JB Review

J. Biochem. **129**, 491–499 (2001)

Participation of Histones and Histone-Modifying Enzymes in Cell Functions through Alterations in Chromatin Structure[1]

Tatsuo Nakayama[2] and Yasunari Takami

Department of Biochemistry, Miyazaki Medical College, 5200, Kihara, Kiyotake, Miyazaki 889-1692

Received February 1, 2001; accepted February 5, 2001

Alterations in the chromatin structure are preferentially involved in the regulation of cell functions, including gene expression, in eukaryotes. Three types of mechanisms, by which the alterations are caused have been reported: (i) variants of histone subtypes, (ii) chromatin remodeling, and (iii) post-translational modification. This review focuses mainly on the first and third mechanisms, especially on the acetylation of core histones, one of the third mechanisms. Using the gene targeting technique for the DT40 chicken B cell line, we systematically generated a number of mutants, respectively, devoid of particular genes encoding histones and histone deacetylase(s) (HDACs). Most of the H1 and core histone variants should be involved positively or negatively in the transcription regulation of particular genes. Of the chicken HDACs (chHDACs), chHDAC-2 controls the amount of the IgM H-chain at the steps of both transcription and alternative pre-mRNA processing, and chHDAC-3 is essential for cell viability, whereas chHDAC-1 merely affects gene expression in DT40 cells. These results indicate that HDAC family members should participate, in combination with one another, and/or histone acetyltransferase(s) (HATs), in the acetylation of core histones that regulates gene expression through alterations in the chromatin structure.

Key words: chromatin structure, DT40 cells, histones, histone-modifying enzymes, transcription regulation.

In eukaryotes genomic DNA is highly organized and packaged into the nucleus (*1*). The organization and packaging are achieved through the addition of proteins, including histones, which form a complex structure, the chromatin, together with DNA. A typical model for the stratum of the chromatin structure is as follows. Approximately 146 base pairs of DNA wrap around a histone octamer, comprising two molecules each of core histones H2A, H2B, H3, and H4, to form the basic structural unit of the chromatin, the nucleosome. Upon the addition of a variable length of linker DNA (0–80 base pairs) and linker histone H1 (in higher eukaryotes), the nucleosome constitutes the fundamental repeating unit of the chromatin, and then the nucleosome arrays are assembled into a higher order chromatin structure, with the assistance of a number of proteins. DNA folds around nucleosomes to form 10 nm fibers that fold helically into 30 nm chromatin fibers. These 30 nm fibers form loops observed in the prophase chromosome axis that coils to form the fully condensed metaphase chromosome.

Because histones (H2A, H2B, H3, H4, H1, and/or H5) are essential for maintenance of the chromatin structure, a number of each histone subtype must be rapidly accumulated in nuclei prior to the division of cells. To ensure a supply of a large amount of every histone subtype, firstly, the genes encoding them should be present in multiple copies in most higher eukaryotes, ranging from several dozen to hundreds, although yeast has two genes for each of the core histones (*2*). Secondly, much of the control of the amounts of histone mRNAs has been reported to be post-transcriptional (*3*). Thirdly, there is an attractive mechanism, compensatory regulation, by which the mRNA levels of the histone subtypes are precisely maintained in a stoichiometric balance. In a *Saccharomyces cerevisiae* mutant devoid of one of two H2A/H2B gene pairs, the levels of H2A and H2B mRNAs derived from the remaining pair increase to compensate for the mutation (*4*). In homozygous mutant mice devoid of $H1^0$, other H1 genes appear to compensate for the deficiency (*5*). Using the gene targeting technique for the DT40 chicken B cell line, which incorporates foreign DNA through targeted integration at frequencies similar to those seen for random integration (*6*), we systematically generated a number of homozygous DT40 mutants. In the mutant DT40 cell lines devoid of H3-IV/H3-V and H2B-V, respectively, expression of the remaining nine H3 and seven H2B genes increased to compensate for the mutations (*7, 8*). Moreover, in two DT40 mutants devoid of one allele of the major histone gene cluster of 110 kb carrying 39 of the 44 histone genes and two alleles of an approximately 57 kb segment of the cluster carrying 21 of the 39 genes (*9*), respectively, the mRNA levels of each of gene families H1, H2A, H2B, H3, and H4 remained constant. As a result, the amounts of histones H2A, H2B, H3, and H4 (and also H1) do not change in any of these yeast, mouse, and chicken mutant cell lines.

In general, the chromatin structure has been thought to

[1] This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Tel: 81-985-85-3127; Fax: 81-985-85-6503, E-mail: tnakayam@post1.miyazaki-med.ac.jp.

© 2001 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

participate repressively in DNA-utilizing processes, such as replication, recombination, repair, and gene expression. On the other hand, alterations in the chromatin structure have been thought to be preferentially involved in these DNA-utilizing processes, especially in gene expression. Three types of mechanisms for alterations in the chromatin structure have been reported: (i) variants of each histone subtype, (ii) chromatin remodeling, and (iii) post-translational modification. In this review, we mainly give an outline of each of these three mechanisms.

Histone variants

If the role of H1 and core histones is restricted to the maintenance of the chromatin structure, a unique amino acid sequence should be sufficient for each histone subtype. On the contrary, several different variants with amino acid substitution(s) have been reported for most histone subtypes (*10*). For instance, six H1, at least three H2A, four H2B and at least two H3 variants exist in the chicken, for which the nucleotide sequences of almost all histone genes have been determined (*11*). These observations easily led us to suppose that the histone variants are involved in regulation of gene expression, in addition to their vital role in the chromatin organization. In fact, information concerning the nature of histone variants as to regulation of gene expression has accumulated, for example, in the cases of *Saccharomyces cerevisiae* and *Drosophila melanogaster* (*12–15*). In addition, in *Xenopus*, the overexpression of H1 during early development selectively inhibits the expression of the oocyte 5S rRNA gene, with no influence on that of somatic 5S rRNA genes, but depletion of H1 specifically causes increased expression of the oocyte 5S rRNA gene (*16*). Analysis of a macronuclear histone H1 knockout *Tetrahymena thermophila* strain revealed not only that linker H1 does not have a major effect on global transcription, but also that it is required for both the normal basal repression of a gene (*ngoA*) in growing cells and the active expression of another gene (*CyP*) in starved cells (*17*). Using the gene targeting technique, we generated a number of homozygous DT40 mutants devoid of particular histone genes. Our systematic analyses involving 2D-PAGE established not only that the protein patterns remained unchanged in the mutants with no changes in the compositions of any variants of any histone subtypes, but also that they were definitely altered in the mutants with changes in the quality of the H1 and/or H2B variants (*8*, *9*, *18*, *19*). Thus, most of the H1 and core histone variants should participate positively or negatively in transcription regulation of particular genes, probably through alterations in the chromatin structure.

Chromatin remodeling

As mentioned above, the chromatin structure should act as a transcriptional repressor *in vivo* because it inhibits the binding of transactivator proteins to their binding sites. A first step in gene activation possibly involves alterations in the chromatin (nucleosome) structure, "remodeling" at active promoters and/or enhancers of DNA, which allows binding of transcription factors. In this chromatin remodeling process, a particular enzymatic activity has been reported to be necessary for transcription regulation. Many different chromatin-remodeling complexes have been identified independently with different assays in various organisms, *i.e.* nucleosome remodeling factor (NURF), chromatin accessibility complex (CHRAC), and ATP-dependent chromatin assembly and remodeling factor (ACF) in *Drosophila melanogaster*, SWI/SFF in yeast and mammals, RSC, ISW1, and ISW2 in yeast, and RSF and WCRF in mammals. They are functionally and biochemically distinct from each other but ubiquitously exhibit ATPase activity that disrupts DNA-histone interaction. Detailed insights concerning the chromatin remodeling have been reviewed elsewhere (*20–22*).

Post-translational modifications

Post-translational modifications, such as phosphorylation, methylation, and acetylation, occur at histone tails (see later). Topics concerning the former two have been reviewed elsewhere (*23*). Here we mainly discuss the potential functional impact of alterations in the chromatin structure, based on the acetylation of core histones. The molecules of core histones have been grouped into three types of motifs (*24*): (i) histone-fold regions, (ii) diversed extensions, and (iii) histone tails, which extend outside the nucleosome core particle. On the other hand, the chemical modification of core histones with acetyl groups was first proposed more than 30 years ago to be of fundamental importance as to transcriptional activation in eukaryotes (*25*). Acetylated core histones were preferentially associated with transcriptionally active chromatin (*26*). Subsequently, it was established that acetylation occurs at conserved Lys residues in the N-terminal tails of core histones, such as Lys-9 and Lys-14 of histone H3, and Lys-5, Lys-8, Lys-12, and Lys-16 of histone H4 (*23*). The positions of acetylation at Lys residues, with some changes, have remained nearly invariant throughout eukaryotic evolution.

In the recent few years, knowledge concerning the involvement of acetylation of core histones in the regulation of gene expression has rapidly accumulated (*27*, *28*). The chemical modification of core histones has been thought to be of fundamental importance as to conformational changes of the chromatin (*29*). The level of acetylation is related to transcription activity, and then the acetylation induces an open chromatin conformation that allows the transcription machinery access to promoters. This modification that reduces how tightly histones associate with DNA in the chromatin should be precisely controlled with a cooperative function of histone acetyltransferase(s) (HATs) and deacetylase(s) (HDACs).

Histone acetyltransferases (acetylases) (HATs)

A number of HATs, which are quite different in terms of their enzymatic activity and regulation, have been identified in the past several years (Table I) (*29*, *30*). The HAT family is classified into two types, HAT-A and HAT-B, *i.e.* the former should be localized in nuclei and involved in the regulation of gene expression, and the latter should be localized in the cytoplasm and involved in the acetylation of newly synthesized core histones (*31*). As to the latter type, we have revealed that chicken HAT-1, chHAT-1, binds to a C-terminal region of approximately 30 amino acids of the chicken p46 polypeptide (*32*). In addition, the interaction of chHAT-1 with the p48 subunit of chicken chromatin assembly factor-1, chCAF-1p48, does not require the proper propeller structure of the latter, based on its WD repeat motifs, but the leucine zipper motif of chHAT-1 is essential for the interaction with chCAF-1p48 (*33*). Interestingly, both yeast

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

TABLE I. **Substrate specificities and functions of histone acetyltransferases.**

Catalytic subunit	Complex	Substrates	Functions
HAT1	HAT1/p46	H2A/H4	Acetylates newly synthesized histones
PCAF	PCAF complex	H3/H4 (nucleosomal H3)	Interacts with CBP/p300 and SCR1/ACTR, and stimulates transcription *in vivo*
GCN5L	STAGA/TFTC	H3/H4 (nucleosomal H3)	Can substitute for TFIID in transcription
GCN5	SAGA/ADA	H3/H4 (nucleosomal H3/H2B)	Interacts with acidic activation domains and TBP
ESA1	NuA4	H2A/H4 (nucleosomal H2A/H4)	Interacts with acidic activation domains Essential for viability
TIP60	TIP60 complex	H2A/H3/H4	Transcriptional regulator
SAS3		H2A/H3/H4	Regulator of silencing in yeast
p300/CBP		H2A/H2B/H3/H4	Global transcriptional regulator
SRC1/ACTR		H3/H4	Mediates nuclear receptor–dependent transcription
TAF(II)250	TFIID	H3/H4	Subunit of general transcription complex TFIID
TFIIIC (p90, p110, p220)	TFIIIC	nucleosomal H2A/H3/H4	Essential for PolII transcription
ELP3	Elongator	H2A/H2B/H3/H4	Transcription elongation

HAT-1 and chHAT-1 are not essential for cell viability.

This review focuses on recent evidence concerning the HAT-A type. First, Gcn5 was discovered in *S. cerevisiae* as a general transcriptional coactivator or adaptor for regulatory proteins affecting DNA-binding activator activity (*34*). Subsequently, it has been shown to be a component of a complex containing Ada2 and Ada3 bridging activation domains and the TATA-box binding protein (TBP) component of the basal transcriptional machinery. Next, unexpectedly, nuclear HAT enzyme activity in *Tetrahymena* was reported to be very similar to that of yeast Gcn5p (*35*). This finding was an epoch-making in the field of the regulation of gene expression through alterations in the chromatin structure. Many investigations revealed that they are members of the GCN5-related *N*-acetyltransferase (GNAT) superfamily found in all kingdoms of life, and share conserved motifs of N-acetyltransferase(s) (NATs) in the HAT domain (*36, 37*). Subsequently, GNAT-unrelated coactivators, such as a large cellular protein (p300/CBP) that forms a complex with certain transcription factors, a p300/CBP-associated factor (PCAF), and a protein ($TAF_{II}230/250$) that is part of a large transcription factor (TFIID) and ACTR/SRC-1 (activator of retinoid receptors), have been found to possess the ability to acetylate core histones (*38–41*).

Moreover, SAS2 has been reported to be one of the SAS-like genes known as the MYST family and to be involved in the regulation of transcription silencing in yeast (*42*). SAS3, a homolog of SAS2, has also been found to possess HAT activity and to require a zinc finger motif for its activity (*43*). MOZ was isolated as a chimeric gene fused to CBP through chromosomal translocation in an acute leukemia case (*44*). MOF was identified as the gene necessary for male-specific hypertranscription and X-linked dosage compensation in *Drosophila* (*45*). TIP60 was isolated as a gene encoding an HIV-1–Tat interactive protein (*46*).

These HATs exhibit somewhat different substrate specificities from assay to assay (Table I) (*23, 47, 48*). When free core histones are used as substrates, p300/CBP can acetylate all core histones (H2A, H2B, H3, and H4), and TIP60 can acetylate H2A, H3, and H4, whereas all of Gcn5, PCAF, TAFII250, and SRC-1/ACTR can only add acetyl groups to core histones H3 and H4, and HAT-1 can acetylate histones H2A and H4. In addition, Gcn5/ADA complexes show distinct specificities, *i.e.* they can acetylate histones H3 and H2B. On the other hand, interestingly, when nucleosomal core histones are used as acetylation targets, the substrate specificities of the enzymes are slightly distinct. For instance, SAGA and ADA complexes can acetylate histones H3 and H2B, and PCAF can only add acetyl groups to histone H3, although p300/CBP and SRC-1/ACRT cause no changes, *i.e.* the former can add acetyl groups to all of core histones H3, H4, H2A, and H2B, and the latter to histones H3 and H4. Provocatively, recent observations indicate that HATs are capable of acetylating non-histone substrates, including several interesting transcription factors, such as tumor suppressor and DNA-binding activator p53, and two basal transcription factors, TFIIE and TFIIF. On reflection, most members of the HAT family were originally found to have distinct, particular functions. Almost all true connections between their physiological roles and HAT activities are still open to question.

Histone deacetylase(s) (HDACs)

A number of HDAC family members have been identified in the past several years (Fig. 1). Prior to a breakthrough in the connection between deacetylation of acetylated histones and transcription regulation (*49*), a number of reagents, including trichostatin A (TSA), trapoxin and sodium butyrate, were known to cause hyperacetylation of histones for about 20 years. The former two are effective as to hyperacetylation at a nanomolar concentration, indicating that they are specific inhibitors of the HDAC activity, whereas the effective concentration of the latter was of the millimolar level (*50*). TSA or trapoxin appeared to reversibly or irreversibly inhibit the HDAC activity. Also, it is known that yeast transcriptional corepressors Sin3 and Rpd3 negatively regulate the global expression of genes (*51*).

Using an affinity column of trapoxin, cDNA encoding human HDAC-1 was cloned, and its deduced amino acid sequence exhibited extensive similarity to that of yeast Rpd3 (*49*). Following this epoch-making discovery, knowledge concerning the participation of HDACs in the regulation of gene expression has rapidly accumulated (*27, 28, 52*). Transcriptional repression by Mad-Max heterodimers requires interaction of Mad with mSin3, the mammalian homolog of Sin3 (*53–55*). Corepressor mSin3 exists in a complex with HDAC-1 and 2 (*56*). Both the Mad repression and HDAC activity of the mSin3 immunocomplexes are abolished by TSA. Transcriptional corepressors SMRT and N-CoR act as silencing mediators for retinoid and thyroid hormone receptors (*57–59*). Transcriptional repression by nuclear receptors is mediated by a complex containing

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

SMRT or N-CoR, mSin3 and HDAC. Mammalian HDAC-1 and 2 have been reported to be recruited by the retinoblastoma protein (Rb) to repress transcription (*60*, *61*). In parallel, methylation of CpG islands has long been thought to be linked to gene silencing, and then Mecp2, a protein that specifically binds to methylated CpG, was reported to participate in CpG silencing. Recent findings revealed that Mecp2 recruits HDACs and Sin3 to repress gene expression (*62*, *63*). Thus, HDACs are involved in transcription repression through methylation of CpG islands, and de-

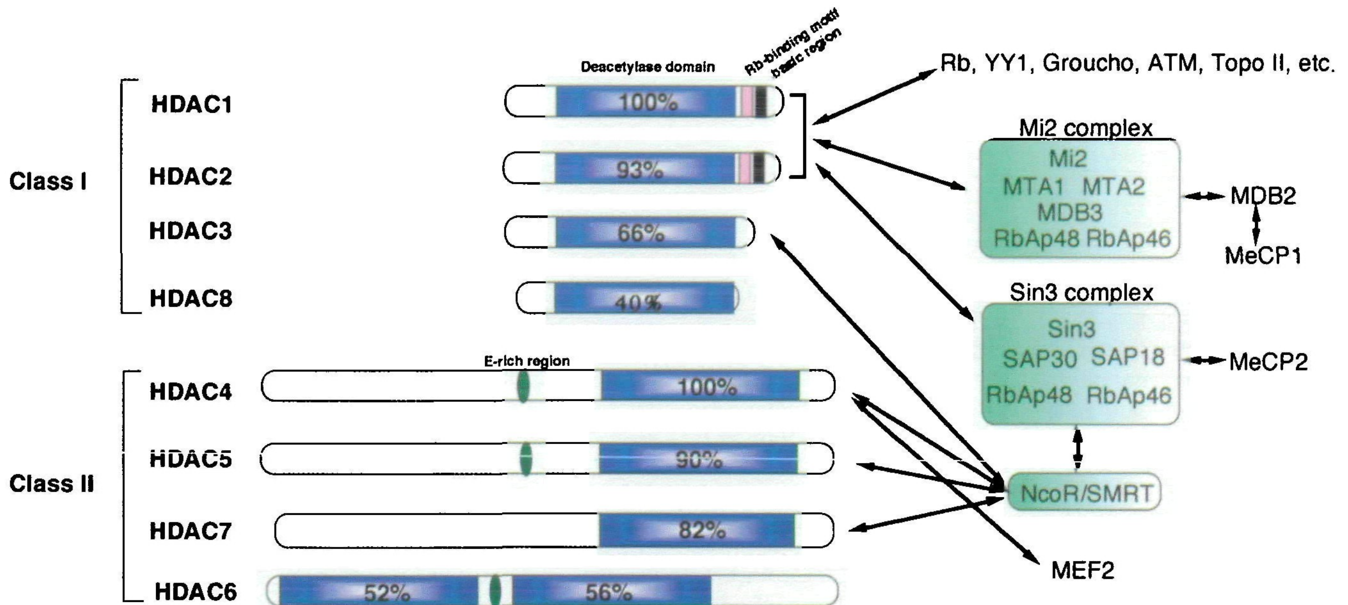

Fig. 1. **Classification of histone deacetylases.** The HDAC family members are grouped into classes I and II. Arrows indicate their interactions with proteins or their involvement in complexes that are discussed in the text.

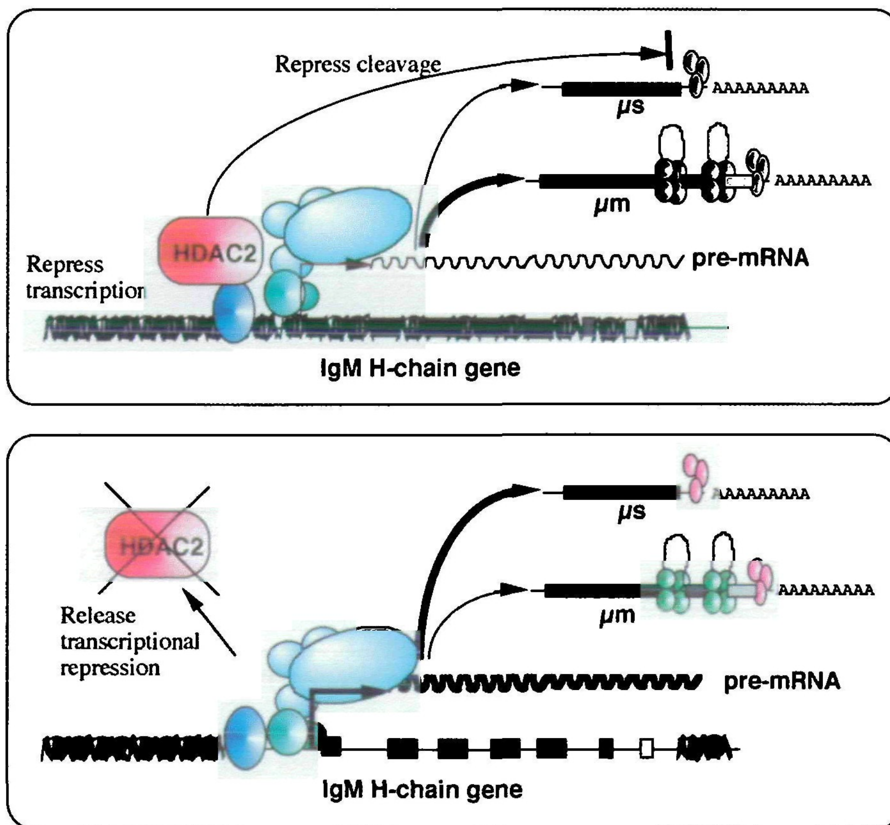

Fig. 2. **A model for a key role of chHDAC-2 in the control of the amount of the IgM H-chain.**

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

acetylation of acetylated histones is a common mode of repression.

Interestingly, most DNA sequences in which HDAC complexes assemble should be involved in opposite functions in the regulation of gene expression. For instance, E2F activates transcription in part by recruiting a p300/CBP complex, when it binds to an E2F site (*64*). Conversely, when Rb is activated, it binds to E2F and then recruits HDAC to repress transcription. On the other hand, Rb does not appear to require the HDAC activity to inactivate E2F in the *in vitro* transcription assay (*65*). Thus, these DNA sequences participate as enhancers or silencers, depending on whether the HAT complex or HDAC complex is assembled on them.

Another type of HDACs, which exhibits a low sequence homology to Rpd3, has also been identified in a variety of eukaryotes, and therefore multiple forms of HDACs exist, there being at least eight HDACs (Fig. 1) (*66*). These HDAC family members are divided into two classes, I and II. Class I of the HDAC family comprises four members, HDAC-1, 2, 3, and 8, each of which contains a deacetylase domain exhibiting from 45 to 93% identity in amino acid sequence. Interestingly, both the Rb-binding motif and the basic region are present within the C-terminal regions of HDAC-1 and 2, but not within those of HDAC-3 and 8. Class II of the HDAC family comprises HDAC-4, 5, 6, and 7, the molecular weights of which are all about twofold larger than those of the class I members, and the deacetylase domains are present within the C-terminal regions, except that HDAC-6 contains two copies of the domain, one within each of the N-terminal and C-terminal regions. Compared with the deacetylase domain of HDAC-4, which shows about 37% identity to HDAC-1 (class I), the domains of HDAC-5, 7, and 6 exhibit 52 to 90% identity. In addition, Glu-rich regions are present upstream of the deacetylase domains of HDAC-4 and 5, and in the middle of the two deacetylase domains of HDAC-6, but not in HDAC-7.

Although the deacetylase activity of the three human HDACs has been examined with free histones and nucleosomes as substrates *in vitro*, no apparent difference in substrate specificity has been reported among them, i.e. the human HDACs deacetylated all four acetylated core histone subtypes. Human HDAC-1, 2 and 3 were expressed in various tissues, but the others (HDAC-4, 5, 6, and 7) showed tissue-specific expression patterns (*66*). These results suggested that each member of the HDAC family exhibits a different, individual substrate specificity and function *in vivo*, when core histones packaged into nucleosomes are targeted as substrates. Also, this led us to expect that HDAC family members play individual, particular roles *in vivo*, and then participate, in combination with one another, and/or with HAT family members, in the acetylation of core histones. To understand the overall picture of transcription regulation, through alterations in the chromatin structure based on the acetylation of core histones, it is essential to determine the role of each of the multiple HDACs, like HATs, *in vivo*.

Characteristics of chicken HDACs

We have cloned cDNAs encoding the three chicken HDACs, chHDAC-1, 2, and 3, respectively, which comprise 480, 488, and 428 amino acids, and exhibit 93.8, 97.1, and 97.0% identity to human HDAC-1, 2, and 3, respectively (*67, 68*). The consensus sequence for the Rb-binding motif, which is conserved in *Xenopus* to human HDACs, is located in the C-terminal regions of chHDAC-1 and 2. A considerable difference was observed in the C-terminal sequences of approximately 50 amino acids of these two enzymes. Compared with chHDAC-1 and 2, chHDAC-3 lacks 6-7 amino acids and approximately 50–60 amino acids at its N-terminal and C-terminal ends, respectively. In addition, chHDAC-3 is noticeably different from the other two in the N-terminal region of approximately 140 amino acids and the C-terminal region of approximately 100 amino acids. Interestingly, a typical nuclear export signal (NES) is present at positions 29–41 of chHDAC-3, and is somewhat different in the corresponding regions of chHDAC-1 and 2. Several other polypeptides were co-precipitated with the anti–chHDAC-1 or 2 antiserum, but no other polypeptides were co-precipitated with the anti–chHDAC-3 antiserum. chHDAC-3 was localized in both nuclei and the cytoplasm, although chHDAC-1 and 2 were preferentially localized in nuclei. These results should agree with that a typical NES is localized in chHDAC-3, but not in chHDAC-1 or 2.

Generation of homozygous *chHDAC-1*– and *2*–deficient mutants, and the conditional *chHDAC-3*–deficient mutant

We sequentially transfected DT40 cells with the pchHDAC-1/neo and pchHDAC-1/hisD constructs, and generated *chHDAC-1*–deficient mutants. To generate *chHDAC-2*–deficient mutants, the pchHDAC-2/neo and pchHDAC-2/hisD constructs were sequentially introduced into DT40 cells (*67*). The levels of chHDAC-1 mRNAs in the heterozygous 1/2ΔchHDAC-1 mutant were about 50% those in DT40 cells. In the homozygous ΔchHDAC-1 mutant, no chHDAC-1 mRNAs were detected. On the other hand, the levels of chHDAC-2 mRNAs in the heterozygous 1/2ΔchHDAC-2 mutant were about 90%, indicating that the *chHDAC-2* gene should have the ability to compensate for the disruption of one allele of the gene. No transcript of *chHDAC-2* was detected in the homozygous ΔchHDAC-2 mutant.

We introduced the pΔchHD-3/neo construct into DT40 cells, and could easily generate heterozygous *chHDAC-3*–deficient mutants. However, all attempts to obtain a homozygous *chHDAC-3*–deficient mutant were unsuccessful, suggesting that the disruption of both alleles of *chHDAC-3* was lethal. The heterozygous mutant (1/2ΔchHDAC-3) was transfected with the tet-responsive chHDAC-3 expression vector, and then 1/2ΔchHDAC-3/FHDAC3 clones carrying the construct bearing the FLAG sequence integrated randomly on the chromosomes were obtained. Finally, we transfected the pΔchHD-3/hisD construct into 1/2Δ-chHDAC-3/FHDAC3 to generate the conditional homozygous ΔchHDAC-3/FHDAC3 mutant (*68*).

chHDAC-2 controls the amount of the IgM H-chain

To clarify the difference in the roles of chHDAC-1 and 2, we compared total cellular proteins of the two mutants, ΔchHDAC-1 and 2, with those of the wild-type cell line (*67*). The protein patterns on 2D-PAGE definitely changed for ΔchHDAC-2, but the changes were insignificant for ΔchHDAC-1. Interestingly, the amounts of the IgM H and L-chains increased in ΔchHDAC-2. Taken together, these results revealed that chHDAC-1 and 2 are definitely distinct in their roles, *i.e.* the participation of the former in

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

protein pattern changes is slight, but the latter predominantly participates in the accumulation of the IgM H and L-chains. Both chHDAC-1 and 2 exhibit extensive homology (~94%) in approximately 430 N-terminal amino acids, but considerably low homology (~50%) in approximately 50 C-terminal amino acids. The difference in the involvement of chHDAC-1 and 2 in the accumulation of the IgM H and L-chains should be due to the difference in their C-terminal sequences.

Gene expression in eukaryotes can be controlled through multiple steps, including transcription and alternative pre-mRNA processing. The regulation of IgM H-chain synthesis during B cell development is known as an example of the latter mechanism (*69*). There is a regulated switch from the membrane-bound (μm) to secreted (μs) form of IgM H-chain mRNA. Several factors, including CstF-64, participate cooperatively in this alternative processing of IgM H-chain pre-mRNA (*70*). The *chHDAC-2* mutation influenced the expression of the *IgM H-chain* gene, and predominated the switch from μm to μs mRNA. This participation of chHDAC-2 was confirmed by the findings that treatment with TSA caused increases in both the level of total IgM H-chain mRNA and the switch from μm to μs mRNA in DT40 cells. These influences of TSA should be related only to chHDAC-2, since *chHDAC-1*– and *-3*–deficiencies had no effects on the amounts of the IgM H-chain and its mRNA (*67*, *68*).

chHDAC-3 is essential for the viability of DT40 cells

Inhibition of the expression of the *chHDAC-3* transgene on the addition of tet caused the disappearance of FHDAC3 in less than 24 h, but ΔchHDAC-3/FHDAC3 grew normally by day 2 (*68*). Thereafter, the growth was delayed. The number of mutant cells in the S or G_2/M phase of the cell cycle decreased, cells containing less than a diploid amount of DNA appeared by 72 h, and then the number of dying cells increased. Inspection of bulk chromosomal histone preparations from DT40 cells in the absence of tet and ΔchHDAC-3/FHDAC3 after treatment with the drug by Western blotting (involving antibodies against particular Lys residues, Lys-8 and Lys-12, of histone H4) revealed insignificant changes in the deacetylation levels of chromosomal core histones. Therefore, the *chHDAC-3* mutation did not cause alterations in the global deacetylation of chromosomal core histones, suggesting that the mutation should only affect the deacetylation of core histones, in particular, narrow regions of chromatin, if chHDAC-3 targets chromosomal core histones. In addition, the *chHDAC-3* depletion caused no alterations in the expression of a number of cell cycle-related genes.

Both the 1-23 N-terminal amino acids and the 389–417 C-terminal amino acids of chHDAC-3 are essential for the viability of DT40 cells. The essential C-terminal region of chHDAC-3 may facilitate cell viability through its deacetylation activity, depending on the region of amino acids 323–428. Moreover, a complementation experiment [involving two single missense mutations (H135A) and (H193F)] revealed that substitution of the essential His residues at positions 135 and 193, respectively, with Ala and Tyr resulted in a decrease or complete loss of the deacetylation activity. In parallel, these two single missense mutations caused a lack of the complementation ability, indicating that the deacetylation activity of chHDAC-3 based on the conserved active domain is also certainly necessary for its viability function.

Interestingly, the double substitution (L29I and L31M) of the conserved Leu residues at positions 29 and 31 within the typical NES, even with Ile and Met, respectively, which are identical to the amino acids at the corresponding positions of both chHDAC-1 and 2, resulted in extensive loss of the complementation ability but not the deacetylation activity. In addition, over-expressed FHDAC3 was localized in nuclei and the cytoplasm, but exhibited no influence on cell growth, indicating that the reduced complementation ability of the double missense mutation should be due to decreased nuclear export rather than increased accumulation in nuclei. Therefore, the proper NES of chHDAC-3 should be required for both nuclear export and the viability function. Furthermore, the Leu residues at definite positions could not be completely compensated for by even the amino acids located at the corresponding positions of other HDACs.

Possible function of the chHDAC family members

We propose a molecular basis for the differences in the functional specificities of these multiple chHDACs *in vivo*. The highly conserved internal domains of the chHDAC family members should participate in their general roles, i.e. all of them should catalyze universally the removal of acetyl group(s) of Lys residue(s) of core histones, and then interact with other proteins, for instance, the p48 subunit of CAF-1 and the p46 polypeptide (see Fig. 1) (*32*, *71*). Conversely, the variable N-terminal and C-terminal domains of the enzymes should be involved in their individual, particular roles.

For instance, chHDAC-2 controls the amount of the IgM H-chain at the steps of both transcription of its gene and alternative processing of its pre-mRNA through alterations in the chromatin structure (Fig. 2), although chHDAC-1 merely affects gene expression in DT40 cells (*67*). The transcription regulation mediated by chHDAC-2 probably occurs in conjunction with hypothetical signal(s), and the chHDAC-2 activity is reduced or completely abolished by it. The decreased activity should change the chromatin structure restricted to the narrow region surrounding the *IgM H-chain* gene, resulting in increased transcription of this target gene. In addition, the increased transcription of the *IgM H-chain* gene is possibly accompanied by that of the gene(s) encoding putative switch-related factor(s), including CstF-64 (*70*). The putative factor(s) should promote the switch from μm to μs mRNA. Most or all of the specific functions of chHDAC-2 can not be compensated for by any remaining chHDAC enzymes, all of which should be different from the former in substrate specificity, *i.e.* in core histone subtypes, histone variants or deacetylatable Lys residues. Furthermore, chHDAC-2 should be predominantly involved in the control of the transcription of genes encoding a set of B cell–specific proteins (*67*). The difference in the participation of these enzymes should be mainly due to the differences in both the 50–60 C-terminal amino acids and the complex formation with certain transcription factors.

On the other hand, chHDAC-3 is essentially involved in cell viability (*68*). This particular participation is probably mainly due to the N-terminal and C-terminal regions, which are considerably distinct from those of the other two members, chHDAC-1 and 2. As an example, the ability of

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

only chHDAC-3, *i.e.* not chHDAC-1 and 2, to be exported from nuclei to the cytoplasm should be due to the proper NES in the variable N-terminal region. As a possible result, chHDAC-3, as a scavenger in the cytoplasm but not in nuclei, even if it acts differently in the two subcellular fractions, probably deacetylates acetyl groups from the acetylated Lys residue(s) of core histones, before they assemble to form the nucleosome. However, the disruption of yeast Rpd3, which is homologous to mammalian HDAC-3, as well as other types of yeast HDACs, resulted in no alterations in cell viability (*72*). On the other hand, a strong hypomorphic mutation in the *Drosophila* Rpd3 gene (homologous to both mammalian HDAC-1 and 2, but not HDAC-3) caused embryonic lethality and a specific pair-rule segmentation phenotype (*73*). The discrepancy and/or differences regarding yeast, *Drosophila* and DT40 cells (and probably most higher eukaryotic cell types) are still questionable.

Conclusions

Alterations in the chromatin structure are involved in DNA-utilizing processes, including gene expression, in eukaryotes. Acetylation of core histones preferentially participates in these alterations. This chemical modification of core histones with acetyl groups is precisely controlled by HATs and HDACs through complex formation with certain transcription factors. Each member of the HAT and HDAC families should play an individual, particular role, and then should act in combination with each other in the acetylation of core histones, the level of which is related to transcription activity. For instance, chHDAC-2 controls the amounts of the IgM H and L-chains, and chHDAC-3 is essential for the viability of DT40 cells. Most or all of the specific roles of particular members of the HAT and HDAC families in cell functions are not compensated for by any remaining enzymes, probably because they are distinct from each other in substrate specificity, complex formation, and location for assembly on the chromatin.

REFERENCES

1. Wolffe, A.P. (1995) *Chromatin: Structure and Function*, 2nd ed., Academic Press, San Diego, CA
2. van Holde, K.E. (1989) *Chromatin* Springer-Verlag, New York
3. Sariban, E., Wu, R.S., Erickson, L.C., and Bonner, W. M. (1985) Interrelationships of protein and DNA syntheses during replication of mammalian cells. *Mol. Cell. Biol.* **5**, 1279–1286
4. Osley, M.A. and Hereford, L.M. (1981) Yeast histone genes show dosage compensation. *Cell* **24**, 377–384
5. Sirotkin, A.M., Edelmann, W., Cheng, G., Klein-Szanto, A., Kucherlapati, R., and Skoultchi, A.I. (1995) Mice develop normally without the $H1^0$ linker histone. *Proc. Natl. Acad. Sci. USA* **92**, 6434–6438
6. Buerstedde, J.-M. and Takeda, S. (1991) Increased ratio of targeted to random integration after transfection of chicken B cell lines. *Cell* **67**, 179–188
7. Takami, Y., Takeda, S., and Nakayama, T. (1995) Targeted disruption of an H3-IV/H3-V gene pair causes increased expression of the remaining H3 genes in the chicken DT40 cell line. *J. Mol. Biol.* **250**, 420–433
8. Takami, Y., Takeda, S., and Nakayama, T. (1995) Targeted disruption of H2B-V encoding a particular H2B histone variant causes changes in protein patterns on two-dimensional polyacrylamide gel electrophoresis in the DT40 chicken B cell line. *J. Biol. Chem.* **270**, 30664–30670
9. Takami, Y., Takeda, S., and Nakayama, T. (1997) An approximately half set of histone genes is enough for cell proliferation and a lack of several histone variants causes protein pattern changes in the DT40 chicken B cell line. *J. Mol. Biol.* **265**, 394–408
10. Coles, L.S., Robins, A.J., Madley, L.K., and Wells, J.R.E. (1987) Characterization of the chicken histone H1 gene complement. Generation of a complete set of vertebrate H1 protein sequences. *J. Biol. Chem.* **262**, 9656–9663
11. Takami, Y., Higashio, M., Fukuoka, T., Takechi, S., and Nakayama, T. (1996) Organization of the chicken histone genes in a major gene cluster and generation of an almost complete set of the core histone protein sequences. *DNA Res.* **3**, 95–99
12. Norris, D. and Osley, M.A. (1987) The two gene pairs encoding H2A and H2B play different roles in the *Saccharomyces cerevisiae* life cycle. *Mol. Cell. Biol.* **7**, 3473–3481
13. Norris, D., Dunn, B., and Osley, M.A. (1988) The effects of histone gene deletions on chromatin structure in *Saccharomyces cerevisiae. Science* **242**, 759–761
14. Grunstein, M. (1990) Histone function in transcription. *Annu. Rev. Cell Biol.* **6**, 643–678
15. van Daal, A. and Elgin, S.C.R. (1992) A histone variant, H2AvD, is essential in *Drosophila melanogaster. Mol. Biol. Cell* **3**, 593–602
16. Bouvet, P., Dimitrov, S., and Wolffe, A.P. (1994) Specific regulation of *Xenopus* chromosomal 5S rRNA gene transcription *in vivo* by histone H1. *Genes Dev.* **8**, 1147–1159
17. Shen, X. and Gorovsky, M.A. (1996) Linker histone H1 regulates specific gene expression but not global transcription *in vivo. Cell* **86**, 475–483
18. Takami, Y. and Nakayama, T. (1997) A single copy of linker H1 genes is enough for proliferation of the DT40 chicken B cell line, and linker H1 variants participate in regulation of gene expression. *Genes Cells* **2**, 711–723
19. Takami, Y., Nishi, R., and Nakayama, T. (2000) Histone H1 variants play individual roles in transcription regulation in the DT40 chicken B cell line. *Biochem. Biophys. Res. Commun.* **268**, 501–508
20. Armstrong, J.A. and Emerson, B.M. (1998) Transcription of chromatin: these are complex times. *Curr. Opin. Genet. Dev.* **8**, 165–172
21. Gregory, P.D. and Horz, W. (1998) Life with nucleosomes: chromatin remodelling in gene regulation. *Curr. Opin. Cell Biol.* **10**, 339–345
22. Varga-Weisz, P.D. and Becker, P.B. (1998) Chromatin-remodelling factors: machines that regulate? *Curr. Opin. Cell Biol.* **10**, 346–353
23. Davie, J.R. (1998) Covalent modifications of histones: expression from chromatin templates. *Curr. Opin. Genet. Dev.* **8**, 173–178
24. Luger, K. and Richmond, T.J. (1998) The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.* **8**, 140–146
25. Allfrey, V., Faulker, R,M., and Mirsky, A.E. (1966) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci. USA* **51**, 786–794
26. Hebbers, T.R., Thorne, A.W., and Crane-Robinson, C. (1988) A direct link between core histone acetylation and transcriptionally active chromatin. *EMBO J.* **7**, 1395–1402
27. Wolffe, A.P. and Pruss, D. (1996) Targeting chromatin disruption: transcription regulators that acetylate histones. *Cell* **84**, 817–819
28. Pazin, M.J. and Kadonaga, J.T. (1997) What's up and down with histone deacetylation and transcription? *Cell* **89**, 325–328
29. Cheung, W.L., Briggs, S.D., and Allis, C.D. (2000) Acetylation and chromosomal functions. *Curr. Opin. Cell Biol.* **12**, 326–333
30. Brown, C.E., Lechner, T., Howe, L., and Workman, J.L. (2000) The many HATs of transcription coactivators. *Trends Biochem. Sci.* **25**, 15–19
31. Kleff, S., Andrulis, E.D., Anderson, C.W., and Sternglanz, R. (1995) Identification of a gene encoding a yeast histone H4 acetyltransferase. *J. Biol. Chem.* **270**, 24674–24677
32. Ahmad, A., Takami, Y., and Nakayama, T. (2000) Distinct

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

regions of the chicken p46 polypeptide are required for its *in vitro* interaction with histones H2B and H4 and histone acetyltransferase-1. *Biochem. Biophys. Res. Commun.* **279**, 95–102
33. Ahmad, A., Nagamatsu, N., Kouriki, H., Takami, Y., and Nakayama, T. (2001) Leucine zipper motif of chicken histone acetyltransferase-1 is essential for *in vivo* and *in vitro* interactions with the p48 subunit of chicken chromatin assembly factor-1. *Nucleic Acids Res.* **29**, 629–637
34. Georgakopoulos, T. and Thireos, G. (1992) Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.* **11**, 4145–4152
35. Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996) *Tetrahymena* histone acetyltransferase A: a homolog of yeast Gcn5p linking histone acetylation to gene activation. *Cell* **84**, 843–851
36. Yang, X.J., Ogryzko, V.V., Nishikawa, J.I., Howard, B., and Nakatani, Y. (1996) A p300/CBP-associated factor that competes with the adenoviral E1A oncoprotein. *Nature* **382**, 319–324
37. Kuo, M.-H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* **383**, 269–272
38. Mizzen, C.A., Yang, X.J., Kokubo, T., Brownell, J.E., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., Nakatani, Y., and Allis, D. (1996) The TAFII250 subunit of TFIID has histone acetyltransferase activity. *Cell* **87**, 1261–1270
39. Bannister, A.J. and Kouzarides, T. (1996) The CBP co-activator is a histone acetyltransferase. *Nature* **384**, 641–643
40. Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87**, 953–959
41. Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y., and Evans, R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**, 569–580
42. Reifsnyder, C., Lowell, J., Clarke, A., and Pillus, L. (1996) Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat. Genet.* **14**, 42–49
43. Takechi, S. and Nakayama, T. (1999) Sas3 is a histone acetyltransferase and requires a zinc finger motif. *Biochem. Biophys. Res. Commun.* **266**, 405–410
44. Borrow, J., Stanton, V.P., Jr., Anderson, J.M., Bechner, R., Behm, F.G., Chaganti, R.S., Civin, C.I., Disteche, C., Dube, I., Frischauf, A.M., Horsman, D., Mitelman, F., Volinia, S., Watmore, A.E., and Housman, D.E. (1996) The translocation t(8; 16) (p11; p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat. Genet.* **14**, 33–41
45. Hilfiker, A., Hilfiker-Kleiner, D., Pannuti, A., and Lucchesi, J. C. (1997) Mof, a putative acetyltransferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in *Drosophila*. *EMBO J.* **16**, 2054–2060
46. Kamine, J., Elangovan, B., Subramanian, T., Coleman. D., and Chinnadurai, G. (1996) Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. *Virology* **216**, 357–366
47. Berger, S.L. (1999) Gene activation by histone and factor acetyltransferases. *Curr. Opin. Cell Biol.* **11**, 336-341
48. Luo, R. X. and Dean, D. C. (1999) Chromatin remodeling and transcriptional regulation. *J. Natl. Cancer Inst.* **91**, 1288–1294
49. Taunton, J., Hassig, C.A., and Schreiber, S.L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* **272**, 408–411
50. Yoshida, M., Kijima, M., Akita, M., and Beppu, T. (1990) Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by Trichostatin A. *J. Biol. Chem.* **265**, 17174–17179
51. Vidal, M. and Gaber, R.F. (1991) RPD3 encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **11**, 6317–6327
52. Pennisi, E. (1997) Opening the way to gene activity. *Science* **275**, 155–157
53. Hassig, C.A., Fleischer, T.C., Billin, A.N., Schreiber, S.L., and Ayer, D.E. (1997) Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell* **89**, 341–347
54. Kadosh, D. and Struhl, K. (1997) Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell* **89**, 365–371
55. Zhang, Y., Iratni, R., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (1997) Histone deacetylases and SAP18, a novel polypeptide, are components of a human Sin3 complex. *Cell* **89**, 357–364
56. Laherty, C.D., Yang, W.-M., Sun, J.-M., Davie, J.R., Seto, E., and Eisenman, R.N. (1997) Histone deacetylases associated with the mSin3 corepressor mediate Mad transcriptional repression. *Cell* **89**, 349–356
57. Alland, L., Muhle, R., Hou Jr., H., Potes, J., Chin, L., Schreiber-Agus, N., and Depinho, R.A. (1997) Roles for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. *Nature* **387**, 49–55
58. Heinzel, T., Lavinsky, R.M., Mullen, T.-M., Soderstrom, M., Laherty, C.D., Torchia, J., Yang, W.-M., Brard, G., Ngo, S.D., Davie, J.R., Seto, E., Eisenman, R.N., Rose, D.W., Glass, C.K., and Rosenfeld, M.G. (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387**, 43–48
59. Nagy, L., Kao, H.-Y., Chakravarti, D., Lin, R.J., Hassig, C.A., Ayer, D.E., Schreiber, S.L., and Evans, R.M. (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89**, 373–380
60. Brehm, A., Miska, E.A., McCance, D.J., Reid, J.L., Bannister, A.J., and Kouzarides, T. (1998) Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* **391**, 597–601
61. Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J.P., Troalen, F., Trouche, D., and Harel-Bellan, A. (1998) Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature* **391**, 601–605
62. Jones, P.L., Veensta, G.J., *et al.* (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat. Genet.* **19**, 187–191
63. Nan, X., Ng, H.H., *et al.* (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386–389
64. Trouche, D., Cook, A., and Kouzarides, T. (1996) The CBP co-activator stimulates E2F1/DP1 activity. *Nucleic Acids Res.* **24**, 4139–4145
65. Ross, J.F., Liu, X., and Dynlacht, B.D. (1999) Mechanism of transcriptional repression of E2F by the retinoblastoma tumor suppressor protein. *Mol. Cell* **3**, 195–205
66. Cress, W.D. and Seto, E. (2000) Histone deacetylases, transcriptional control, and cancer. *J. Cell. Physiol.* **184**, 1–16
67. Takami, Y., Kikuchi, H., and Nakayama, T. (1999) Chicken histone deacetylase-2 controls the amount of the IgM H-chain at the steps of both transcription of its gene and alternative processing of its pre-mRNA in the DT40 cell line. *J. Biol. Chem.* **274**, 23977–23990
68. Takami, Y. and Nakayama, T. (2000) N-terminal region, C-terminal region, nuclear export signal and deacetylation activity of histone deacetylase-3 are essential for the viability of the DT40 chicken B cell line. *J. Biol. Chem.* **275**, 16191–16201
69. Peterson, M.L. (1994) RNA processing and expression of immunoglobulin genes in *Handbook of B and T Lymphocytes* (Snow, E.C. ed.) pp. 321–342, Academic Press, San Diego
70. Takagaki, Y., Seipelt, R.L., Peterson, M.L., and Manley, J.L. (1996) The polyadenylation factor CstF-64 regulates alternative processing of IgM heavy chain pre-mRNA during B cell differ-

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

entiation. *Cell* **87**, 941–952
71. Ahmad, A., Takami, Y., and Nakayama, T. (1999) WD repeats of the p48 subunit of chicken chromatin assembly factor-1 required for *in vitro* interaction with chicken histone deacetylase-2. *J. Biol. Chem.* **274**, 16646–16653
72. Rundlett, S.E., Carmen, A.A., Kobayashi, R., Bavykin, S., Turner, B.M., and Grunstein, M. (1996) HD1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc. Natl. Acad. Sci. USA* **93**, 14503–14508
73. Mannervik, M. and Levine, M. (1999) The Rpd3 histone deacetylase is required for segmentation of the *Drosophila* embryo. *Proc. Natl. Acad. Sci. USA* **96**, 6797–6801

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012