺/NADH Redox Status—It was earlier shown that in an NE-based transcription system in which the pre-existing redox components were removed, the H2B transcription was stimulated by exogenous NAD⁺ and inhibited by NADH (tested up to 0.4 mM; Ref. 10). The current study used the B cell NE-based transcription mixture, in which the pre-existing redox components were also removed chromatographically (“Experimental Procedures”). We tested a broader NAD⁺ titration and observed a biphasic response: the H2B transcription was stimulated by higher dosages of NAD⁺ at higher dosages (Fig. 1A). NADH always inhibited (Fig. 1B). Mixing 1 mM NAD⁺ with additional NAD⁺ or with NADH repressed the NAD⁺-stimulated H2B transcription (Fig. 1C).

In vivo, (free) NAD⁺/NADH ratios define the redox status, prompting us to study whether a range of NAD⁺/NADH ratios may modulate H2B transcription in vitro. Increasing NAD⁺ on top of 0.4 mM NADH stimulated H2B transcription (up to a 4:1
correlate with the H2B promoter occupancy by OCA-S (through a direct Oct-1-p38/GAPDH interaction; see Fig. 2). With the same set of NAD+/NADH ratios used for in vitro transcription (Fig. 1E), we tested the H2B promoter occupancy by the OCA-S complex in an immobilized H2B promoter pull-down assay. As seen (Fig. 1F), while the recruitment of Oct-1 to the H2B promoter was largely constant, that of the OCA-S components exhibited a biphasic pattern. This is in line with the biphasic H2B transcription pattern (Fig. 1E). Hence, the NAD+/NADH ratio (redox status) may modulate the H2B transcription via regulating the OCA-S recruitment to the H2B promoter through the octamer-bound Oct-1.

p38/GAPDH, microinjected into Xenopus oocytes, was shown to activate an ectopic H2B gene (19). This offers a more physiological assay to examine an NAD+/NADH response for Oct-1-dependent H2B promoter co-activation. We microinjected an H2B promoter-luciferase reporter, p38/GAPDH, and NAD+/NADH into the oocytes, and recapitulated a biphasic luciferase reporter expression in responding to increasing NAD+ (Fig. 1G).

Results in Fig. 1 prompted us to conclude that optimal H2B transcription in vitro requires a proper NAD+/NADH ratio (redox status) and that maximal H2B transcription in Xenopus oocytes is ensured by a proper NAD+ level.

A Nucleating Role of p38/GAPDH for the OCA-S Function—The role(s) of NAD(H) in H2B transcription in vitro is consistent with co-existing p38/GAPDH and p36/LDH in OCA-S (Ref. 10; Fig. 1). p38/GAPDH directly interacts with p38/GAPDH and p36/LDH in OCA-S (Ref. 10; Fig. 1). p38/GAPDH directly interacts with OCA-S and may be central in regulating H2B transcription by sensing the redox changes that modulate an Oct-1-OCA-S interaction (Fig. 1, also see below).

First, we immunodepleted p38/GAPDH from the B cell NE-based transcription mixture under 500 mM KCl high salt conditions, which allowed for depletion of one OCA-S component while keeping others intact in the depleted samples (Ref. 10 and Fig. 2A; also see below). Then we carried out a subsequent...

FIGURE 1. H2B transcription in vitro (A–E) or in Xenopus oocytes (G), and OCA-S recruitment to the H2B promoter (F). A, effects of NAD+ on H2B transcription in the B cell NE-based transcription mixture. The H2B transcription activities were quantified using a densitometer (see bottom panel) and normalized to the largely constant transcription activities of the redox-insensitive IgH template (10) as an internal control (also see B–E). B, NADH-inhibited H2B transcription. C, H2B transcription stimulated by NAD+ (1 mM) was reduced by NADH or additional NAD+. D, H2B transcription in an NAD+ titration on top of 0.4 mM NADH. E and F, H2B transcription and H2B promoter occupancy by OCA-S (an immobilized H2B promoter pull-down assay) responded in a biphasic manner to an NAD+/NAD+ ratio titration. In F, the bound proteins were examined by immunoblot with IgGs against Oct-1 or the OCA-S components with available antibodies: p38/GAPDH, p36/LDH, p60/Sti1 (stress inducible protein 1), p20/nm23-H1 (non-metastatic protein 23 in human, 1), and p18/nm23-H2 (non-metastatic protein 23 in human, 2). The three panels below the immunoblot images show the largely constant recruitment of Oct-1 and the varied recruitment of p38/GAPDH and p36/LDH representing the OCA-S complex, as quantified by densitometry of relevant signals. G, H2B promoter activity in oocytes. The H2B promoter-luciferase reporter gene was microinjected into oocytes with p38/GAPDH (+) or BSA (−), without (−), or with NAD+ (0.04, 0.2, 1, 2, 25 mM).
immobilized H2B promoter pull-down assay (Fig. 2B). The binding of Oct-1 to the H2B promoter was constant (all lanes) and that of OCA-S components was inhibited by NADH and stimulated by NAD^+ (1 mM) in the control (lanes 1–3). In the p38/GAPDH-depleted B cell NE-based transcription mixture, however, the binding of other components was impeded (lanes 4–6). In line with the above promoter recruitment patterns, H2B transcription in vitro was similarly modulated by exogenous NAD(H) at 1 mM level (Fig. 2C, top panel).

Given that an octamer mutant (H2B Mut) template was not redox-sensitive (Fig. 2C, bottom panel), the NAD(H) redox effects did not target the basal H2B transcription. In addition, NAD(H) were not converted to other metabolites nor interconverted in the in vitro reactions (Fig. 2D). This suggests primary or direct, and NAD(H)-specific, redox effects.

The above results suggest that, by sensing the NAD^+ /NADH redox, p38/GAPDH nucleates an Oct-1-OCA-S interaction as a key regulatory step for the H2B transcription in vitro. The redox effect is on an Oct-1-p38/GAPDH interaction, but not on an Oct-1-octamer interaction or the H2B basal transcription machinery. In living cells, the Oct-1-p38/GAPDH interaction is likely subject to direct redox-modulation as well, which determines the H2B transcription output in vivo (see below).

Essential and Redox-modulator Roles of p36/LDH for H2B Transcription—p38/GAPDH (10), p36/LDH (Fig. 3A) and other tested OCA-S components (not shown) are essential for H2B transcription in living cells. Given a cytoplasmic interaction between GAPDH and LDH (20), a similar p38/GAPDH-p36/LDH interaction within OCA-S may help tether an intact OCA-S complex to the H2B promoter. This might help explain the essential role of p36/LDH for H2B expression in vivo. To support the above notion, we carried out immobilized H2B promoter pull-down assays in the B cell NE-based transcription mixture. In the p36/LDH-depleted sample, promoter occupancy by p20/nm23-H1, p18/nm23-H2, and p60/St1 was

![FIGURE 2. The Oct-1-OCA-S interaction is a switch for H2B transcription and is nucleated by p38/GAPDH. A, immunodepletion of p38/GAPDH in the B cell NE-based transcription mixture. The mock- and p38/GAPDH-depleted samples were examined by immunoblot with antibodies against Oct-1 or OCA-S components. B, immunodepleted H2B promoter pull-down that showed the recruitment of Oct-1 or OCA-S components without or with 1 mM of NAD[H]. With an octamer mutant promoter, neither Oct-1 nor p38/GAPDH (10) or the other OCA-S-components (data not shown) was recruited. C, mock- and p38/GAPDH-depleted samples with the wild-type (H2B WT; top panel) or mutant (H2B Mut; middle panel) promoter template in the transcription reactions without or with 1 mM of NAD(H). The exposure time to obtain the H2B Mut image was three times longer than that to obtain the H2B WT image. Thus, without an OCA-S function (lane 4) and reflecting a 3-fold activation potential of Oct-1 (14), the H2B WT template was transcribed ~3 times more actively than the H2B Mut template, which was transcribed at a basal level. The transcription activities from the H2B WT template were quantified using a densitometer (bottom panel) and normalized to the largely constant transcription activities of the H2B Mut template. D, in vitro, exogenous NADH and NAD^+ were not interconverted or converted to other metabolites. The coenzyme levels were measured after (+) or before (−) transcription reactions.

![FIGURE 3. Essential and redox-modulator roles of p36/LDH. A, HeLa cells were treated with control (Random), or p36/LDH-specific (#1 and #2) siRNAs to silence the p36/LDH expression by RNA interference (RNAi, Ref. 13). p36/LDH deficiency (top panel, immunoblot with p38/GAPDH as loading control) abolished H2B expression (middle panel, RT-PCR with β-actin as internal control) at a transcriptional level (bottom panel, an H2B promoter-luciferase assay). B, mock- or p36/LDH-depleted (lanes 1 and 2) B cell NE-based transcription mixture was used in an immobilized H2B promoter pull-down assay without lanes (3 and 4) or with 1 mM NAD(H) (lanes 5 and 6), C, an immobilized H2B promoter pull-down assay with the mock or p60/St1i-depleted B cell NE-based transcription mixture. D, an immobilized H2B promoter pull-down assay. The bound OCA-S was examined by immunoblot with indicated amounts of input (the B cell NE-based transcription mixture). Amounts of bound p38/GAPDH and p36/LDH (lane 1) were similar to 1 milliunit of human erythrocyte enzymes (lane 6). E, enzyme assays for input or bound OCA-S using 1 milliunit of enzymes as controls. F, NAD(H)-NAD^+ conversion by LDH and pyruvate (bottom panel) relieved and stimulated NADH-inhibited H2B transcription (top panel, lanes 2–7). Lane 8, stimulatory effect of 1 mM NAD^+ (compare with lane 1). The exposure time to obtain the H2B Mut image (middle panel) was ~3 times longer than that to obtain the H2B WT image.
Redox Status and Histone Expression

impeded as opposed to the normal and NAD\(^+\)-enhanced recruitment of OCA-S in the mock-depleted sample (Fig. 3B). In the p60/Stil-depleted sample, the recruitment of p36/LDH and p38/GAPDH to the H2B promoter was normal but the recruitment of p20/nm23-H1 and p18/nm23-H2 was impeded (Fig. 3C). Hence, p36/LDH is essential as an OCA-S component that helps tether an intact OCA-S complex to the H2B promoter. This is most likely through a direct interaction between p38/GAPDH and p36/LDH within OCA-S; however, the possibility that the interaction is mediated by other untested OCA-S component(s) is not yet excluded.

p38/GAPDH along with p36/LDH did not exercise glycolytic activities in the reactions in the B cell NE-based transcription mixture, because NAD(H) was not converted to other metabolites or interconverted (Fig. 2D); however, we cannot rule out the possibility that the two essential OCA-S components, while associated in OCA-S, might have catalytic activities that are able to feedback to H2B transcription if provided substrates.

Under optimized conditions, immobilized H2B promoter pull-down assays allowed for 10–20% recruitment of OCA-S (Fig. 3D). We found that the H2B promoter-bound OCA-S exhibited an enzyme activity for p36/LDH, which was as potent as the input (the B cell NE-based transcription mixture); however, we have failed to detect any intrinsic enzyme activity for p38/GAPDH, which was marginal even in the input (Fig. 3E). This suggests that the OCA-S intrinsic enzyme activity for p36/LDH, which uses NADH as a coenzyme (to be re-oxidized to NAD\(^+\)) to convert pyruvate to lactate, can impact H2B transcription. Indeed, the H2B transcription in vitro inhibited by 1 mm NADH was relieved and stimulated by pyruvate in a dose-dependent manner (Fig. 3F, top panel), accompanied by the pyruvate-facilitated NADH to NAD\(^+\) re-oxidation (Fig. 3F, bottom panel).

Disrupting NAD\(^+\) Biosynthesis Inhibits H2B Expression in Living Cells—The belief that optimal H2B transcription in vitro requires a proper NAD\(^+\)/NADH (redox status) (Fig. 1) prompted studies on the roles of the NAD\(^+\)/NADH redox status for H2B expression in living cells.

~80% mammalian NAD\(^+\) is derived from a two-step biosynthesis (21): the cytoplasmic nicotinamide phosphoribosyltransferase (Nampt) converts precursor nicotinamide to nicotinamide mononucleotide, which is converted to NAD\(^+\) by the enzyme family nicotinamide mononucleotide adenyllyltransferases (Nmnat, of which Nmnat-1 is a nuclear isozyme). Therefore, silencing the expression of Nampt or Nmnat-1 can deplete the cellular or nuclear NAD\(^+\) pool, allowing us to study a role of NAD\(^+\) for H2B expression in vivo.

We then silenced the Nmnat-1 expression, which has been implicated in certain functions by maintaining a nuclear NAD\(^+\) pool (22, 23). HeLa cells treated with Nmnat-1-specific siRNAs (nos. 1 or 2) exhibited just partial silencing (Fig. 4E) with reduced H2B mRNA levels (Fig. 4F) and free cellular NAD\(^+\)/NADH ratios (Fig. 4G). However, the control or Nmnat-1-depleted cells exhibited no difference in the total cellular NAD\(^+\) levels (Fig. 4H). This is in line with the fact that the normal, Nmnat-1-null, or Nmnat-1-overexpressing cells behaved similarly in this regard (22, 23).

Because of certain technical limitations, there is no direct assessment of the free NAD\(^+\)/NADH ratios, or the total NAD\(^+\) (or NADH) levels, in the nuclear compartment. The measured free cellular NAD\(^+\)/NADH ratios and total NAD\(^+\) (or NADH) levels, therefore, might partially reflect the nuclear ratios and levels. Nmnat-1-depleted cells might have more reduced free NAD\(^+\)/NADH ratios in the nuclei than the measured cellular ratios (Fig. 4G), which might be comparable to the nuclear ratios in Nampt-depleted cells. Indeed, although reduction of
The histone expression is tightly coupled with S-phase progression (7). Thus, there could be a possibility that NaN3 and CoCl2 might adversely affect S-phase progression, which then fed back to H2B expression; however, the treatment by NaN3 or CoCl2 inhibited the H2B expression (Fig. 5, B and E) but did not grossly change cell cycle profiles (Fig. 5G). This implies that the observed H2B expression defects (Fig. 5, B and E) were most likely primary defects due to the redox changes, but not secondary defects caused by a potentially defective S-phase progression. This is in line with primary or direct redox effects in vitro (Fig. 2).

In vitro, p38/GAPDH nucleates an Oct-1-OCA-S interaction subject to redox-modulation to determine the H2B transcriptional outputs, in close correlation with the H2B promoter occupancy by p38/GAPDH (Figs. 1 and 2). Such an interaction in living cells should be reflected by H2B promoter occupancy by p38/GAPDH. Indeed, ChIP assays revealed that in the NaN3- or CoCl2-treated cells, H2B promoter occupancy by p38/GAPDH (OCA-S) was reduced (Fig. 5, C and F). This parallels the H2B expression defects in the NaN3- or CoCl2-treated cells (Fig. 5, B and E), which are (primarily) at the transcriptional level (see below).

The NAD+/NADH redox status reflects cellular metabolic states, hence raising a concern of whether the H2B expression defects, as a result of NaN3 and CoCl2 treatments or of disrupting NAD+ biosynthesis, were due to a severe shortage of ATP (an RNA building block) because of the changed NAD+/NADH ratios. We feel that this is highly unlikely for several reasons. First, the β-actin expression (internal control) was unaffected by the changed redox status (Figs. 4, B and F and 5, B and E). Second, the overall ATP levels in control or redox-perturbed cells did not differ significantly. For example, NaN3 treatment led to an only slight decrease of ATP levels at ~16 h (Fig. 6A); however, H2B expression defects were already manifested within 2 h of the treatment by NaN3 (Fig. 5B) or even in a shorter period of time (see below).

NaN3 and CoCl2 perturb the redox status with reduced free cellular NAD+/NADH ratios but sustain a constant NAD+ plus NADH level (24). To reaffirm this, we measured redox-related levels in the redox-perturbed cells; Fig. 6B offers an example with the NaN3 treatment (1 mM) in a 20-h time course. Obviously, the total (NAD+ plus NADH) levels did not significantly change over the 20-h time course, and the total NAD+/NADH ratios (calculated from the total NAD+ and NADH pools) were not significantly reduced at 2 or 4 h. However, the free cellular NAD+/NADH ratio was reduced by ~50% in 2 h of NaN3 treatment (Fig. 5A) with reduced H2B expression (Fig. 5B) and reduced H2B promoter occupancy by OCA-S (p38/GAPDH; Fig. 5C). The reduced free cellular NAD+/NADH ratios were also a common feature upon blocking the NAD+ biosynthesis, which also led to H2B expression defects (Fig. 4).

Thus, the H2B expression in vivo is linked to a proper NAD+/NADH ratio, which defines a proper redox status; perturbing this status inhibits H2B expression (Fig. 5, B and E) by disrupting Oct-1-p38/GAPDH interaction (Fig. 5, C and F). To provide the support that H2B expression defects in redox-perturbed cells are (primarily) at a transcriptional level, we transfected an H2B promoter-luciferase reporter.
Redox Status and Histone Expression

**FIGURE 6.** Primary (or direct) NAD(H) redox effects on H2B expression at a transcriptional level. A, short-term NaN3 treatment did not reduce the overall cellular ATP levels in HeLa cells treated with 1 mM NaN3 for the indicated hours. B, short-term NaN3 treatment did not reduce the overall cellular total NAD+ plus NADH levels or the total NAD+/NADH ratios in HeLa cells treated with 1 mM NaN3 for the indicated hours. C, H2B promoter-luciferase activities in control or NaN3-treated (0.5 mM, overnight) HeLa cells. D, rapid onset of H2B expression defects in NaN3-treated cells. Cells were treated with 1 mM NaN3 for the indicated hours, and the H2B expression levels quantified by real-time PCR. E, swift recovery of H2B expression upon NaN3 withdrawal. H2B expression levels in untreated cells, or cells treated with 1 mM NaN3 for 1 or 2 h, or for 1 h but allowed to recover for 1 h, were quantified by real-time PCR.

into HeLa cells and found the expression of the ectopic gene to be much reduced in NaN3-treated cells (Fig. 6C). All above in vivo results are in line with the patterns of H2B transcription and the H2B promoter occupancy by p38/GAPDH (OCA-S) in vitro (Figs. 1 and 2).

**Primary (or Direct) Redox Effects on H2B Transcription—** It was shown that NAD(H) directly exerted redox effects on H2B transcription in vitro (Fig. 2). H2B expression defects (Fig. 5, B and E) in vivo upon NaN3 or CoCl2 treatment manifested without a change in cell cycle profiles (Fig. 5G). This is in agreement with a primary effect and argues against an indirect cell cycle effect. To further support that the redox effects on H2B transcription in vivo were primary, we studied the onset of H2B expression defects caused by NaN3 treatment and found the defects to manifest very swiftly (in 30 min of the NaN3 treatment, Fig. 6D). In parallel with this swift onset, reversing redox perturbations by withdrawing NaN3 allowed swift H2B expression recovery (Fig. 6E). If the effects of redox perturbations on H2B transcription are not primary but secondary to the redox changes (e.g. through the cell cycle or other physiological changes), the above onset or recovery might not be as swift as so observed.

The above results support primary (or direct) NAD(H) effects on H2B transcription in vitro and in vivo, and all the results (Figs. 1–6) support the notion that redox changes are directly sensed by p38/GAPDH to impact upon an Oct-1-OCA-S (p38/GAPDH) interaction to determine the H2B transcriptional output (see “Discussion”).

**DISCUSSION**

p38/GAPDH, essential for in vivo H2B transcription (10), represents the enzymes with the coenzyme-modulated gene-switching functions (10–12, 25) and acts as a redox sensor (Fig. 2, B and C) to regulate an Oct-1-OCA-S interaction. This interaction is a key regulatory step determining the H2B transcriptional output. p36/LDH is essential for tethering an intact OCA-S complex to the H2B promoter in vitro, explaining the essential role for H2B expression in vivo (Fig. 3, A–C).

The sum of the molecular masses of the OCA-S components approximates the native size (~300 kDa) of the OCA-S complex (10). This is consistent with monomer presence. Generally, monomeric dehydrogenases, while retaining full NAD(H) binding capacities, lack catalytic abilities (26); however, p36/LDH within OCA-S seems to be an exception (Fig. 3E) that circumvented the NADH-inhibited H2B transcription in vitro (Fig. 3F). A similar redox modulator role might operate as part of an essential role for H2B expression in living cells (Fig. 3A) to circumvent physiological redox constraints.

Coexisting p38/GAPDH and p36/LDH in OCA-S might represent an advantage in which the NAD+/NADH redox status plays multiple roles. For instance, in addition to an Oct-1-p38/GAPDH interaction, a proper NAD+/NADH redox status might play a role in the assembly of the OCA-S complex by allowing p38/GAPDH or p36/LDH to assume proper conformation(s). Future structural studies may provide clues regarding this notion.

The redox-modulated H2B expression is most likely a direct (or primary) response of H2B transcription to varied NAD+/NADH redox status. First, in vitro, the H2B transcription and Oct-1-OCA-S interaction were redox-modulated (Figs. 1 A–F, 2, B–C, and 3B) in which the exogenous NAD(H) were not converted to other metabolites or interconverted (Fig. 2D), which otherwise might exert indirect effects. Second, the H2B promoter and Oct-1-OCA-S specificity (Figs. 1, A–E and 2C) argue against a nonspecific redox effect. Third, in vivo upon redox-perturbations, H2B expression defects (Fig. 5, B and E) manifested without changing the cell cycle profiles (Fig. 5G). Fourth, the onset of H2B expression defects upon redox-perturbations was very swift, and H2B expression recovered swiftly upon withdrawal of the redox perturbations (Fig. 6, D and E). Taken together, these results are in accord with the primary (or direct) NAD(H) redox effects on H2B transcription in vitro and in vivo, and with the notion that the redox target is the Oct-1-OCA-S interaction that determines H2B transcriptional output.

The biphasic responses (Fig. 1, A, C–E, and G), and that H2B transcription was sensitive to the NAD+ depletion (Fig. 4), suggest that the H2B transcription favors higher NAD+ levels or higher NAD+/NADH ratios within a certain range that defines a proper redox status. DNA damage/repair generally consumes the nuclear NAD+ pool (27), which may render the H2B expression sensitive to NAD+ depletion in a similar fashion as scenarios in Fig. 4. This and a coordination mechanism (10) may contribute to a global histone expression inhibition upon DNA damage, in addition to the activated cell cycle checkpoint (e.g. Ref. 28).
In view of the in vitro biphasic responses (see Fig. 1, A and C–E), physiological or pathological redox perturbations that can potentially elevate the NAD\(^+\)/NADH ratios in vivo, if beyond certain threshold, are expected to inhibit H2B expression. This is in line with the H2B transcription requiring a proper cellular redox status (redox balance).

Direct links between gene switching and the metabolic state of a cell are quite common in prokaryotes but rarely reported in eukaryotes (12). The current study shows that the activity of p38/GAPDH as an OCA-S component in histone transcription, and potentially other aspects of the OCA-S function, can be modulated by the redox status. Given that the cellular redox status reflects the cellular metabolic state, our study suggests a direct link between cellular metabolism and gene switching in higher eukaryotes. Because S-phase events are tightly coupled to ensure an orderly S-phase progression (1–7), the metabolic states of the cell might also feedback to other coupled S-phase events in addition to histone (H2B) expression.

Acknowledgments—We thank L. Yaw, P. Wong, and Y. Foo for technical assistance, and M. Lee for critical reading of the manuscript. We are grateful to B. Edil who helped us with English.

REFERENCES
1. Ma, T., van Tine, B. A., Wei, Y., Garrett, M. D., Nelson, D., Adams, P. D., Wang, J., Qin, J., Chow, L. T., and Harper, J. W. (2000) Genes Dev. 14, 2298–2313
2. Zhao, J., Kennedy, B. K., Lawrence, B. D., Barbie, D. A., Matera, A. G., Fletcher, J. A., and Harlow, E. (2000) Genes Dev. 14, 2283–2297
3. Ewen M. E. (2000) Genes Dev. 14, 2265–2270
4. Osley M. A. (1991) Annu. Rev. Biochem. 60, 827–861
5. Marzluff W. F., and Duronio R. J. (2002) Curr. Opin. Cell Biol. 14, 692–699
6. Luo, Y., and Roeder, R. G. (1999) Cold Spring. Harb. Symp. Quant Biol. 63, 119–131
7. Nelson, D. M., Ye, X., Hall, C., Santos, H., Ma, T., Kao, G. D., Yen, T. J., Harper, J. W., and Adams, P. D. (2002) Mol. Cell. Biol. 22, 7459–7472
8. Segil, N., Roberts, S. B., and Heintz, N. (1991) Science 254, 1814–1816
9. Fletcher, C., Heintz, N., and Roeder R. G. (1987) Cell 51, 773–781
10. Zheng, L., Roeder, R. G., and Luo, Y. (2003) Cell 114, 255–266
11. McKnight S. L. (2003) Cell 114, 150–152
12. Shi, Y., and Shi, Y. (2004) Trends Genet. 20, 445–452
13. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001) Nature 411, 494–498
14. Luo, Y, and Roeder R. G. (2002) Mol. Cell. Biol. 22, 7459–7472
15. Hedge, N., and Heintz, N. (1991) Science 254, 1814–1816
16. Vy, C., Heintz, N., and Roeder R. G. (1987) Cell 51, 773–781
17. Luo, Y., and Roeder, R. G. (1999) Curr. Op. Cell Biol. 114, 255–266
18. Zhang, Q., Piston, D. W., and Goodman R. H. (2002) Science 295, 1895–1897
19. Shi, Y., Sawada, J., Sui, G., Affar el, B., Whetstone, J. R., Lan, F., Ogawa, H., Luke, M. P., Nakatani, Y., and Shi, Y. (2003) Nature 422, 735–738
20. Sirov, M. A. (1999) Biochim. Biophys. Acta 1432, 159–184
21. Kim, M. Y., Zhang, T., and Kraus, W. L. (2005) Genes Dev. 19, 1951–1967
22. Su, C., Gao, G., Schneider, S., Helt, C., Weiss, C., O’Reilly, M. A., Bohmann, D., and Zhao, J. (2004) EMBO J. 23, 1133–1143