Chromatin Remodeling and Circadian Control: Master Regulator CLOCK Is an Enzyme


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The molecular machinery that governs circadian rhythmicity is based on clock gene products organized in regulatory feedback loops. Recently, we have shown that CLOCK, a master circadian regulator, has histone acetyltransferase activity essential for clock gene expression. The Lys-14 residue of histone H3 is a preferential target of CLOCK-mediated acetylation. As the role of chromatin remodeling in eukaryotic transcription is well recognized, this finding identified unforeseen links between histone acetylation and cellular physiology. Indeed, we have shown that the enzymatic function of CLOCK drives circadian control. We reasoned that CLOCK’s acetyltransferase activity could also target nonhistone proteins, a feature displayed by other HATs. Indeed, CLOCK also acetylates a nonhistone substrate: its own partner, BMAL1. This protein undergoes rhythmic acetylation in the mouse liver, with a timing that parallels the down-regulation of circadian transcription of clock-controlled genes. BMAL1 is specifically acetylated on a unique, highly conserved Lys-537 residue. This acetylation facilitates recruitment of the repressor CRY1 to BMAL1, indicating that CLOCK may intervene in negative circadian regulation. Our findings reveal that the enzymatic interplay between two clock core components is crucial for the circadian machinery.

INTRODUCTION

The eukaryotic genome is organized in a nucleoprotein structure, chromatin, that enables a large variety of central processes such as regulation of gene expression, DNA repair, apoptosis, and cell division (Luger 2003; Felsenfeld and Groudine 2003). Changes in chromatin organization have a central role in ensuring that the storage, organization, and readout of the genetic information occurs in a proper spatial and temporal sequence during all biological processes. The basic repeating unit of chromatin, the nucleosome core particle, consists of 147 bp of DNA organized in approximately two superhelical turns of DNA wrapped around an octamer of core histones (two copies each of histone H2A, H2B, H3, and H4). Nucleosomes become organized in higher-order structures when interacting with H1 linker histones and more loosely with nonhistone chromatin-associated proteins (Luger 2003). A number of enzymatic processes lead to remodeling of the architecture of chromatin. These are dynamic changes, essential for the transition of chromatin from a condensed to a decondensed state, and vice versa, each state being permissive to specific cellular functions. These states somewhat correlate with the definition of “euchromatin” versus “heterochromatin,” which most times identifies with “active” versus “inactive” states of gene expression, respectively (Strahl and Allis 2000; Felsenfeld and Groudine 2003).

Various cellular mechanisms operate that lead to modifications in chromatin structure: One type involves the active participation of ATP-dependent remodeling factors, such as Swi/Snf and NURF (Peterson and Workman 2000), and others implicate posttranslational covalent modifications of histones by specific enzymes. The amino-terminal tails of the core histones H2A, H2B, H3, and H4 are highly conserved and represent the protein domains where most of the covalent histone modifications occur. Since histone tails are thought to confer secondary and more flexible contacts with DNA, allowing for dynamic changes in the accessibility of the underlying genome, their modifications have been shown to contribute to chromatin remodeling and thereby to the control of a large array of nuclear processes (Cheung et al. 2000b; Felsenfeld and Groudine 2003). The amino-terminal domains of histones are subjected to a large variety of covalent modifications, such as acetylation (Grunstein 1997; Gregory et al. 2001; Roth et al. 2001), phosphorylation (Mahadevan et al. 1991; Sassone-Corsi et al. 1999), and methylation (Bannister et al. 2002), but also ADP-ribosylation (Huletsky et al. 1985) and ubiquitination (Sun and Allis 2002). In several cases, the combinatorial association of some specific modifications has been reported and coupled to unique nuclear functions (Cheung et al. 2000a; Lo et al. 2000), strongly suggesting that the coordinate action of multiple enzymes converges to promote physiological changes in chromatin organization (Lo et al. 2001; Merienne et al. 2001).

CHROMATIN REMODELING AND GENE EXPRESSION: AN ENZYMATIC AFFAIR

The four core histones have amino-terminal tails whose primary sequences are highly conserved from yeast to mammals. Remarkably, histone tails have a high density of residues that are prone to posttranslational modifications. For the most part, the enzymes that elicit these modifications are also conserved among species. Various position-specific modifications have been associated with distinct chromatin-based outputs (Strahl and Allis 2000; Felsenfeld and Groudine 2003). For example, modifications in the histone H3 amino-terminal tail have been cou-
Histone phosphorylation is remarkable because it is directly linked to intracelluar signaling pathways. Indeed, activation of a specific transduction system by an extracellular stimulus results in the stimulation of kinase cascades and phosphorylation at distinct sites on histones. Various serine-threonine kinases have been implicated for phosphorylating potential serine phosphoacceptor sites on histone H2A (Ser-1 and Ser-18), H2B (Ser-14 and Ser-32), H3 (Ser-10 and Ser-28), and H4 (Ser-1). The distance between the serine residues in H2A, H2B, and H3 is conserved among all histones, with a constant spacing of 18 residues. The significance of this intriguing feature is unclear. Phosphorylation of Ser-10 in histone H3 has been studied in detail (Sassone-Corsi et al. 1999; Clayton et al. 2000) and found to be elicited by a number of kinases, all belonging to the AGC branch of cyclic nucleotide-regulated protein kinase, including RSK-2, MSK1, PKA, and IKKα (Nowak and Corces 2004).

Histone acetylation is believed to have a pivotal role in the modulation of chromatin structure associated with transcriptional activation (Grunstein 1997; Wade and Wolffe 1998; Kuo and Allis 1998; Struhl 1998; Workman and Kingston 1998). In support of this notion, a wide variety of nuclear proteins involved in transcriptional control have been demonstrated to possess intrinsic histone acetyltransferase (HAT) activity (Kouzarides 1999; Sterner and Berger 2000; Roth et al. 2001). In particular, a number of transcriptional coactivators, including GCN5 (Brownell et al. 1999), PCAF (Yang et al. 1996), CBP/p300 (Bannister and Kouzarides 1996; Ogryzko et al. 1996), SRC-1 (Spencer et al. 1997), and ACTR (Chen et al. 1997) are known to acetylate histones, thereby facilitating the trans-activation exerted by a number of DNA-binding transcription factors. Furthermore, HAT function has been ascribed also to TAFII250, a component of the TATA-box-binding TFID complex of the basal transcription machinery (Mizzen et al. 1996), and to ATP-2, a sequence-specific DNA-binding transcription factor (Kawai et al. 2000). Thus, HATs constitute a family of proteins with remarkably diverse features.

Amino acid sequence analyses of HAT proteins reveal an important feature: HATs fall into distinct families that share relatively poor sequence similarity. For example, ACTR/SRC-1 is thought to constitute a unique class of HAT (Chen et al. 1997; Spencer et al. 1997), whereas p300/CBP displays only limited homology with the GCN5-related N-acetyltransferase superfamily (Martinez-Balbas et al. 1998). The MYST family of HATs is particularly interesting as these proteins show similarity with other acetyltransferases exclusively within the acetyl-coenzyme-A-binding motif (denominated “motif A”) (Yamamoto and Horikoshi 1997). Accumulating evidence indicates that histone acetylation exerted by various classes of HATs contributes to plasticity in transcriptional control by increasing the dynamic changes in chromatin structure (Fischle et al. 2003).

Histone methylation has been linked mostly to gene silencing, although some examples of its role in transcriptional activation have been reported. Specifically, methylation of the Lys-4 of H3 has been found to be a positive mark for gene activation, possibly in combination to acetylation at Lys-14 (Berger 2007). Three forms of histone methylation exist, each one of them promoting a different regulatory function. Indeed, mono-, di-, and tri-methylated lysines are obtained by the action of different types of histone methyltransferases (HMTs). Some of the enzymes devoted to the removal of the methyl groups, the demethyltransferases, have been implicated in neurological disorders (Shi 2007). Importantly it is the combined association of different posttranslational modifications that appears to bring unique biological functions. Thus, the identification of the enzymes involved in histone modifications, and their regulatory interplays, has far-reaching physiological consequences.

PERIPHERAL VERSUS CENTRAL CLOCKS: WHERE IS THE DIFFERENCE?

The finely controlled transcriptional regulation within the circadian system is absolutely remarkable. Indeed, more than 10% of all mammalian transcripts undergo circadian fluctuations in their expression levels (Akhtar et al. 2002; Duffield et al. 2002; Panda et al. 2002), underscoring that genome-wide mechanisms must operate in order to ensure such global transcriptional regulation. The highly specialized, temporarily based regulation of gene expression that characterizes circadian oscillations elects the cellular clock as a prominent model for the study of dynamic regulations of chromatin remodeling (Crosio et al. 2000). Moreover, as circadian function is intimately coupled to physiological and metabolic control (Dunlap 1999; Rutter et al. 2001; Schibler and Naef 2005), clock-controlled chromatin reorganization is likely to reveal yet unexplored pathways linking histone modifications to cellular metabolism. In this context, the finding that peripheral tissues also contain independent clocks is of fundamental importance (Whitmore et al. 2000; Giebultowicz 2001; Stokkan et al. 2001; Schibler and Sassone-Corsi 2002). Peripheral clocks are not fully self-sustained and autonomous and, differently from the suprachiasmatic nucleus (SCN), require specific stimuli in order to sustain their circadian rhythms (Cermakian and Sassone-Corsi 2000; King and Takahashi 2000; Reppert and Weaver 2002). We favor a scenario where peripheral clocks are affected by physiological stimuli that may originate from the SCN and/or may be the result of SCN-mediated messages (Schibler and Sassone-Corsi 2002). This view has been substantiated by a number of studies that have identified growth factors, some steroids such as glucocorticoids, and retinoic acid to induce oscillations of clock genes and clock-controlled genes in cultured fibroblasts or peripheral tis-
These observations underscore the presence of possible differences at the molecular level between the organization of the clock mechanism in the SCN and that in peripheral tissues. These may operate following some unique tissue-specific regulatory pathways since restricted access to food has an effect on peripheral rhythms without affecting the central pacemaker function of the SCN (Schibler and Sassone-Corsi 2002).

**PLASTICITY IN CIRCADIAN REGULATION AND CHROMATIN REMODELING**

How is the oscillatory expression of clock-controlled genes regulated, so that transcription-permissive chromatin states are dynamically established in a circadian time-specific manner? Interestingly, the activation of clock-controlled genes (CCGs) by CLOCK:BMAL1 has been shown to be coupled to circadian changes in histone acetylation at their promoters (Etchegaray et al. 2003; Curtis et al. 2004; Naruse et al. 2004; Nakahata et al. 2007). Specifically, histone H3 is acetylated in chromatin that encompasses the Per1, Per2, and Cry1 promoters when these genes are actively transcribed. The molecular dissection of the CLOCK:BMAL1-mediated trans-activation mechanism is likely to provide significant information on how circadian regulation of histone acetylation is achieved. Several lines of genetic evidence also indicate that the carboxy-terminal glutamine-rich region of CLOCK exerts a central function in the circadian trans-activation of target genes in flies and mice (Antoch et al. 1997; King et al. 1997; Allada et al. 1998; Gekakis et al. 1998), has some important features. Except for a polyglutamine stretch, no characteristic structural motifs were previously described in this region. A closer look revealed that the carboxy-terminal glutamine-rich region of CLOCK displays a significant sequence homology with the carboxy-terminal domain of ACTR, a domain shown to have intrinsic HAT activity (Chen et al. 1997). In this region, at least six independent amino acid residues common to CLOCK and ACTR are evolutionarily conserved in both proteins. Importantly, the amino acid residues common to CLOCK and ACTR are evolutionarily conserved in both proteins. It is noteworthy that CLOCK and ACTR share a number of other structural features outside of the carboxy-terminal glutamine-rich region. These include the highly conserved basic helix-loop-helix (bHLH)-PAS domain at the amino termini, a NRID (nuclear receptor interaction domain), as well as serine-rich regions within the middle portion of both proteins. Although CLOCK is a significantly smaller protein as compared to ACTR, these common features result in a

**THE PROTEIN CLOCK IS AN ENZYME**

The carboxy-terminal glutamine-rich region of CLOCK, a region implicated in trans-activation function (Allada et al. 1998; Gekakis et al. 1998), has some important features. Except for a polyglutamine stretch, no characteristic structural motifs were previously described in this region. A closer look revealed that the carboxy-terminal region of CLOCK displays a significant sequence homology with the carboxy-terminal domain of ACTR, a domain shown to have intrinsic HAT activity (Chen et al. 1997). In this region, at least six independent amino acid regions are found to share significant sequence similarity between the two proteins. Importantly, the amino acid residues common to CLOCK and ACTR are evolutionarily conserved in both proteins. It is also noteworthy that CLOCK and ACTR share a number of other structural features outside of the carboxy-terminal glutamine-rich region. These include the highly conserved basic helix-loop-helix (bHLH)-PAS domain at the amino termini, a NRID (nuclear receptor interaction domain), as well as serine-rich regions within the middle portion of both proteins. Although CLOCK is a significantly smaller protein as compared to ACTR, these common features result in a
From homozygous based on mouse embryonic fibroblast (MEF) cells derived al. 2006). Most importantly, using an experimental system H3 Lys14 is where most of the acetylation occurs (Doi et al. 2006). Detailed sequence comparison between the acetyl-CoA-binding motifs of various HATs revealed that CLOCK contains a motif within the carboxy-terminal glutamine-rich region. This amino acid sequence stretch shares significant similarity to the so-called “motif A” in the HAT family denominated MYST (for its founding members MOZ, Ybf2/Sas3, Sas2, and Tip60). In particular, CLOCK shows high sequence similarity to yeast Sas1 and other MYST members, including yeast Sas3, fly MOF, and human Tip60. Importantly, residues that have been demonstrated to be involved in acetyl-CoA interaction by crystal structure analysis of the Esal protein (Yan et al. 2000) are shared with CLOCK. It is significant that these residues are all fully conserved in CLOCK proteins of various species. One intriguing feature of the motif A in CLOCK, when compared to MYST family members, is the insertion of five amino acids, also fully conserved among species. As compared to Esal, the additional five amino acids would lengthen the loop comprised between β9 and ε2, a region that is demonstrated to be exposed at the protein surface (Yan et al. 2000). Intriguingly, the putative acetyl-CoA-binding motif in Drosophila CLOCK lacks the five extra amino acids present in the vertebrate counterparts, which increases its similarity to Esal and other MYST family members. Finally, a number of acetyl-CoA-binding motifs from various N-acetyltransferases also show similarities. In conclusion, the structural features of CLOCK appear to identify it as a novel type of DNA-binding HAT. Indeed, the general organization is similar to a SRC/ACTR-type of HAT, but the acetyl-CoA-binding motif is more related to the MYST class. Thus, the unique combination of protein domains in CLOCK seems to form a somewhat hybrid HAT.

CIRCADIAN PHYSIOLOGY NEEDS A HAT

Our biochemical analyses have established that CLOCK is indeed a bona fide HAT with high specific enzymatic activity for H3 and H4, as it does not acetylate H2A and H2B. Site-directed mutagenesis indicated that H3 Lys14 is where most of the acetylation occurs (Doi et al. 2006). Most importantly, using an experimental system based on mouse embryonic fibroblast (MEF) cells derived from homozygous Clock mutant (c/c) mice (Vitaterna et al. 1994), we showed that the HAT function of CLOCK is essential for the circadian control of CCGs (Doi et al. 2006). As Clock is essential for circadian rhythm (Antoch et al. 1997; King et al. 1997), MEF c/c cells show no cyclic expression of clock genes (Pando et al. 2002). Importantly, ectopic expression of CLOCK is able to rescue the circadian expression of endogenous target genes. MEF c/c cells stably transfected with a CLOCK expression plasmid were subjected to a serum shock, a stimulus commonly used to trigger circadian gene transcription in a variety of cell lines (Balsalobre et al. 1998; Pando et al. 2002). Although the MEF c/c cells had no functional circadian clock, CLOCK ectopic expression restored circadian oscillation of the endogenous Per1 gene and of the Dhp gene, an E-box-regulated circadian CCG output gene. On the contrary, ectopic expression of a HAT-deficient CLOCK failed to restore the circadian trans-activation of mPer1 and Dhp, demonstrating that the HAT activity of CLOCK is necessary for circadian gene expression (Doi et al. 2006). These findings underscore the importance of chromatin remodeling in circadian regulation and reveal the molecular pathways by which such essential control is achieved (Nakahata et al. 2007).

BMAL1 IS ACETYLATED BY CLOCK

The finding that CLOCK is a HAT (Doi et al. 2006) suggested that the acetyltransferase enzymatic activity could also target other nonhistone proteins. This feature is displayed by other HATs (Glozak et al. 2005; Zhang and Dent 2005) and is demonstrated to have profound physiological significance. In a search for putative targets, we have found that CLOCK mediates the acetylation of its own heterodimerization partner, BMAL1 (Hirayama et al. 2007).

In a survey aimed at identifying proteins that may be acetylated rhythmically in vivo, we analyzed various clock proteins, such as BMAL1, CLOCK, and PER1 in the mouse liver at different zeitgeber times. Although, as expected, these proteins oscillate in abundance and phosphorylation levels (Lee et al. 2001; Matsu et al. 2003), acetylation of BMAL1 displays a robust circadian oscillation with a peak at ZT15 (Hirayama et al. 2007). Significantly, the other clock proteins are not acetylated. Ongoing studies in our laboratory on a variety of nuclear proteins and transcription factors indicate that BMAL1 is one of the few substrates for CLOCK, underscoring the specificity of the assay. Importantly, CLOCK is directly responsible for BMAL1 acetylation in cultured mammalian cells (Hirayama et al. 2007).

High specificity: CLOCK acetylates a single lysine in BMAL1

In addition to acetylation, BMAL1 is posttranslationally modified by phosphorylation (Kondratov et al. 2003; Hirayama and Sassone-Corsi 2005) and SUMOylation
CLOCK AND CHROMATIN REMODELING

SUMOylation, as acetylation, occurs on lysine residues and thus we investigated whether an interplay converging on BMAL1 may exist between these two enzymatic pathways. We had previously identified four candidate lysines, K223, K229, K259, and K272, as subjected to SUMOylation in mouse BMAL1. Our analysis then demonstrated that K259 is the major in vivo SUMOylation site in BMAL1 (Cardone et al. 2005). We have found that none of these lysines are acetylated by CLOCK (Fig. 2), demonstrating that the target lysine residues for the two modifications are different. Thus, we embarked in the search for the lysines that are specifically acetylated by CLOCK. We first generated two carboxy-terminally truncated BMAL1 proteins (amino acids 1–282 and 1–469) (Fig. 2A). Both of these mutant proteins interact with CLOCK but are not acetylated, indicating that the target lysines for CLOCK-dependent acetylation must be located in the carboxyl terminus of mouse BMAL1 (amino acids 470–631; Fig. 2B). Somewhat conveniently, this region contains only four potential target lysines at positions 475, 494, 537, and 538, rendering our analysis quite accessible. Each lysine residue was mutated to arginine and tested for CLOCK-mediated acetylation. Strikingly, all mutant proteins are acetylated at levels comparable to wild-type BMAL1, with the exception of K537R (Fig. 2C). Specificity was confirmed also in an in vitro acetylation assay using bacterially purified GST-BMAL1 and a mutant GST-BMAL1-K537R in the presence of 3H-labeled acetyl-CoA. Thus, K537 is the major acetylation site for CLOCK (Hirayama et al. 2007), and it is remarkable that this lysine is highly conserved among all vertebrate BMAL1s (Fig. 2A).

**BMAL1 ACETYLATION IS ESSENTIAL FOR CIRCADIAN RHYTHMICITY**

The unique acetylation profile and the remarkable specificity of CLOCK in targeting one single lysine in BMAL1 prompted us to establish the physiological relevance of BMAL1 acetylation. To investigate the requirement for circadian rhythmicity of BMAL1 acetylation, we performed rescue experiments using MEFs generated from Bmal1 null mice. Lack of BMAL1 expression in these MEFs results in a dysfunctional circadian clock and arrhythmic gene expression (Kondratov et al. 2003; Cardone et al. 2005). We used retroviruses expressing either wild-type BMAL1, an acetylation-deficient BMAL1 (K537R), or green fluorescent protein (GFP), and infected Bmal1−/− MEFs (Fig. 3). To synchronize the infected MEFs, we used dexamethasone (DEX), and circadian oscillation was monitored by a real-time bioluminescence assay based on an mPer2 promoter-driven luciferase reporter vector (Nagoshi et al. 2004; Sato et al. 2006). Although wild-type BMAL1 rescued circadian mPer2 expression, the BMAL1 (K537R) mutant was unable to do so. This experiment demonstrates that the acetylation of BMAL1 is essential for circadian gene regulation (Fig. 3).

Our interest then turned to the molecular mechanism by which BMAL1 acetylation influences circadian rhythmicity: What we found revealed a regulatory pathway highly significant for circadian physiology. Our analyses

**Figure 2.** Highly specific acetylation of BMAL1. (A) Schematic representation of mBMAL1 protein showing the positions of bHLH, PAS A (A), PAS B (B), and CRY-binding site (CRY binding) (Hirayama and Sassone-Corsi 2005). Numbers indicate the amino acid residues in the mouse protein. The extent of two deletion mutants (amino acids 1–282 and 1–469) is shown as two gray bars on the top. The sequence alignment of BMAL1 with the target lysine for acetylation and its surrounding amino acids from various species is shown. (Bold) Target lysines. (B,C) Identification of the target lysine for acetylation. Cells were cotransfected with expression vectors for Myc-CLOCK and Flag-Myc-BMAL1 wild type or for each BMAL1 mutant described. Flag-Myc-BMAL1 proteins were immunoprecipitated, and acetylation was determined by western blotting using anti-pan-acetyl-Lys. Expression of precipitated BMAL1 was determined by western blotting using anti-BMAL1 (B, right panel) or anti-Myc (C, bottom panel) antibodies.

**Figure 3.** Acetylation of BMAL1 is essential to rescue the circadian rhythmicity in BMAL1−/− MEFs. Retrovirus-infected Bmal1−/− MEFs were used to rescue circadian rhythmicity by expressing either a wild-type BMAL1 protein (light gray dots) or the BMAL1 (K537R) mutant (gray dots) and compared with the lack of circadian oscillation in the noninfected Bmal1−/− MEFs (black dots). The mPer2 promoter luciferase reporter plasmid was transfected into the retrovirus-infected cells, and mPer2 promoter activity was monitored by a real-time bioluminescence assay. Expression levels were plotted as arbitrary units.
show that the BMAL1(K537R) mutant is recruited to the mPer2 promoter with an efficiency equivalent to that of BMAL1. In addition, the K537R mutation has no effect on BMAL1 protein stability, capacity of association with CLOCK, and subcellular localization of the protein. Thus, we reasoned that BMAL1 acetylation could be involved in modulating CRY-mediated repression. This possibility would rationalize the results obtained in the rescue circadian experiments (Fig. 3) and is supported by the notion that impairment of CRY-mediated repression of the CLOCK-BMAL1 complex leads to loss of circadian rhythmicity (Sato et al. 2006). Indeed, the BMAL1(K537R) mutant showed a drastically reduced sensitivity to CRY1-mediated repression compared to wild-type BMAL1 (Fig. 4). In other terms, our findings indicate that acetylation of BMAL1 by CLOCK may be an essential regulatory switch as it facilitates CRY-dependent repression. On the basis of association studies, our working model hypothesizes that acetylation of BMAL1 at K537 induces a conformational switch that generates a better “docking site” for CRY.

CONCLUSIONS AND PERSPECTIVES

The notion that at least 10% of all cellular transcripts oscillate in a circadian manner underscores the importance of chromatin remodeling in the control of the circadian gene expression (Nakahata et al. 2007). The finding that CLOCK is a HAT pointed to unforeseen links between histone acetylation and cellular physiology. Indeed, because of these multiple implications, a number of exciting avenues of research are now evident. One of them relates to the search and finding of nonhistone targets for CLOCK. Our findings demonstrate that CLOCK exerts its enzymatic activity on another core component of the clock machinery in vivo in a circadian manner. As we demonstrated that BMAL1 enhances the intrinsic HAT activity of CLOCK (Doi et al. 2006), it is tempting to speculate that BMAL1 regulates its own acetylation by reciprocally controlling CLOCK enzymatic activity. Acetylation of proteins is recognized as an essential regulatory mechanism having both stimulatory and inhibitory effects on transcription (Workman and Kingston 1998; Sterner and Berger 2000). We have demonstrated that acetylation may operate at yet another level of control, because BMAL1 acetylation serves to increase the repressive function of another regulator, CRY. Our results also indicate that CLOCK enzymatic activity has a dual regulatory function. Indeed, we have shown that it contributes to the negative limb of the circadian feedback loop (Hirayama et al. 2007), whereas CLOCK-mediated acetylation of histones participates in the transcriptional stimulation of CCGs, acting within the positive limb of the loop (Doi et al. 2006). Thus, CLOCK enzymatic function contributes in multiple ways to the time-dependent regulation of transcription.

Future studies aimed at deciphering the structural determinants of HAT function, its regulation by BMAL1, and the possible intervention of additional regulators are on the way. Like other HATs, CLOCK is likely to associate with a number of nuclear proteins in a chromatin complex. The study of this complex will reveal additional control elements for circadian transcription, as well as putative histone deacetyltransferases (HDACs) involved in the regulation of transcription.

Figure 4. The enzymatic function of CLOCK governs the circadian machinery. Shown is a schematic model of CLOCK-mediated histone and nonhistone acetylation and its role within the physiological pathways of circadian rhythmicity. The HAT function of CLOCK regulates promoters of CCGs and clock genes (such as Per1) by inducing locally open organization of the chromatin. For example, acetylation on Lys-14 of histone H3 is thought to elicit chromatin remodeling by inducing a transcription-permissive state. Acetylation at Lys-14 could also be favored by additional concerted modifications of the histone tails. For example, phosphorylation at Ser-10, an event directly coupled to activation of intracellular signaling pathways, such as light-induced signals in SCN neurons (Crosio et al. 2000), induces a more efficient acetylation at Lys-14. We envisage a scenario where circadian control of chromatin remodeling by CLOCK may be influenced by the dynamic assembly of a multiprotein regulatory complex. In addition, CLOCK is responsible for BMAL1 acetylation at K537, an event that increases the repression potential by CRY of the CLOCK:BMAL1 complex. Thus, the acetyltransferase enzymatic activity of CLOCK has a dual function, by regulating the circadian machinery by targeting both histone and nonhistone proteins. Indeed, although acetylation of histone H3 results in the positive regulation of CCGs, acetylation of BMAL1 is involved in the negative repression by CRY.
CLOCK AND CHROMATIN REMODELING

in enzymatically countering the acetylation. Thus, many questions remain to be addressed. The exciting part is now to design experiments and gather the results that will elucidate the intricacies of how circadian control is linked to metabolism, cell cycle, and physiology. Uncovering the intimate links between chromatin remodeling and the circadian clock constitutes a conceptually novel challenge that will provide more excitement for future studies.

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