Phosphorylation is a major regulatory mechanism controlling circadian clocks. In the Neurospora circadian clock, the PER-ARNT-SIM (PAS) domain-containing transcription factor, WHITE COLLAR (WC)-1, acts both as the blue light photoreceptor of the clock and as a positive element in the circadian negative feedback loop in constant darkness, by activating the transcription of the frequency (frq) gene. To understand the role of WC-1 phosphorylation, five in vivo WC-1 phosphorylation sites, located immediately downstream of the WC-1 zinc finger DNA binding domain, were identified by tandem mass spectrometry using biochemically purified endogenous WC-1 protein. Mutations of these phosphorylation sites suggest that they are major WC-1 phosphorylation sites under constant conditions but are not responsible for the light-induced hyperphosphorylation of WC-1. Although phosphorylation of these sites does not affect the light function of WC-1, strains carrying mutations of these sites show short period, low amplitude, or arrhythmic conidiation rhythms in constant darkness. Furthermore, normal or slightly higher levels of frq mRNA and FRQ proteins were observed in a mutant strain containing mutations of all five sites despite its low WC-1 levels. Together, these data suggest that phosphorylation of these sites negatively regulates the function of WC-1 in the circadian negative feedback loop and is important for the function of the Neurospora circadian clock.

Eukaryotic circadian oscillators consist of autoregulatory transcription/translation-based negative feedback loops (1, 2). In these negative feedback loops, the positive elements activate the transcription of the negative elements, whereas the negative elements inhibit their own transcription by inhibiting the activity of the positive elements. In the Neurospora circadian negative feedback loop, such as those in Drosophila and mammals, the positive element is a heterodimeric complex made of two PER-ARNT-SIM (PAS) domain-containing transcription factors, WHITE COLLAR (WC)-1 and WC-2. In the dark, the two WC proteins form a heterodimer through their PAS domains and activate the transcription of the frequency (frq) gene by directly binding to its promoter (3–7). When the amount of FRQ protein reaches a certain level, the homodimeric FRQ in complex with FRH, a FRQ-interacting RNA helicase, represses frq transcription by interacting with the WC complex and preventing its binding to the frq promoter, thus closing the negative feedback loop (3, 8–14).

In addition to their essential role in the circadian negative feedback loop in the dark, WC-1 and WC-2 are required for all known light responses in Neurospora, including the entrainment of the circadian clock (4, 7, 15–21). WC-1 binds to chromophore FAD through its photosensory LOV (light, oxygen, or voltage) domain, a specialized PAS domain, and functions as the blue light photoreceptor for light responses (22–24). Light triggers the formation of a large WC complex and its binding to the promoters of light-inducible genes (23), resulting in light-induced transcription and light responses.

Regulations of circadian clock proteins by phosphorylation are critical for clock functions in all eukaryotic systems examined (9, 25–35). In Neurospora, FRQ, WC-1, and WC-2 proteins are all phosphorylated in vivo (9, 36, 37). Extensive studies of FRQ phosphorylation revealed that its phosphorylation status is determined by both kinases and phosphatasas. Casein kinases I and II, and a calcium/calmodulin-dependent kinase have been shown to be the kinases that phosphorylate FRQ (38–41), whereas protein phosphatase 1 and protein phosphatase 2A counter the effects of the kinase by dephosphorylating FRQ (42). Phosphorylation of FRQ promotes its degradation through the ubiquitin/proteasome pathway mediated by FWD-1, an F-box/WD40 repeat-containing protein, which is part of an SCP-type ubiquitin E3 ligase (29, 43). In addition, the phosphorylation of FRQ regulates the FRQ-WC interaction and is important for the role of FRQ to repress the activity of the WC complex (39, 40, 42). Thus, regulation of FRQ phosphorylation states by its kinases and phosphatasas is critical for circadian periodicity and proper function of the clock.

Like the positive elements in the circadian negative feedback loops of Drosophila and mammals (31, 44), WC-1 and WC-2 proteins are phosphorylated under constant conditions (36, 37). Similar to other blue light photoreceptors, such as the plant cryptochromes and phototropins (32, 45, 46), the WC proteins become hyperphosphorylated after light exposure. The functions of these WC phosphorylation events are not known. Previously, pharmacological studies and in vitro phosphorylation assay suggested that protein kinase C (PKC) might be a kinase that phosphorylates WC-1 (47). Treatment of Neurospora cells by PKC inhibitors led to increased and prolonged light-induced
transcription, suggesting that PKC may be a negative regulator of light responses in *Neurospora*. On the other hand, the transient nature of WC-1 hyperphosphorylation after light exposure and faster degradation rate of WC-1 under light than in the dark suggest that light-induced phosphorylation may lead to WC-1 degradation (36, 48).

WC-1 and WC-2 form WC complexes through the PASC region of WC-1 and the PAS domain of WC-2 (6, 7). Even though the WC complexes activate gene transcription both in the dark and after light exposure, these two functions can be separated molecularly. The deletion of the WC-1 LOV domain abolishes the light function of the protein, but not its dark function (22). On the other hand, deletion of the zinc finger DNA binding domain of WC-1 eliminates the dark function of the protein, but not its light function (7). Thus, WC-1 uses different domains of the protein to carry out its roles in gene activation in the dark and after light exposure. Therefore, regulation of different domains of WC-1 is likely to have a different impact on these two functions.

In this study, to understand the role of WC-1 phosphorylation in the regulation of circadian clock and light responses, we identified five in *vivo* WC-1 phosphorylation sites by mass spectrometry analyses. These sites are located immediately downstream of the zinc finger DNA binding domain. Mutation of these phosphorylation sites showed that although they are not required for the light function of the protein, they negatively regulate the activity of WC-1 in the dark and are important for the function of the circadian clock.

**EXPERIMENTAL PROCEDURES**

**Strains, Culture Conditions, and Race Tube Assay**—The *bd* strain was used as the wild-type strain in this study. A *wc-1(bd)* strain was used as the host strain for various *his-3* targeting *wc-1* constructs (22). Liquid culture conditions were as described previously (8). Race tube assay media contained 1× Vogel’s, 0.1% glucose, 0.17% arginine, and 50 mg/ml biotin. Densitometric analyses of race tubes and calculations of period length were performed as previously described using Chrono II version 11.1 (49). Cultures were harvested by filtration either under red safety light or after light treatment (160 lux). For rhythmic experiments, the *Neurospora* cultures were moved from LL to DD at time 0 and were harvested in constant darkness at the indicated times (hours).

**Identification of in Vivo WC-1 Phosphorylation Sites by Tandem Mass Spectrometry**—Purification of the WC complex was as previously described (22). The SDS-PAGE gel was stained with colloidal blue (Invitrogen). Phosphorylation sites were identified by a combination of precursor ion scanning and nano-electrospray tandem mass spectrometry (MS/MS). The colloidal blue-stained WC-1 protein bands were excised from SDS gels and subjected to in-gel digestion with trypsin essentially as described previously (50). The dried protein digests were dissolved in 5% formic acid and loaded onto a packed capillary filled with POROS R2 resin. After washing three times with 5% formic acid, the peptides were eluted into a nanoelectrospray needle with 1–2 μl of nanoelectrospray sample solutions for either precursor ion scanning in negative ion mode or MS/MS in positive ion mode.

All mass spectrometry analyses were performed on a QSTAR Pulsar-I quadrupole time-of-flight tandem mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with a nanoelectrospray ion source (MDS Proteomics, Odense, Denmark). For precursor ion scanning experiments, the instrument was set in negative ion mode, with the quadrupole Q2 pulsing function turned on, to detect the PO3 acidic fragment ion at m/z ~ 79. The optimum collision energies were determined for each experiment by gradually increasing the voltage of Q0 in steps corresponding to one-twentieth of the m/z value of the precursor ion. After data acquisition by precursor ion scanning, the instrument was switched to positive ion mode, and the phosphopeptide sequence and sites of phosphorylation were identified by nanoelectrospray MS/MS. In the MS/MS scan mode, precursor ions were selected in quadrupole Q1 and fragmented in the collision cell (q2), using argon as the collision gas.

**Plasmid Constructs and Neurospora Transformation**—All point mutations of the WC-1 phosphorylation sites were constructed by using the Transformase site-directed mutagenesis kit (BD Biosciences) using pcw1-3 as the template. The mutagenic primers used were WC1.990A (Ser-990 to Ala), WC1.988.2A (Ser-988 and Ser-990 to Ala), WC1.992.5A (Ser-992, Ser-994, and Ser-995 to Ala), and WC1.988.3A (Ser-988, Ser-992, Ser-994, and Ser-995 to Ala). After mutagenesis, the NsiI-AgeI-digested *wc-1* fragments from the constructs were inserted into the Nai-AgeI-digested pcw1-2 construct. All resulting constructs were confirmed by DNA sequencing and then targeted to the *his-3* locus of the *wc-1* strain by transformation.

**Protein and RNA Analyses**—Protein and RNA analyses were as previously described (6, 8, 11). Equal amounts of total protein (50 μg) were loaded in each protein lane, and the blots were developed by chemiluminescence (ECL, Amersham Biosciences). For Northern blot analysis, equal amounts of total RNA (20 μg) were loaded onto agarose gels for electrophoresis, and the gels were blotted and probed with RNA probe specific for *vdg*, *al-3*, and *frq*. Densitometric analysis of the data was performed using National Institutes of Health IMAGE 1.61. For phosphatase treatments, 50 μg of total protein was digested in 1× phosphatase buffer and treated with 1000 units of a phosphatase (New England Biolabs) for 30 min at 30 °C.

**RESULTS**

**Identification of Five in Vivo WC-1 Phosphorylation Sites by Tandem Mass Spectrometry**—Previously, we created a *Neurospora* strain (Myc-His-WC-2) in which WC-2 was tagged by both c-Myc and His6 epitope tags (22). We showed that the Myc-His-WC-2 can form a functional complex with WC-1 and can rescue the light and circadian clock defects of a WC-2-null strain. Using this strain, we developed a biochemical purification protocol that can obtain pure WC complex. To identify WC-1 phosphorylation sites in *vivo*, large-scale purifications were carried out using extracts of the Mys-His-WC-2 strain grown in DD. In DD, the phosphorylation profile of WC-1 is similar to that of the LL condition, lacking the hyperphosphorylated WC-1 species induced by a light pulse (36, 37). The left panel of Fig. 1A shows the colloidal blue-stained SDS-PAGE gel of the purification products. Similar to our previous result, the WC complex was purified to near homogeneity. The minor bands are mostly degradation products of the WC proteins, except for one contaminating band (labeled with an asterisk). To confirm that the purified WC proteins are still phosphorylated after the purification procedure, a small portion of the purification products were subjected to λ-phosphatase treatment. As shown in the right panel of Fig. 1A, the phosphatase treatment led to the disappearance of the slow mobility WC-1 species, indicating that they were phosphorylated WC-1. No significant gel mobility difference was observed with Myc-WC-2 after the treatment, suggesting that the majority of WC-2 was not phosphorylated under this condition (36). Thus, we only used the purified WC-1 protein for phosphorylation site mapping studies.

The colloidal blue-stained protein bands corresponding to WC-1 were excised from SDS gels and subjected to trypsin digestion. The resulting peptides were then analyzed by nanoelectrospray MS/MS (50). Most of the identified phosphopeptides matched to one trypsin-digested fragment, corresponding to amino acids 988–999 of WC-1. Fig. 1B shows a representative result of the MS/MS analysis for one of the phosphopeptides, which carries a single phosphorylated serine. In this peptide, Ser-990 was identified as the phosphorylation site. Additional mass spectrometry results indicate that, in some of the phosphopeptides, all five serines in this fragment are phosphorylated. Thus, Ser-988, Ser-990, Ser-992, Ser-994, and Ser-995 are WC-1 phosphorylation sites in *vivo*. The protein fragment containing these five phosphorylation sites is located immediately downstream of the zinc finger DNA binding domain (Fig. 1C). Interestingly, two of the phosphorylated serines are followed by a proline residue, suggesting that these phosphorylation events may be mediated by a proline-directed kinase.

**Phosphorylation of These Sites is Light-independent**—Although mass spectrometry can identify *in vivo* phosphorylation
sites, it does not provide information on the extent of phosphorylation of the identified sites in vivo. To confirm that these five residues represent major WC-1 phosphorylation sites in vivo under different conditions, constructs were created in which these sites were mutated to alanines either singly or in combination, and the constructs were transformed into a wc-1-null strain (wc-1IRI) (22). As shown in Fig. 2, A and B, mutation of three of the sites (3A; S988A/S990A/S992A) or all five sites (5A; all five sites were mutated to alanines) led to mostly hypophosphorylated WC-1 forms in LL and DD, as indicated by the significantly reduced phosphorylated WC-1 species and the result of the phosphatase treatment. These data indicate that these sites are major WC-1 phosphorylation sites under constant conditions.

To examine whether the phosphorylation of these sites is responsible for the light-induced hyperphosphorylation of WC-1, phosphorylation profiles of WC-1 of the wild-type and 5A strains were monitored after a transition from DD to LL. As shown in Fig. 2C, after 15–30 min of light treatment, the light-induced hyperphosphorylation of WC-1 was similar in both the wild-type and 5A strains. Together with the results in Fig. 2, A and B, these data suggest that these five phosphorylation sites are not involved in the light-induced hyperphosphorylation of WC-1 and that their phosphorylation is light-independent.

To exclude the possibility that the normal light-induced WC-1 hyperphosphorylation profile observed in the 5A mutant was due to phosphorylation of other unidentified sites in this region, we monitored the light-induced WC-1 phosphorylation in two additional mutant strains, bNLS (deletion of the putative nuclear localization signal and the WC-1 C-terminal region) and NLSa (deletion of the zinc finger and the C-terminal region) (7), in which the entire zinc finger region and C-terminal part of the protein were deleted. As shown in Fig. 2D, the light-induced hyperphosphorylation of WC-1 was maintained in these two strains, indicating that the light-dependent hyperphosphorylation of WC-1 is due to phosphorylation of unidentified sites in the N-terminal part of WC-1. Because the size of WC-1 in these deletion mutants is smaller than the wild-type WC-1, their light-induced phosphorylation appeared to be more extensive.

In addition to the hypophosphorylation of WC-1 in the 3A and 5A mutants, their WC-1 levels were significantly reduced compared with the wild-type strain. The levels of WC-1 in the 3A and 5A mutants were about 30% and 20% of the wild-type levels, respectively, suggesting that the phosphorylation of the 5A strains was reduced at all levels.
these sites may be important for maintaining the steady-state levels of WC-1. The low levels of the WC-1 proteins in the mutants are not due to their expression at the his-3 locus because the wild-type wc-1 construct (wc1-2) was expressed at normal level (Fig. 2A). In addition, Northern blot analysis showed that the expression levels of wc-1 mRNA in the mutants were comparable with that of the wild-type strain (data not shown). When WC-1 stability was measured in the presence of the protein synthesis inhibitor cycloheximide, the degradation rates of WC-1 were not significantly different between the wild-type and the 5A strains (Fig. 2E), suggesting that the phosphorylation of these sites does not promote WC-1 degradation. Thus, it is likely that these mutations may affect WC-1 protein folding after its synthesis (improperly folded protein would be quickly degraded), leading to low levels of WC-1 without affecting the stability of the properly folded protein.

Light-induced Transcription in the 5A Mutant—To examine whether the phosphorylation of these five sites regulates the light function of WC-1, light-induced transcription in the 5A mutant was compared with that in the wild-type strain. Light induction of al-3 was significantly reduced (Fig. 3A), whereas the light induction of vvd was only modestly affected (Fig. 3B). For frq, induction in the mutant was similar to that of the wild-type strain. Because of the different WC-1 requirements for light induction of these three genes, the changes in light-induced transcription observed for al-1 and vvd are most likely due to the reduction of WC-1 levels in the 5A mutant rather than changes in WC-1 activity. Thus, phosphorylation of these five sites does not appear to regulate the light function of WC-1. This interpretation is consistent with our previous results that normal light-induced transcription was observed in strains lacking the WC-1 zinc finger domain and the C-terminal region including these sites (7).

Short Period, Low Amplitude, or Arrhythmic Conidiation Rhythms in DD When WC-1 Phosphorylation Sites Are Mutated—Because the zinc finger DNA binding domain of WC-1 is required for its dark function but not its light function (7) and the identified phosphorylation sites are immediately downstream of the zinc finger domain, it is likely that these phosphorylation events regulate the function of WC-1 in the circadian feedback loop in the dark. Thus, we examined the circadian conidiation rhythms of the wc-1RIP strains carrying a construct with either a single or multiple WC-1 phosphorylation site mutations in DD. In addition to its arrhythmicity on race tubes, our wc-1RIP strain showed a faster growth rate (~15%) than the wild type on race tubes, similar to other...
previously described wc-1 mutants (20). As shown in Fig. 4A, a wild-type wc-1 construct (WC1-2) (6) was able to rescue the robust circadian conidiation rhythmicity of the wc-1RIP strain with a period that was ~1 h longer than that of the wild type. Mutation of a single phosphorylation site (1A; S990A) was also able to rescue the conidiation rhythm; however, its period was 1.6 h shorter than that of the WC1-2 strain. A short period conidiation rhythm was also observed in the 2A strain (S988A/S990A), but its conidiation rhythm became less robust than the 1A strain, as indicated by its broad conidiation bands. When three (3A; S988A/S990A/S992A) or all five sites (5A) were mutated, even though the conidiation bands can be observed in the first 2 days, conidiation became arrhythmic or oscillated with a low amplitude in the following days in DD. The growth rates of these mutants resemble that of the wc-1RIP strain, probably due to their low WC-1 levels. Previously, we have shown that high WC-1 levels lead to a modestly shorter period length of the circadian rhythm in DD (Fig. 2), even though the conidiation bands can be observed in the first 2 days in DD. The low amplitude FRQ rhythm of the 5A strain (especially after 28 h) is consistent with its race tube phenotype in the first 2 days in DD. The comparison of the phosphorylation profile of FRQ between the two strains indicates that the 5A mutant has a shorter period than the wild type (note the ratio between extensively phosphorylated FRQ and newly synthesized hypophosphorylated FRQ at 12 and 28 h). Because the conidiation of the 5A strain became arrhythmic after 2 days in DD, the molecular rhythms observed here are likely due to the light/dark transition. Immunoprecipitation assays suggest that the interaction between FRQ and the WC complex is not affected by the phosphorylation site mutations (data not shown).

Together, these molecular data indicate that the lack of robust circadian rhythmicity in the phosphorylation site mutants is not due to their low WC-1 levels, suggesting that the phosphorylation of WC-1 at these sites negatively regulates its activity as a positive element in the circadian negative feedback loop.

**DISCUSSION**

Phosphorylation of clock proteins is critical for functions of circadian clocks in eukaryotic systems. WC-1, an essential component of the Neurospora circadian clock, functions both as the blue light photoreceptor mediating light input into the clock and as a positive element in the circadian negative feedback loop in the dark, by activating frq transcription. In this study, using mass spectrometry analyses, we identified five in vivo WC-1 phosphorylation sites near the WC-1 zinc finger DNA binding domain. Mutations of these sites indicate that these
Phosphorylation events are not required for the light function of WC-1, but they are important for the function of WC-1 as an activator of frq expression in the dark. Strains with one or two of the phosphorylation sites mutated showed short period circadian rhythms, whereas strains with three or five sites mutated exhibited arrhythmic or low amplitude circadian conidial rhythms. In the mutant with all five sites mutated, despite its low WC-1 levels, the amounts of frq mRNA and FRQ protein in DD are comparable with or slightly higher than those of the wild-type strain. Because the amounts of WC-1 determine the expression level of frq in the dark in a wild-type strain (5), these data suggest that these phosphorylation events negatively regulate the transcription activator activity of WC-1 in the dark. The short period phenotype of the phosphorylation site mutants, therefore, is likely due to their increased activities of WC-1, resulting in earlier activation of frq transcription than seen in the wild-type strain. This interpretation is consistent with the advanced phases of the frq mRNA and FRQ protein observed in the 5A mutant (Figs. 5 and 6). Thus, the regulation of WC-1 activity by phosphorylation of these sites is important for period determination and proper function of the circadian clock. Because the identified phosphorylation sites reside immediately downstream of the WC-1 zinc finger region, which is essential for the dark function of WC-1 but not required for its light function, it is likely that the phosphorylation of this region negatively regulate the DNA binding ability of WC-1 in the dark. This is the first time that in vivo phosphorylation sites and their function have been revealed for a positive element in a eukaryotic circadian negative feedback loop. Like the WC proteins in Neurospora, the positive elements of the circadian negative feedback loop in Drosophila (dCLOCK and mouse (CLOCK and BMAL1) are phosphorylated in vivo (31, 44), but the sites and roles of their phosphorylation are not known.

Hyperphosphorylation of WC-1 was previously observed to be associated with WC-1 degradation in LL (36, 48), suggesting that phosphorylation probably promotes WC-1 degradation. However, in strains with mutations of the identified phosphorylation sites, WC-1 levels are low, and the stability of WC-1 is not significantly altered. In addition, the phosphorylation of these sites is light-independent. These data suggest that the phosphorylation of these five sites does not lead to WC-1 degradation. Thus, the likely role of phosphorylation in regulating WC-1 stability is due to light-dependent phosphorylation of WC-1 in the N-terminal part of the protein. The sites of these phosphorylation events remain to be identified.

Pharmacological studies previously suggested that PKC acts as a negative regulator of light responses in Neurospora, probably by regulating the function of the WC complex directly or indirectly (47). In vitro, PKC can phosphorylate the zinc finger region of WC-1, but it is unclear whether this phosphorylation is relevant in vivo. Although the phosphorylation sites we identified here are located in the zinc finger region, their phosphorylation is unlikely to be due to PKC phosphorylation. First, the amino acid sequence surrounding these five sites does not resemble those of the PKC consensus phosphorylation sites (S/TXXKR), although there are putative sites similar to the PKC sites in the zinc finger region. Second, mutations of these sites or deletions of the zinc finger region do not appear to affect the light-dependent WC-1 phosphorylation and the function of WC-1 in light-induced transcription (7). Thus, the function of PKC in regulating light responses is probably not through its phosphorylation of the zinc finger region of WC-1.

The proline residues next to two of the phosphorylation sites suggest that their phosphorylation is likely mediated by a proline-directed kinase. Interestingly, glycogen synthase kinase-3, a known proline-directed kinase (51, 52), has been shown to regulate the circadian clock in Drosophila (33). Because of the conservation of known clock kinases from Neurospora to animal circadian systems (27, 30, 35, 39, 41, 53), it will be interesting to examine to the role of the Neurospora glycogen synthase kinase-3 in WC-1 phosphorylation.

REFERENCES

1. Dunlap, J. C. (1999) Cell 96, 271–290
2. Young, M. W., and Kay, S. A. (2001) Nat. Rev. Genet. 2, 702–715
3. Froehlich, A. C., Loros, J. J., and Dunlap, J. C. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 5914–5919
4. Crosthwaite, S. K., Dunlap, J. C., and Loros, J. J. (1997) Science 276, 763–769
5. Cheng, P., Yang, Y., and Liu, Y. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7408–7413
6. Cheng, P., Yang, Y., Gardner, K. H., and Liu, Y. (2002) Mol. Cell. Biol. 22, 517–524
7. Cheng, P., Yang, Y., Wang, L., He, Q., and Liu, Y. (2003) J. Biol. Chem. 278, 3801–3808
8. Aronson, B., Johnson, K., Loros, J. J., and Dunlap, J. C. (1994) Science 263, 1578–1584
9. Garreau, N., Liu, Y., Loros, J. J., and Dunlap, J. C. (1997) Cell 89, 489–496
10. Liu, Y., Garreau, N. L., Loros, J. J., and Dunlap, J. C. (1997) Cell 89, 747–748
11. Cheng, P., Yang, Y., Heinzen, C., and Liu, Y. (2001) EMBO J. 20, 101–108
12. Denault, D. L., Loros, J. J., and Dunlap, J. C. (2001) EMBO J. 20, 109–117
13. Merrow, M., Franchi, L., Dragovic, Z., Gorl, M., Johnson, J., Brunner, M., Macino, G., and Roenneberg, T. (2001) EMBO J. 20, 307–315
14. Cheng, P., He, Q., He, Q., Wang, L., and Liu, Y. (2005) Genes Dev. 19, 234–241
15. Ferrari, P., Vittorioso, P., Magrelli, P., Talora, C., Cubillo, A., and Macino, G. (1996) EMBO J. 15, 1650–1657
16. Linden, H., and Macino, G. (1997) EMBO J. 16, 98–109
17. Linden, H., Ballario, P., and Macino, G. (1997) Curr. Top. Cell. Mol. Biol. 22, 141–150
18. Collett, M. A., Garreau, N., Dunlap, J. C., and Loros, J. J. (2002) Genetics 160, 149–158
19. Liu, Y., He, Q., and Cheng, P. (2003) Cell Mol. Life Sci. 60, 2131–2138
20. Lee, K., Dunlap, J. C., and Loros, J. J. (2003) Genetics 163, 103–114
21. Liu, Y. (2003) J. Biol. Rhythms 18, 195–205
22. He, Q., Cheng, P., Yang, Y., Wang, L., Gardiner, K. H., and Liu, Y. (2002) Science 297, 840–843
23. Froehlich, A. C., Liu, Y., Loros, J. J., and Dunlap, J. C. (2002) Science 297, 815–819
24. Cheng, P., He, Q., Yang, Y., Wang, L., and Liu, Y. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 5938–5943
25. Edery, I., Zweibel, L., Dembinska, M., and Roshbash, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2260–2264
26. Kisses, B., Price, J. L., Saez, L., Blau, J., Rothenhuf, A., and Young, M. W. (1998) Cell 94, 97–107
27. Price, J. L., Blau, J., Rothenhuf, A., Adoeeley, M., Kisses, B., and Young, M. W. (1998) Cell 94, 83–95
28. Sugano, S., Andonis, C., Green, R., Wang, Z. Y., and Tobin, E. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11020–11025
29. Liu, Y., Loros, J., and Dunlap, J. C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11020–11025
30. Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamanishi, S., Zemenides, P. D., Ralph, M. R., Menaker, M., and Takahashi, J. S. (2000) Science 288, 483–492
31. Lee, C., Etchegaray, J. P., Cagampang, F. R., Loudon, A. S., and Reppert, S. M. (2001) Cell 107, 855–867
32. Eide, E. J., Vielhaber, E. L., Hinz, W. A., and Virshup, D. M. (2002) J. Biol. Chem. 277, 17248–17254
33. Martinek, S., Izonog, S., Manoukian, A. S., and Young, M. W. (2001) Cell 105, 769–779
34. Tsh, K. L., Jones, C. R., He, Y., Eide, E. J., Hinz, W. A., Virshup, D. M., Ptacek, L. J., and Fu, Y. H. (2001) Science 291, 1040–1043
35. Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M., and Allada, R. (2002) Nature 420, 816–820
36. Talora, C., Franchi, L., Linden, H., Ballario, P., and Macino, G. (1999) EMBO J. 18, 4961–4968
37. Schwerdtfeger, C., and Linden, H. (2000) Eur. J. Biochem. 267, 414–422
38. Yang, Y., Cheng, P., Zhi, G., and Liu, Y. (2002) Genes Dev. 16, 994–1006
39. Yang, Y., Cheng, P., He, Q., Wang, L., and Liu, Y. (2003) Mol. Cell. Biol. 23, 6221–6228
40. Gorl, M., Merrow, M., Huttner, B., Johnson, J., Roenneberg, T., and Brunner, M. (2001) EMBO J. 20, 7074–7084
41. Y., He, Q., Cheng, P., Wrage, P., Yarden, O., and Liu, Y. (2004) Genes Dev. 18, 255–260
42. He, Q., Cheng, P., Yang, Y., He, Q., Yu, H., and Liu, Y. (2003) EMBO J. 22, 4421–4430
43. Lee, C., Bae, K., and Edery, I. (1998) Neuron 21, 857–867
44. Shalitin, D., Yang, H., Meckler, T. C., Maymon, M., Guo, H., Whitelam, G. C., and Lin, C. (2002) Nature 417, 763–767
45. Christie, J. M., Reymond, P., Powell, G. K., Bernasconi, P., Rappakas, A. A., Liscum, E., and Briggs, W. R. (1998) Science 282, 1698–1701
46. Arpaia, G., Cerri, F., Baima, S., and Macino, G. (1999) Mol. Gen. Genet. 262, 314–322
47. Lee, K., Loros, J. J., and Dunlap, J. C. (2000) Science 290, 107–110
48. Roenneberg, T., and Taylor, W. (2000) Methods Enzymol. 305, 104–119
49. Shu, H., Chen, S., DeCamp, D., Huse, R. C., Mumbir, M., and Brekken, D. (2000) ACS Research Reports [url]
50. Frame, S., and Cohen, P. (2001) Biochem. J. 359, 1–16
51. Sasaki, T., Tanaka, M., Ishiguro, K., Uchida, A., Saito, T., Isobe, T., and Hisanaga, S. (2002) J. Biol. Chem. 277, 36632–36639
52. Akten, B., Jauch, E., Genova, G. K., Kim, E. Y., Edery, I., Raabe, T., and Jackson, F. R. (2003) Nat. Neurosci. 6, 251–257