

Report

Post-Translational Regulation of Circadian Transcriptional CLOCK(NPAS2)/BMAL1 Complex by CRYPTOCHROMES

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Original manuscript submitted: 03/02/06

Manuscript accepted: 03/07/06

Previously published online as a *Cell Cycle* E-publication:

<http://www.landesbioscience.com/journals/cc/abstract.php?id=2684>

KEY WORDS

circadian, posttranslational modifications, CLOCK, BMAL1, CRYPTOCHROME, transcriptional regulation

ABBREVIATIONS

BMAL1	brain-and-muscle ARNT-like protein
NPAS2	neuronal PAS-domain protein 2
CRY	cryptochrome
SCN	suprachiasmatic nucleus
CT	circadian time
HAT	histone acetyl transferase
LMP-B	Leptomycin B

NOTE

Supplementary Figures can be found at: <http://www.landesbioscience.com/journals/cc/supplement/kondratovCC5-8-sup.pdf>

ACKNOWLEDGEMENTS

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ABSTRACT

Mammalian CLOCK(NPAS2), BMAL1 and CRYPTOCHROMES are core components of the circadian oscillatory mechanism. The active CLOCK/BMAL1 or NPAS2/BMAL1 complexes regulate expression of numerous genes including two *Cryptochromes*. The products of these genes, CRY1 and CRY2, in turn repress CLOCK/BMAL1 transcriptional activity by an unknown mechanism. We have examined the effect of CRYPTOCHROMES on posttranslational modifications and intracellular distribution of endogenous and ectopically expressed CLOCK(NPAS2) and BMAL1 proteins. We found that ectopic coexpression with CRY led to stabilization and nuclear accumulation of unphosphorylated forms of the proteins, which directly correlated with the inhibition of their transcriptional activity. This effect was CRY-specific, as other known repressors of CLOCK/BMAL1 and NPAS2/BMAL1 transcriptional activity were not able to induce similar effects. CRYs had no effect on CLOCK(NPAS2)/BMAL1 complex formation or its ability to bind DNA. Altogether, these results demonstrate that CRYs regulate the functional activity of circadian transcriptional complex at the posttranslational level. Importantly, the posttranslational modifications and intracellular distribution of CLOCK and BMAL1 proteins were critically impaired in the tissues of mice with targeted disruption of both *Cry* genes, thus confirming the suggested role of CRY in clock function in vivo. Based on these findings we propose a modified model of the circadian transcriptional control, which implies CRY-mediated periodic rotation of transcriptionally active and inactive forms of CLOCK/BMAL1 on the promoter. This model provides mechanistic explanation for previously reported dual functional activity of CLOCK/BMAL1 and highlights the involvement of the circadian system in modulating the organism's response to various types of genotoxic stress, including chemotherapy and radiation.

INTRODUCTION

Circadian rhythms are generated by an intracellular clock mechanism that involves a network of transcriptional feedback loops that drive rhythmic RNA and protein expression of key clock components.¹⁻³ In the SCN - the master pacemaker of the mammalian clock system—as well as in some peripheral tissues, circadian transcription is controlled by the bHLH-PAS-domain transcription factors CLOCK and BMAL1. In brain regions other than the SCN and in the vasculature, the major circadian loop operates through BMAL1 interaction with the closest CLOCK paralog NPAS2.⁴⁻⁶ Both CLOCK and NPAS2 form complexes with BMAL1; the heterodimers directly bind to DNA and activate transcription from E box containing promoters.^{7,8}

Circadian system plays an important role in modulating various biological responses, including the organism's response to genotoxic stress induced by chemotherapy and irradiation. At the molecular level, this response directly correlates with the transcriptional activity of the CLOCK/BMAL1 complex.^{9,10} Recently we have demonstrated that CLOCK/BMAL1 complex possesses dual functional activity working both as activator and repressor of transcription.¹¹ Such a transition from a transactivating to a transrepressing state occurs upon the interaction with CRYPTOCHROMES—transcriptional targets of CLOCK/BMAL1 complex that suppress CLOCK/BMAL1 and NPAS2/BMAL1-mediated transcription.^{12,13} However, the exact molecular mechanism of this suppression has not been yet defined.

Posttranslational modifications play an important role in the regulation of numerous transcription factors, including components of the circadian system.¹⁴⁻¹⁶ Transcriptional activity of CLOCK/BMAL1 complex depends on its phosphorylation status and is closely coupled with CLOCK and BMAL1 degradation.¹⁵ Therefore, we hypothesized that CRYs

might inhibit CLOCK(NPAS2)/BMAL1 transcriptional activity by affecting post-translational regulation of the complex. To test this hypothesis, we ectopically expressed CLOCK(NPAS2), BMAL1 and CRY1 in different combinations and examined them biochemically and functionally in a way similar to our previous studies.^{11,15} Here we report that CRY expression leads to a nuclear accumulation of unphosphorylated CLOCK(NPAS2)/BMAL1 complex, which remains bound to responsive promoter thus blocking its activation. We also present the evidence that CRY controls CLOCK and BMAL1 in similar way in vivo as both the stability and the intracellular distribution of both proteins are impaired in the tissues of *Cry1^{-/-}Cry2^{-/-}* knockout animals. Based on these results we propose a model, which involves an additional level of regulation within circadian transcription-translation feedback loop.

MATERIALS AND METHODS

Mammalian expression plasmids. Luciferase reporter plasmids with *Per1* and *Cry1* promoter regions were described in reference 17; CRY1 and CRY2 expressing plasmids in reference 13; DEC1 and DEC2 expressing plasmids in reference 18 and HA-*Clock* and HA-*Bmal1* in reference 15. *Npas2* was amplified with 5'-GCAGATCTGACGAAGATGAGAAGGAT-3' and 5'-ATACTC-GAGCTATCCCCGGCTGCTGGG-3' and cloned in BamHI/XhoI sites of *pcDNA3-HA*. CLOCK and BMAL1 were fused with YFP from Clontech.

Cell culture and transient transfection. HEK293 cells were maintained in DMEM with 10% of fetal calf serum. Transfection was done using LipofectAMINE PLUS reagents (Invitrogen) according to manufacturer's protocol. Cells were collected for analysis 36 hours after transfection. All transient transfection experiments were normalized by coexpression of CMV-LacZ plasmid. β -Galactosidase and luciferase activity detection were performed as previously described.¹⁵

Western blotting, gel retardation assay and detection of YFP fused proteins. Cells were collected 36 hours after transfection. Lysis procedure, electrophoretic separation, transfer and detection with anti-HA antibody (Santa Cruz Biotechnology) as well as EMSA analysis were performed as previously described.¹⁵ CLOCK-YFP and BMAL1-YFP fused proteins were detected 36 hours after transfection. Cells were fixed with 10% formaldehyde and nuclei were stained with DAPI for visualization. Immunoblotting and fractionation of liver lysates were performed as described previously.¹⁴ Primary antibodies used for CLOCK, BMAL1, CRY1 and CRY2 were CLK-1-GP, BM1-2-GP, C1-GP and C2-GP, respectively.^{14,19}

Animal handling and tissue collection. *Cry1^{-/-}Cry2^{-/-}* double-knockout mice originally obtained from Dr. Sancar's laboratory were backcrossed to C57BL/6J strain (10 backcross generations). Animals were maintained on a 12:12 light:dark cycle for 2 weeks prior to transfer to constant darkness conditions. At the designated times on the second cycle in constant darkness animals were sacrificed, livers were dissected and processed for RNA extraction as previously described.²⁰

Real-time RT-PCR. Analysis of gene expression and RNA abundance calculations were performed by real-time RT-PCR as described (ref. 20). Probes and primers for *Rev-erb α* and *Per2* were described in reference 20, for *Dbp*—in reference 21.

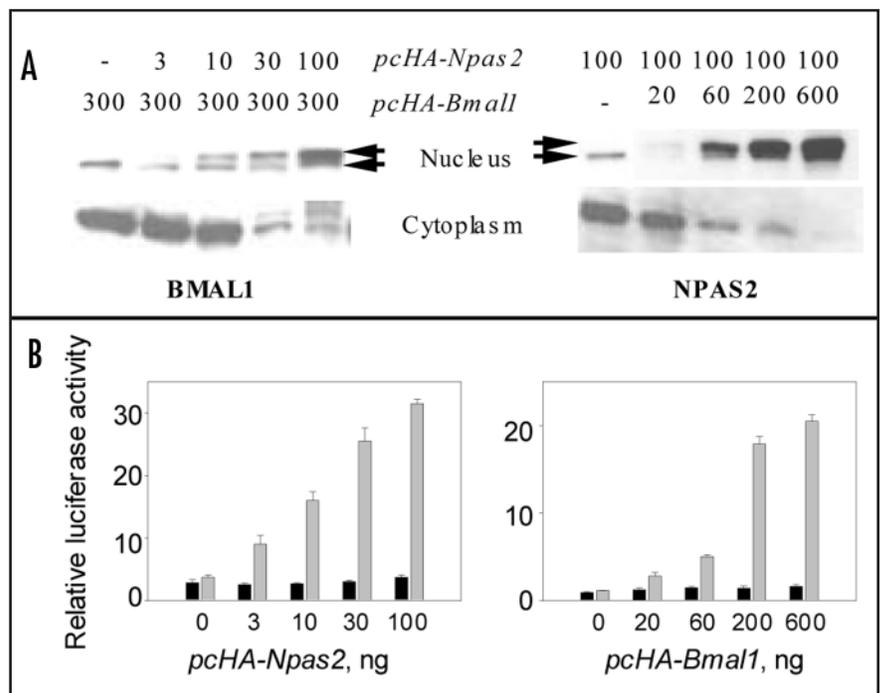


Figure 1. Posttranslational modifications of NPAS2/BMAL1 complex. (A) Western blot analysis of nuclear and cytoplasmic extracts of cells transiently transfected with HA-*Npas2* and HA-*Bmal1* and incubated with HA antibodies; (B) luciferase reporter assay of the cells transiently transfected with indicated constructs.

RESULTS

NPAS2/BMAL1 interaction induces their post-translational modification. Because posttranslational modifications play an important role in the regulation of many transcription factors, including components of the circadian system,^{14,15} we hypothesized that the circadian repressor mechanism might be based on posttranslational regulation of CLOCK/BMAL1 (or NPAS2/BMAL1) induced by their interaction with CRYs. This hypothesis originated from our previous report demonstrating that CLOCK/BMAL1 heterodimerization triggers a series of posttranslational events regulating stability, intracellular distribution and functional activity of the circadian transactivation complex.¹⁵ To check if a similar type of regulation occurs upon dimerization of BMAL1 with NPAS2, we examined their interaction both biochemically and in a transactivation assay. The results of Western blot analysis demonstrate that, similar to CLOCK, when NPAS2 and BMAL1 are expressed individually, they migrate on a gel as single bands; however their coexpression results in the nuclear detection of additional slow-migrating bands (Fig. 1). The fact that these bands are eliminated after the treatment of cell extracts with lambda phage protein phosphatase (data not shown), indicates that they correspond to phosphorylated forms of both proteins previously described for CLOCK and BMAL1.^{14,15} Nuclear accumulation of phosphorylated forms of both proteins was dose-dependent and correlated with the transactivation ability of the complex measured by the luciferase reporter assay performed with the same extracts that were used for the Western blot analysis (Fig. 1). These results allowed us to conclude that upon interaction with BMAL1, NPAS2 is regulated at the post-transcriptional level in a way similar to CLOCK.

CRYPTOCHROMES regulate CLOCK(NPAS2)/BMAL1 complex at the post-translational level: nuclear accumulation of unphosphorylated forms of CLOCK(NPAS2)/BMAL1 correlates with the inhibition of their transcriptional activity. To test the effect of CRY on NPAS2/BMAL1 transactivation complex, we transiently transfected cells with different combinations of NPAS2, BMAL1 and CRY1 expressing plasmids and examined all three proteins' cellular distribution and functional activity. As expected, CRY1

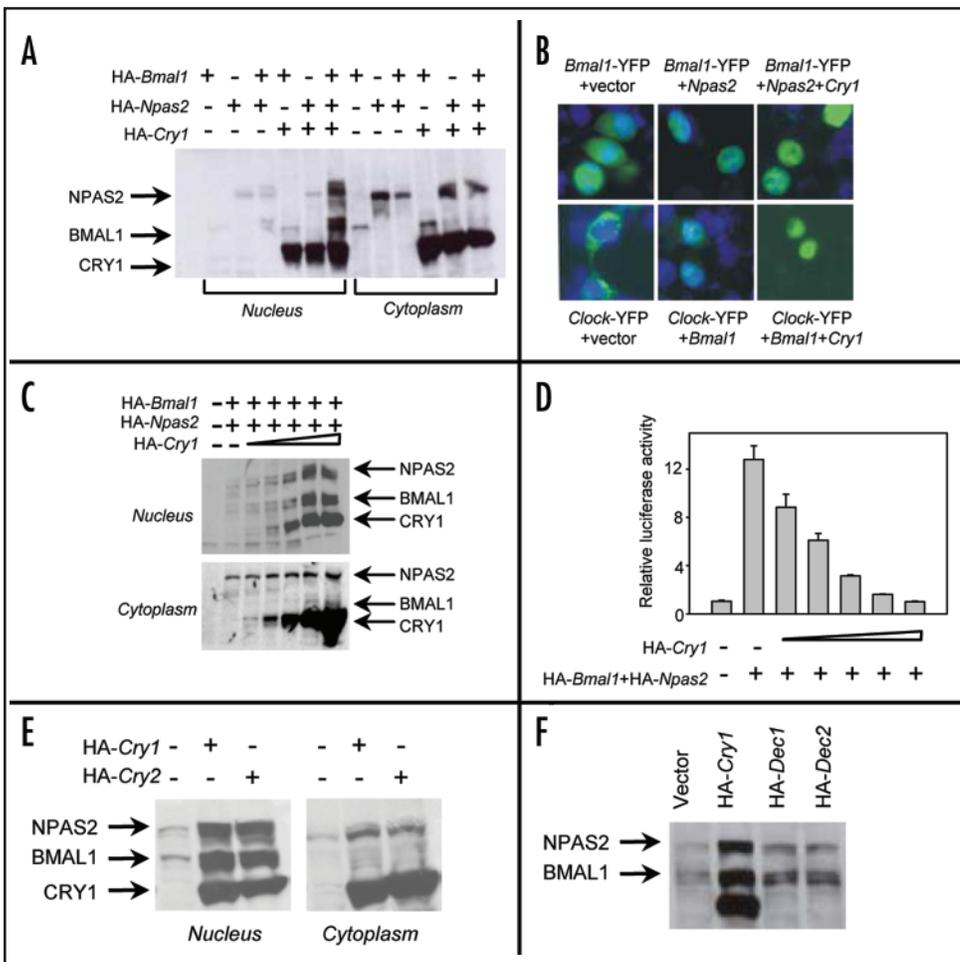


Figure 2. Posttranslational regulation of CLOCK, NPAS2 and BMAL1 by CRYPTOCHROMES. (A) CRY1-induced nuclear accumulation of NPAS2/BMAL1 complex. 293 cells were transfected with indicated plasmids and analyzed by Western blot. (B) In situ detection of CLOCK-YFP (upper panels), BMAL1-YFP (lower panels) in 293 cells upon coexpression of indicated plasmids. Green staining for YFP and blue staining for DAPI (nuclei). (C and D) Dose dependence of CRY1 effect on (C) nuclear accumulation of NPAS2/BMAL1 complex and (D) transactivation of *Per1* promoter. (E) CRY2 acts similar to CRY1. (F) DEC1 and DEC2 have minor effect on NPAS2/BMAL1 nuclear accumulation.

inhibited NPAS2/BMAL1 transcriptional activity (Supplementary Fig. 1). The expression levels of NPAS2 and BMAL1, or their intracellular localization, were not changed when they were individually expressed with CRY1. However, coexpression of all three proteins significantly increased the amount of both phosphorylated and unphosphorylated forms of NPAS2 and BMAL1 in the nucleus but had a relatively minor effect on their cytoplasmic pools (Fig. 2A). CRY1-induced increase in the nuclear accumulation was also confirmed by in situ detection of BMAL1-YFP and CLOCK-YFP in the cells cotransfected with different combinations of expression plasmids (Fig. 2B). The increase in NPAS2 and BMAL1 nuclear accumulation directly correlated with the dose of CRY1 used in transfection (Fig. 2C). Interestingly, coexpression with CRY1 facilitated nuclear accumulation of both phosphorylated and unphosphorylated forms of proteins (Fig. 2C), whereas the expression of NPAS2 and BMAL1 in the absence of CRY1 lead to an increase in abundance of phosphorylated forms only (Fig. 1). Importantly, such CRY1 dose-dependent accumulation of unphosphorylated forms of NPAS2 and BMAL1 correlated with the decrease in their ability to activate the responsive promoter (Fig. 2D). A similar effect was observed when NPAS2 and BMAL1 were coexpressed with CRY2 (Fig. 2E) or when CLOCK expression plasmid was used instead of NPAS2 (Fig. 2B and data not shown). This effect was CRY-specific, as the members of the other class of bHLH proteins, DEC1 and DEC2, which effectively repress

CLOCK/BMAL1- and NPAS2/BMAL1-induced transactivation²² (Supplementary Fig. 2), did not affect nuclear accumulation of the complex (Fig. 2F). This indicates that the posttranslational effect of CRYs on NPAS2/BMAL1 is not just a consequence of suppression of transactivation but must have a functional significance.

To further confirm that CRY1 expression affects CLOCK(NPAS2)/BMAL1 at posttranslational level and has no effect either on the formation of the transactivation complex or its DNA binding activity, we performed EMSA analysis of nuclear extracts of cells expressing different combinations of CLOCK, BMAL1 and CRY1. This analysis showed that, consistent with previous reports,^{14,23} CRY1 had no effect on the DNA binding properties of CLOCK/BMAL1 (Supplementary Fig. 3). CRY1 itself was not detected as part of the complex of CLOCK/BMAL1 with DNA at least in vitro, as anti-HA antibodies in the presence of CRY1 did not induce a supershift. This data also suggests that CRY1 does not disrupt the complex formation. Altogether, these results demonstrate that CRY1 controls functional activity of circadian transactivation complex at posttranslational level. Recently reported increase in the nuclear staining of zCLOCK/zBMAL complex in the presence of zCRY suggests that the similar type of regulation may exist in the zebra fish clock.²⁴

CRYPTOCHROMES' expression leads to stabilization of unphosphorylated form of CLOCK(NPAS2)/BMAL1 complex. CRY may control intracellular distribution of NPAS2/BMAL1 either by modulating its nuclear import/export or by affecting the stability of the complex. To distinguish between these two possibilities, we coexpressed NPAS2 with a BMAL1 mutant, in which both nuclear localization domains in the HLH region were deleted (BMAL1- Δ NLS1,2).

When expressed individually, BMAL1- Δ NLS1,2 demonstrated mostly cytoplasmic localization and was deficient in transactivation (Fig. 3A and Supplementary Fig. 4). Coexpression with NPAS2 induced cytoplasmic accumulation of phosphorylated forms of both proteins, suggesting that upon heterodimerization, phosphorylation of NPAS2/BMAL1 starts in the cytoplasm. In contrast to full-length BMAL1, nuclear accumulation of BMAL1- Δ NLS1,2 in complex with NPAS2 was not stimulated by CRY1 (Fig. 3A). Leptomycin B—the inhibitor of nuclear export—had no significant effect on NPAS2/BMAL1 cellular distribution (Fig. 3B). However, treatment of the cells coexpressing NPAS2 and BMAL1 with proteasome inhibitor MG132 significantly increased the abundance of the complex and mainly of the unphosphorylated forms of both proteins (Fig. 3B).

This observation was further confirmed by the quantitative analysis of the Western blots of cells expressing various combinations of proteins after different periods of cycloheximide treatment. No effect of CRY1 on the half-life of phosphorylated BMAL1 was detected, however the half-life of the unphosphorylated form in the presence of CRY1 increased from 50–60 min to 6–8 hours (Fig. 3C). Similar results were obtained for the half-lives of phosphorylated and unphosphorylated forms of nuclear NPAS2 (data not shown). Thus, nuclear accumulation of NPAS2/BMAL1 complex after CRY1 expression occurs due to stabilization of unphosphorylated forms of NPAS2/BMAL1 rather than control of the nuclear/cytoplasmic transport of the

complex. It is important to note that CRY1 most likely does not prevent phosphorylation, as its expression leads to nuclear accumulation of both phosphorylated and unphosphorylated forms of NPAS2 and BMAL1 proteins (Fig. 1), however CRY1-dependent stabilization is specific to unphosphorylated forms only.

Nuclear accumulation of CLOCK and BMAL1 proteins is impaired in the *Cry1^{-/-}Cry2^{-/-}* cells. To expand our observations made in transient transfection experiments to in vivo interactions of circadian proteins, we analyzed CLOCK and BMAL1 proteins in the liver extracts obtained from wild-type and *Cry1^{-/-}Cry2^{-/-}* double-knockout mice. Animals were placed in constant darkness conditions, tissue samples were collected at the known times of the peak (CT18) and the trough (CT6) of CLOCK and BMAL1 expression^{14,15} and protein extracts were analyzed on Western blots with specific antibodies.

As shown previously,¹⁴ liver extracts contain multiple forms of CLOCK and BMAL1 proteins corresponding to two splice variants of CLOCK as well as to phosphorylated and unphosphorylated forms of both proteins. As expected, the amount of CLOCK and BMAL1 proteins in wild type cells were higher at CT18 when compared to CT6, while in *Cry1^{-/-}Cry2^{-/-}* cells their expression lost rhythmic changes and was significantly reduced at both times tested (below the minimal expression level in the wild type cells) (Fig. 4A). Importantly, nuclear/cytoplasmic fractionation of liver extracts isolated at CT18 demonstrated that the amount of nuclear CLOCK and BMAL1 proteins in *Cry1^{-/-}Cry2^{-/-}* cells were strongly decreased when compared to wild type, while their abundance in the cytoplasm was practically not changed (Fig. 4B). Such a decrease in the total amount of CLOCK and BMAL1 proteins can be partially explained by the observed reduced expression of the corresponding genes in the livers of *Cry1^{-/-}Cry2^{-/-}* animals (Supplementary Fig. 5), which may result from the upregulation of REV-ERB α —a suppressor of the *Bmal1* promoter.²⁵ However, it cannot account for the differential effect of CRY on nuclear and cytoplasmic fractions of both proteins, suggesting that, consistent with the data obtained in the transient transfection experiments (Fig. 2), CRYs are involved in post-translational control of CLOCK/BMAL1 complex in vivo. This effect of CRY on stability and intracellular distribution of circadian transactivators also provides the most compelling explanation for the previously

Figure 4. Post-translational regulation of CLOCK and BMAL1 in *Cry*-deficient mice. (A) The circadian rhythms in CLOCK abundance and phosphorylation are lost and CLOCK levels are greatly reduced in *Cry*-deficient mice. Total liver extracts were prepared from tissue samples of wild-type and *Cry*-deficient mice collected at CT06 and CT18. These extracts were immunoblotted for CLOCK, BMAL1, CRY1 and CRY2. For CLOCK, 1 and 3 are non-phosphorylated isoforms, whereas 2 and 4 represent phosphorylated isoforms. The arrows denote nonspecific bands that ensured equal loading of total proteins from the liver extracts. (B) The reduced levels of CLOCK and BMAL1 are much more pronounced in the nucleus of *Cry*-deficient mouse liver. Total liver extracts isolated from wild-type and *Cry*-deficient mice were fractionated into cytoplasmic (C) and nuclear (N) fractions. These fractions were immunoblotted for CLOCK and BMAL1. Nonphosphorylated and phosphorylated forms of CLOCK are numbered the same as in (A). The arrow denotes a nonspecific band. (C) The maximum abundance of nuclear CLOCK and BMAL1 does not correlate with the maximum of their transcriptional activity. The temporal expression profiles of several known direct targets of CLOCK/BMAL1 transactivation complex in the liver. Tissue samples were collected during second cycle of exposure to constant darkness conditions and processed for RNA extraction and quantitation as described in Materials and Methods.

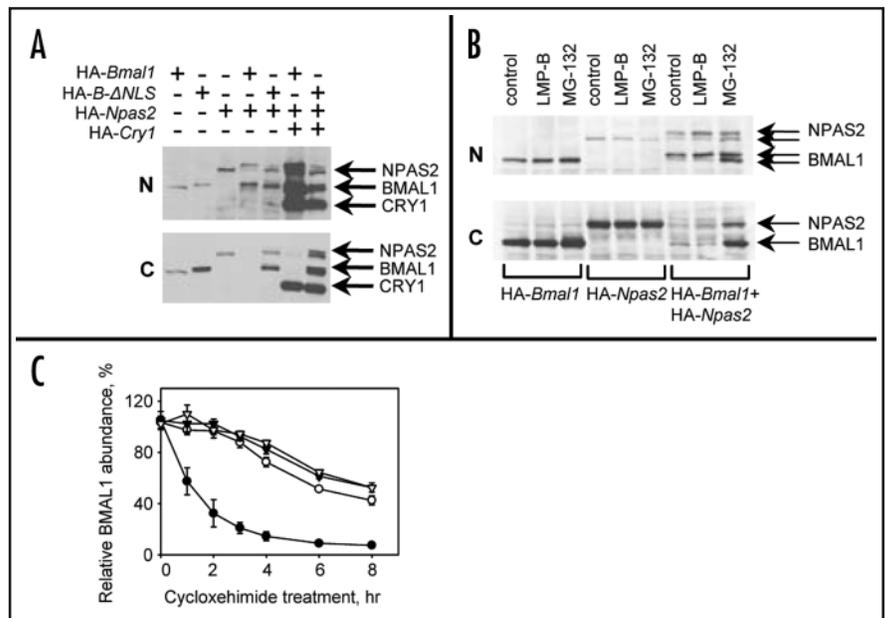
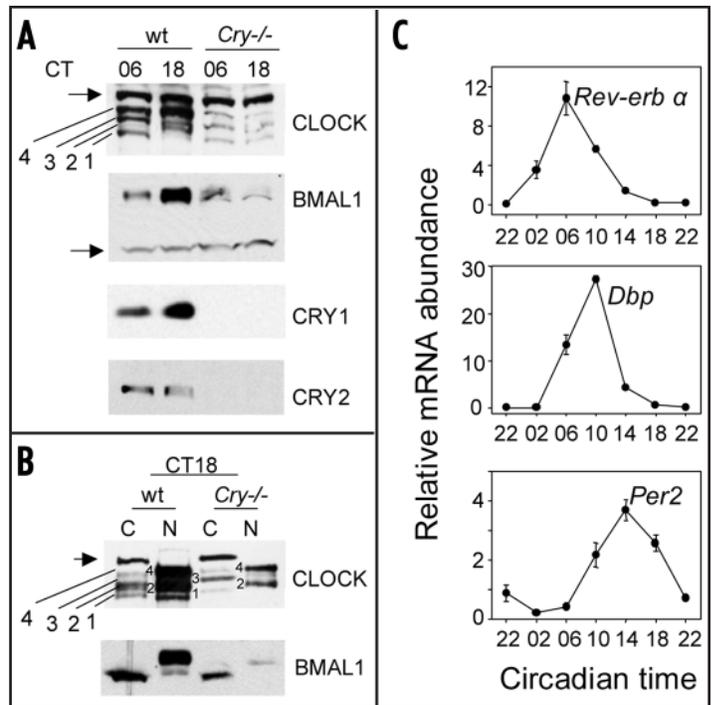


Figure 3. CRYPTOCHROME stabilizes unphosphorylated form of NPAS2/BMAL1 complex. (A) CRY1 does not affect NPAS2/BMAL1 nuclear translocation, Western blot of the 293 cells transfected with indicated expression constructs. (B) Western blot of 293 cells transfected with indicated plasmids and treated with LMP-B or MG132 for 6 hours. (C) CRY1 expression affects half-life of unphosphorylated (closed circles and triangles) and phosphorylated (open circles and triangles) forms of BMAL1. Quantitative analysis of the Western blot of the 293 cells transfected with NPAS2/BMAL1 (closed and open circles) or NPAS2/BMAL1/CRY1 (closed and open triangles) and treated with protein synthesis inhibitor cycloheximide for indicated time periods. At zero time point each protein amount is set to 100%; results are the average of two independent experiments.

made paradoxical observations that the maximum amount of CLOCK and BMAL1 proteins in the nucleus does not directly correlate with the maximum of their transcriptional activity monitored by the expression level of several known and well-characterized direct transcriptional targets of CLOCK/BMAL1 complex¹⁵ (Fig. 4).



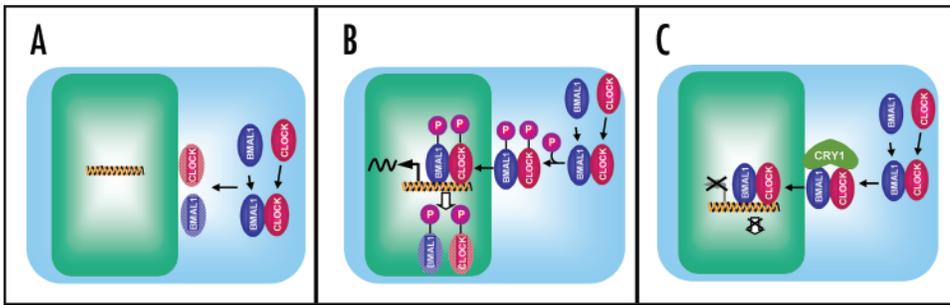


Figure 5. Schematic representation of the circadian transcriptional complex operation. See details in the text.

DISCUSSION

The current view on molecular mechanism of circadian oscillator operation suggests that the negative feedback loop is established through the CRY-mediated inhibition of CLOCK/BMAL1-dependent transactivation of the responsive promoters, including its own (reviewed in Ref. 1). In an attempt to understand the molecular mechanisms of this inhibition, we studied the effect of CRY proteins on posttranslational modifications of CLOCK/BMAL1 and NPAS2/BMAL1 complexes. Such an approach was based on our previous data demonstrating that transcriptional activity of CLOCK/BMAL1 complex depends on its phosphorylation status and that transactivation of responsive promoters correlates with nuclear accumulation of phosphorylated forms of both proteins.¹⁵ Here we present first in vitro and in vivo evidences that CRYs are indeed involved in posttranslational regulation of circadian transactivation complex and that such control is based on CRY-mediated effects on stability and intracellular distribution of CLOCK/BMAL1 and NPAS2/BMAL1.

Based on our previous data¹⁵ and the results presented here, we propose the following modification of the existing model of circadian transcriptional control (Fig. 5). CLOCK/BMAL1 heterodimerization is a signal for both proteins' degradation, which occurs in the cytoplasm (or probably in the process of nuclear transport) (Fig. 5A). If the complex gets phosphorylated by yet unknown kinase(s), it can enter the nucleus, activate transcription of target genes and become degraded after transcriptional initiation to allow the next round of activation (Fig. 5B). Thus, the phosphorylation serves as a stabilization signal during nuclear/cytoplasm translocation and is important for the transactivation process. However, if CLOCK/BMAL1 complex interacts with CRYs, it leads to stabilization of unphosphorylated and, presumably, transcriptionally inactive forms of both proteins, which are translocated to the nucleus and bind to the same elements in the promoters of target genes thus interfering with the activity of phosphorylated circadian transactivation complex and with other transcription factors (Fig. 5C).

The existing model of circadian transcriptional control suggests that CLOCK/BMAL1 complex remains constantly bound to DNA throughout the circadian cycle and that its regulation occurs through rhythmic interaction of the DNA-bound complex with either negative (CRYPTOCHROMES and/or PERIODS) or positive (histone acetylase p300, HAT) elements in a competitive mode.¹⁷ Our data extend this model by suggesting a rotation of transcriptionally active and inactive forms of CLOCK/BMAL1 on the promoter. It postulates that CRY expression leads to stabilization of promoter-bound transcriptionally inactive unphosphorylated forms of CLOCK/BMAL1.

As a result, transcriptionally active complex that interacts with HATs cannot bind to the promoter and recruit HAT to chromatin. Rhythms in the promoter occupation by phosphorylated and unphosphorylated forms result in rhythms in chromatin acetylation/deacetylation described for promoter regions of several circadian genes.^{17,26}

Interestingly, the novel functional role of mammalian CRYs as regulators of CLOCK/BMAL1 stability proposed here is consistent with the known functional activities of CRYs described in other than

mammalian systems. Regardless of species-related differences, in all studied organisms CRYs are involved in proteasome-mediated protein degradation. Thus, *Arabidopsis* CRYs attenuate HY5 degradation;²⁷ *Drosophila* CRY increases light-dependent TIM degradation,²⁸ while in mammals mCRY proteins prevent ubiquitylation and degradation of mPER2 in a light-independent manner.²⁹ Our data allow speculation that rather than developing some novel system for controlling CLOCK/BMAL1 functional activity, mammalian CRYs may utilize the proteasome-related mechanism.

In summary, presented data demonstrate an important role of posttranslational modifications of major clock components in the regulation of circadian clock function. It also shows that circadian transcriptional control in many aspects functions similar to other systems. Thus, the functional coupling of posttranslational modifications and degradation to transactivation has been described for various transcription factors and forms the basis for modern theories of transcriptional control.³⁰ These models imply coordinated dynamic interaction of transcription factors and cofactors with chromatin and assume that ubiquitylation and subsequent degradation of transcription factors is an important step of transcription initiation and/or elongation.

Importantly, if degradation of transcription factors is essential for transcriptional activation of certain genes, the inhibition of this degradation may lead to suppression of the same genes. This view is supported by our recent data, which demonstrate that CLOCK/BMAL1 complex possesses dual functional activity, turning into transcriptional repressor upon interaction with CRY.¹¹ Results presented here suggest that such a change may be achieved through the CRY-dependent stabilization of unphosphorylated CLOCK/BMAL1 complex. Although the molecular details of circadian repressor function require additional studies, it clearly represents an unusual mechanism of transcriptional control based on a switch from transcriptional activation to transcriptional repression depending on the phosphorylation status of the components. The elucidation of the molecular details of such regulation will significantly enhance the development of a rational system for identification of pharmacological modulators of the circadian clock system. Recent data on functional interplay between the circadian and stress response systems provide a rationale for the use of such modulators, in combination with existing genotoxic treatments, to increase their efficiency.

Acknowledgements

We thank Dr. S. Reppert for CRY1 and CRY2 expressing plasmids; Dr. Bingfan Yan for the DEC1 and DEC2 expression plasmids; Rashmi Kannagal for the help with RNA extractions and Elena Vykhovanets for maintaining the mutant mouse colony. We are also grateful for Robert

Simone for technical help on manuscript preparation and Aatur Singhi for helpful comments. This work was supported by NIH grant CA102522 (M.P.A.).

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