

The *Neurospora crassa* White Collar-1 dependent Blue Light Response Requires Acetylation of Histone H3 Lysine 14 by NGF-1[□]

Benedetto Grimaldi,^{*†} Pierluca Coiro,^{*‡} Patrizia Filetici,[§] Emanuela Berge,^{||¶}
Joseph R. Dobosy,^{||#} Michael Freitag,^{||@} Eric U. Selker,^{||} and Paola Ballario^{*§}

^{*}Dipartimento di Genetica e Biologia Molecolare and [§]Istituto di Biologia e Patologia Molecolari del Consiglio Nazionale Delle Ricerche, Università di Roma “La Sapienza,” 00185 Rome, Italy; and ^{||}Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Submitted March 24, 2006; Revised July 31, 2006; Accepted August 4, 2006
Monitoring Editor: William Tansey

Blue light-induced transcription in *Neurospora crassa* is regulated by the White Collar-1 (WC-1) photoreceptor. We report that residue K14 of histone H3 associated with the light-inducible *albino-3* (*al-3*) promoter becomes transiently acetylated after photoinduction. This acetylation depends on WC-1. The relevance of this chromatin modification was directly evaluated in vivo by construction of a *Neurospora* strain with a mutated histone H3 gene (*hH3^{K14Q}*). This strain phenocopies a *wc-1* blind mutant and shows a strong reduction of light-induced transcriptional activation of both *al-3* and *vivid* (*vvd*), another light-inducible gene. We mutated *Neurospora* GCN Five (*ngf-1*), which encodes a homologue of the yeast HAT Gcn5p, to generate a strain impaired in H3 K14 acetylation and found that it was defective in photoinduction. Together, our findings reveal a direct link between histone modification and light signaling in *Neurospora* and contribute to the developing understanding of the molecular mechanisms operating in light-inducible gene activation.

INTRODUCTION

Biochemical signal transduction has been the object of a multitude of studies, mostly devoted to the identification of the molecular components of specific pathways, their epistatic relationships, and their mechanisms of action. Although light signal transduction pathways have been primarily studied in plants (Chen *et al.*, 2004), the filamentous fungus *Neurospora crassa* has become a preferred eukaryotic model to investigate pathways inducible by blue light. Early biochemical and photobiology studies reported that *Neurospora* is responsive only to blue light (Sargent and Briggs, 1967; DeFabo *et al.*, 1976). Genes encoding putative red light photoreceptors have been recently discovered in the *Neurospora* genome (Galagan *et al.*, 2003; Borkovich *et al.*, 2004), but deletion of these genes does not affect any known photoreponses, leaving their function uncertain (Froehlich *et al.*, 2005).

This article was published online ahead of print in *MBC in Press* (<http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E06-03-0232>) on August 16, 2006.

[□] The online version of this article contains supplemental material at *MBC Online* (<http://www.molbiolcell.org>).

Present addresses: [†] University of California, Irvine, CA 92697; [‡] Charité University of Berlin, D-10098 Berlin, Germany; [#] University of Wisconsin, Madison, WI 53792; [¶] MitoSciences Inc., Eugene, OR 97403; [@] Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331.

Address correspondence to: Paola Ballario (paola.ballario@uniroma1.it).

Abbreviations used: HAT, histone acetyltransferase; LRR, light-responsive region; WCC, White Collar complex.

Two proteins are required for blue light perception in *N. crassa*, White Collar (WC)-1 and WC-2 (Ballario *et al.*, 1996; Linden and Macino, 1997). WC-1 is the photoreceptor (He *et al.*, 2002), with a sensor domain called LOV (for Light, Oxygen, and Voltage), homologous to that of plant phototropins (Huala *et al.*, 1997). WC-1 is associated with WC-2 in vivo, forming a nuclear heterodimer, the White Collar complex (WCC; Talora *et al.*, 1999; Schwerdtfeger and Linden, 2001). At various points in the circadian cycle, other proteins associate with WCC, e.g., the oscillator, Frequency (FRQ; Dunlap and Loros, 2004), and protein kinase C (Franchi *et al.*, 2005), but the two WC proteins are the defining, constant components of this light-sensing complex (Cheng *et al.*, 2002). On blue light irradiation, the conformation of the WCC is thought to change to activate light-dependent genes transiently. The early light-inducible genes, such as *albino-3* (*al-3*) and *vivid* (*vvd*), reach their peak induction 15–20 min after a light pulse and are switched off within 1 h (Baima *et al.*, 1991). Initially, WC-1 becomes increasingly phosphorylated and after 1 h the hyperphosphorylated protein begins to turn over (for models, see Talora *et al.*, 1999 and He *et al.*, 2005). As part of a feedback loop, the *wc-1* gene itself is subject to light-induced transcription by the WCC (Ballario *et al.*, 1996; Kaldi *et al.*, 2006).

WC-1 and WC-2 are classified as Per Arnt Sim (PAS) transcription factors, which are characterized by a zinc finger-binding domain similar to that of vertebrate GATA factors (Scazzocchio, 2000; Urnov, 2002). Indeed, the light-responsive region (LRR) of light-inducible promoters, e.g., of the *al-3* and *frq* genes, contain GATA or GATA-derived sequences. These regions are recognized in vitro binding assays by recombinant WC-1 and WC-2 zinc finger domains (Ballario *et al.*, 1996) and by the activated WCC (Froehlich *et al.*, 2002). An in vivo interaction between LRRs and WCC

has been recently demonstrated by He and Liu (2005). In some in vitro assays, however, the zinc finger domains of WC-1, NIT-2, and CYS-4 did not preferentially bind to their specific GATA repeats on naked DNA segments consisting of target promoters (Feng and Marzluf, 1998). This suggests that other chromatin components (e.g., histones) may play a role in the regulation of LRR accessibility and may be important in conferring specificity to the response. Nuclear receptors for steroid hormones are, like WC-1, PAS-containing zinc finger proteins with a sensor domain, and they activate transcription only in the presence of specific coactivators, particularly the histone acetyltransferases (HATs) CBP/P300, PCAF, and GCN5 (Hebbbar and Archer, 2003). One current model suggests that posttranslational histone modifications constitute a "histone code" that is involved in the control of gene expression and other genetic and epigenetic processes (Turner, 2000; Jenuwein and Allis, 2001).

Evidence for involvement of chromatin modifications in light-inducible transcriptional activation is starting to accumulate (Crosio *et al.*, 2000; Etchegaray *et al.*, 2003; Naruse *et al.*, 2004). Rhythmic acetylation of histone H3 at clock-regulated promoters has been correlated with the circadian activation of clock genes in mammals (Etchegaray *et al.*, 2003; Hastings and Herzog, 2004). A transient burst of histone H3 phosphorylation on residue S10 (H3 S10) has been observed in mammalian hypothalamic suprachiasmatic nuclei after a light pulse (Crosio *et al.*, 2000). In addition, cycles of acetylation and deacetylation of histone H3 and interaction of the transcription factor Clock with the histone acetyltransferase have been demonstrated in mouse (Naruse *et al.*, 2004).

Here, we show evidence for light-dependent transient acetylation of the amino-terminal tail of histone H3 associated with the LRR of the *al-3* promoter. The influence of acetylation on light dependent gene expression was confirmed by genetic analyses, both for *al-3* and *vod*. We also demonstrate that light-inducible H3 acetylation is catalyzed by NGF-1, the homologue of the yeast histone acetyltransferase Gcn5p.

MATERIALS AND METHODS

N. crassa Strains and Culture Conditions

A *his-3* strain (*matA his-3*; FGSC 462) obtained from the Fungal Genetics Stock Center (Kansas City, KS) was used as the recipient strain for transformations to generate strain BG3 (Figure 1). Similarly, a *wc-2* strain (allele 234w; FGSC 3817) was used in a control experiment. For chromatin immunoprecipitation (ChIP) experiments, we used *N. crassa* wild-type strain 74OR23-1A (FGSC 987; Figure 2) as a control. The *wc-1* mutant (*matA his-3; bd; wc-1^{null}*; FGSC 3081) was a gift from J. Dunlap (Dartmouth Medical School, Hanover, NH). Strain N644 (*matA; inl am¹³²; [(am/hph/am)^{yc42p112}]^{RIP77}*; Irelan and Selker, 1997) was the transformation host for ectopic insertion of a histone H3 gene bearing a lysine-to-glutamine substitution at residue 14 (*hH3^{K14Q}*).

For dark/light ChIP and Northern experiments, conidia were inoculated in liquid medium and grown in the dark for 18–24 h at 28°C. For dark-grown samples, mycelia were collected by filtration under a red safety lamp and frozen in liquid nitrogen. For illuminated samples, mycelia were photoinduced for 3 min with saturating light (10 W/cm²) and further grown in the dark for different periods. The time intervals indicated in figures are from the beginning of the light pulse to when the cultures were harvested.

Construction of the *hH3^{K14Q}* Mutant

The *hH3^{K14Q}* substitution (AAG replaced by CAG at position 14) was generated by QuikChange site-directed mutagenesis (Stratagene, La Jolla, CA) on pSH12, a plasmid containing the wild-type *hH3* gene (Hays and Selker, unpublished data). N644 was cotransformed with pEB11, carrying the *hH3^{K14Q}* allele and pBT6 (Orbach *et al.*, 1986), a plasmid containing a β -tubulin allele that confers resistance to benomyl (Bml). Transformants were grown en masse in Erlenmeyer flasks on solidified Vogel's sucrose medium containing benomyl. Conidia were plated on media containing no drug, benomyl, or hygromycin (Hyg) to test for reactivation of the *hph* gene as described previously (Tamaru and Selker, 2001). Random Hyg⁺ Bml⁺ transformants were grown in liquid medium to isolate DNA. The presence of ectopic *hH3* copies

was verified by Southern hybridization. One strain, N3095, was used for further studies.

Repeat-induced Point (RIP) Mutagenesis of *ngf-1*

We amplified most of the conserved HAT domain of Neurospora GCN Five (*ngf-1*) with degenerate primers 499 (5'-CTCCCCAAGATGCCCAARGAR-TAYAT-3') and 500 (5'-ATRAARTTYTGTCCCCAAGTGGTTCCTC-3') or 501 (5'-CGCACACCCACCTCATGAAYMANYT-3') and 502 (5'-GGNCTRA-TRATRCTGCAG-TAGTCCCTGGGGT-3') and used this as a hybridization probe to identify corresponding cosmid clones in the Orbach/Sachs cosmid library (<http://www.fgsc.net/craslib.html#mocosx>). Clone G5:H10 contained most of the predicted *ngf-1* gene (Supplemental Figure 1). To generate mutants in the *ngf-1* gene, we inserted the predicted *ngf-1* coding region into pBM60 (Margolin *et al.*, 1997) to yield pBM60-*ngf-1*. This plasmid was used to insert a second copy of the *ngf-1* gene at the *his-3* locus by gene replacement (Margolin *et al.*, 1997). Transformants were crossed to induce the premeiotic mutagenic process RIP (Selker, 1990). Progeny were screened for tell-tale restriction fragment length polymorphisms (RFLPs) and DNA methylation by Southern analyses (our unpublished data). One mutant (*ngf-1^{RIP1}*; N2842) exhibited many RFLPs, and the endogenous copy of the *ngf-1* gene was sequenced to identify mutations (Figure 5 and Supplemental Figure 1).

Construction of the Light-responsive *al-3-hph* Reporter Gene

The promoter region of the *al-3* gene (–332 to –46 from the *al-3* translational start site) was obtained as a BglII-NruI fragment by polymerase chain reaction (PCR) from the pAL3- Δ NruI plasmid (Carattoli *et al.*, 1991), with primers 5'-GCCACAGATAGATCTCTGGCCCTG-3' and 5'-CCGTCGCGATTATTGGAAACCCGTCGGTA-3' (the underlined NruI site was added as a primer tail). The product was inserted upstream of the Hyg resistance gene (*hph*) into BglII + NruI-digested pCH102 carrying a truncated *his-3* gene. This plasmid, pBG3, was used to transform strain FGSC462 by electroporation (Margolin *et al.*, 1997). His⁺ colonies were screened for light-dependent Hyg resistance by spotting equal numbers of conidia of each transformant on minimal medium with or without 100 or 300 μ g/ml Hyg. Duplicate plates were kept for 2 d at 28°C in the dark or in a rhythmic regime of 10 min of light (saturating light, 10 W/cm²) and 1 h of dark, to avoid light adaptation.

ChIP Assays

ChIP assays were performed according to a yeast protocol (Avendano *et al.*, 2005) with some modifications. Conidia (10⁶) were used as inocula, and cultures were incubated in liquid medium for 2 d in the dark at 28°C. Dark- and light-induced mycelia were collected by filtration under a red safety light and in vivo cross-linked (Avendano *et al.*, 2005). Cross-linked chromatin-protein complexes were immunoprecipitated with antibodies against unmodified histone H3 (06–755; Upstate Biotechnology, Charlottesville, VA), acetylated H3 (06–599; Upstate Biotechnology), or acetylated H3 K14 (07–353, Upstate Biotechnology). The recovered DNA was subjected to PCR by using the following primers: 30 (5'-AGATAGATCTCTGGCCCTG-3') and 31 (5'-CGATTATTGGAAACCCGTCGGTA-3') for the promoter region of *al-3*, and ACT1 (5'-CCTCTCTCAGCCAAAGCATC-3') and ACT2 (5'-GAAAGCTTAC-CCCATTGTCCG-3') as the internal standard. PCR products were amplified for 25 cycles and resolved on 3% agarose gels. To ensure that the amplified PCR products were in the linear range, the PCR conditions were calibrated with different amounts of immunoprecipitated samples and input DNA (cross-linked chromatin without immunoprecipitation). Band intensities were quantified by optical density analysis with OptyQuant Software (PerkinElmer Life and Analytical Sciences, Boston, MA). As negative controls, mock precipitations were performed in the absence of antibody. PCR products from these negative control samples were not detectable by ethidium bromide staining. The histograms in Figures 2, 3, and 6 represent the ratios between the values for the *al-3* and *actin* PCR products immunoprecipitated, divided by the same ratio obtained by PCR with input DNA [*al-3/act*]_{IP}/*al-3/act*_{input}. Three ChIP replicates were performed on different preparations for each experiment.

Northern Assays

Growth conditions were as described for ChIP experiments. RNA was isolated according to a previously published method (Baima *et al.*, 1991). For Northern analyses, 20 μ g of total RNA was fractionated on 1.2% agarose gels in 5% formaldehyde-3-(*N*-morpholino)propanesulfonic acid buffer, transferred to positively charged nylon membranes (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) and hybridized according to the manufacturer's instructions. The *al-3* transcript was detected with a PCR fragment amplified with primers AL3F (5'-GTCCCTCCAAGACCTCTC-3') and AL3R (5'-CCAGAGGGCTGTGTGTAGCA-3'). The *vod* transcript was detected with a PCR fragment amplified with primers VVDF (5'-CCAAAC-CCCAAGTAGAAGT-3') and VVDR (5'-GTCCAGTCTCTCTTCGGTCT-3'). For normalization, the membranes were stripped in 0.1% SDS at 95°C and rehybridized with an *actin-1* probe obtained by PCR amplification with primers ACTF (5'-GCCTTCTACGTCTCCACCA-3') and ACTR (5'-GTCC-

GAGAGACCAGGGTACA-3'). Band intensities were quantified by optical density analysis with OptyQuant Software. The mRNA expression values shown in the histograms of Figures 4 and 6 were calculated as ratios between *al-3* or *vvd* to *actin* signals in three independent experiments.

Sequence Analysis

Predicted amino acid sequences for NGF-1 and related sequences were compared and aligned with GeneBee Multialignment software (<http://www.genebee.msu.su>). Sequences used in the alignment were *Saccharomyces cerevisiae* Gcn5p (AAT93234), *Magnaporthe grisea* GCN5 (XP_361134), and *Gibberella zeae* GCN5 (XP_380456). DNA sequences were submitted to the GenBank database (*ngf-1*, DQ431713; *ngf-1^{RIP1}*, DQ431714).

RESULTS

The LRR of *al-3* Is Sufficient for Light Control of a Reporter Gene

As a first step to investigate the possible involvement of chromatin modifications in light-inducible gene expression in *Neurospora*, we tested a portion of the *al-3* promoter that was predicted to be directly involved in light-dependent transcriptional modifications (Carattoli *et al.*, 1994, 1995). This LRR contains a GATA-X₁₅-GATA motif comparable to binding sites recognized by known GATA factors (Scazzocchio, 2000) and was found to bind recombinant WC-1 or WC-2 zinc-finger domains in an electrophoresis mobility shift assay (Ballario *et al.*, 1996; Linden and Macino, 1997).

We fused the *al-3* LRR to the *hph* reporter gene, which encodes hygromycin phosphotransferase and can result in resistance to Hyg B in *Neurospora* (Figure 1). Our expression plasmid, pBG3, carries *his-3* as a selectable marker for gene targeting. The untransformed recipient wild-type strain grew equally well in the dark and light in the absence of Hyg, but it failed to grow in the presence of Hyg (Figure 1). In contrast, the otherwise isogenic *al-3-hph* transformant BG3 grew equally well with or without Hyg in the light, showing that the previously defined *al-3* LRR is sufficient to confer light inducibility.

Light-induced Acetylation of Histone H3

Previous studies showed that the *al-3* gene responds to a light pulse with a transient rapid increase in the level of its mRNA (Baima *et al.*, 1991). Induction is also transient under continuous illumination, but expression decreases more slowly than after a pulse; basal levels are reached after 2 h

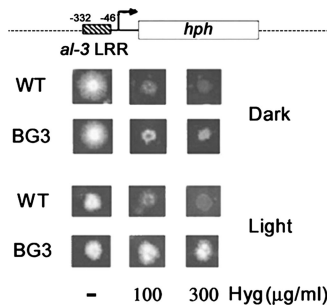


Figure 1. The *al-3* LRR is sufficient to confer light inducibility on the *hph* reporter gene. Top, schematic representation of the reporter region of pBG3 plasmid, containing the LRR of the *al-3* promoter fused to *hph*, a gene that confers resistance to hygromycin B. The sequence coordinates are relative to the transcription start site. Bottom, host wild-type strain (WT; FGSC462) and a representative transformant (BG3) obtained by transformation with plasmid pBG3 were grown on solid medium plus increasing amounts of Hyg in the dark or light (see *Materials and Methods*). Only the BG3 strain is able to grow in the presence of Hyg in the light.

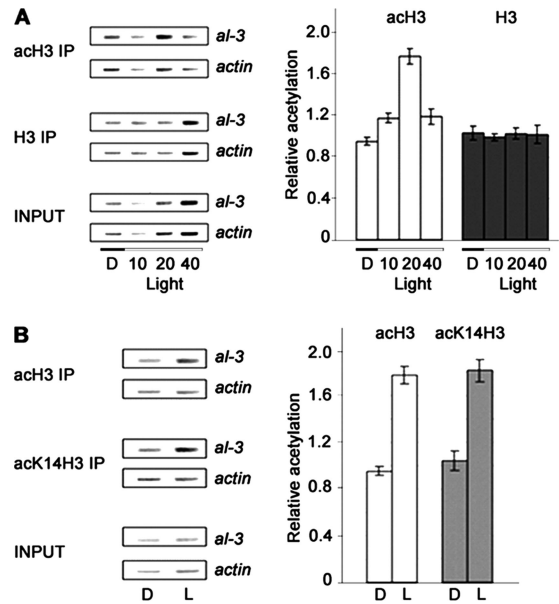


Figure 2. Light induces a transient increase in acetylation of histone H3. (A) Acetylated histones accumulate 20 min after a light pulse. Left, representative PCR coamplifications of the *al-3* LRR and the *actin* promoter (internal control) from chromatin immunoprecipitations of *Neurospora* WT (FGSC987) with antibodies directed against acH3 IP or H3 IP. INPUT represents the sample before immunoprecipitation. Right, histograms derived from three independent immunoprecipitations with two independent immunoprecipitations. (B) H3 K14 is the target for transient acetylation after a light pulse. Left, representative PCR coamplifications of the *al-3* and *actin* promoter regions 20 min after a light pulse (see A) after immunoprecipitation with antibodies directed against acH3 IP or H3 acetylated specifically on K14 (acK14H3 IP). Right, histograms derived from three independent amplifications with two independent immunoprecipitations.

(Baima *et al.*, 1991; Liu *et al.*, 2003). In our hands, induction of *al-3* is maximal at 20 min after the light pulse and transcription returns to basal levels at 60 min. To determine whether a light pulse can influence the degree of acetylation of histone H3 in nucleosomes associated with the LRR, we carried out a series of ChIP experiments (Figure 2). Cross-linked chromatin samples from uninduced (dark; D) and induced (10, 20, or 40 min in light; see *Materials and Methods*) mycelia were sonicated and used directly (INPUT) or immunoprecipitated with antibodies against histone H3 (H3 IP) or against its acetylated form (acH3 IP). DNA fragments associated with either of the two immunoprecipitations were analyzed by semiquantitative PCR with primers specific for the *al-3* LRR and for the promoter of the *actin-1* gene, which is not regulated by light. We observed increased acetylated H3 associated with the *al-3* LRR 20 min after a light pulse. This acetylation was transient and showed kinetics comparable to that of the *al-3* mRNA after induction (Baima *et al.*, 1991). The constant level of histone H3 associated with the LRR suggests that nucleosomes are not depleted from the LRR upon irradiation. The ChIP experiments described above used antibodies directed against H3 peptides acetylated at positions K9 and K14. Acetylation of H3 K14 has been correlated with transcriptional activation in several systems (Carrozza *et al.*, 2003). We therefore considered the possibility that K14 is the target for increased acetylation in the *al-3* LRR upon light induction. We compared the levels of *al-3* LRR associated with nonspecifically acH3

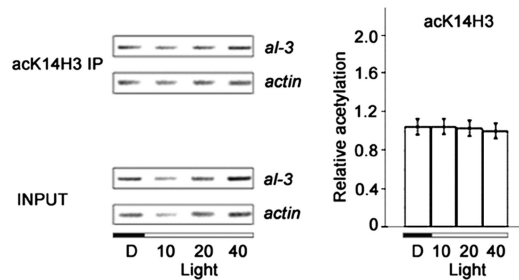


Figure 3. Light-inducible acetylation of H3 K14 requires the presence of WC-1, the blue light photoreceptor. Left, representative PCR coamplifications of *al-3* and *actin* promoter regions (see Figure 2A) after chromatin immunoprecipitation from a *wc-1* mutant strain (FGSC3081) with antibodies directed against histone H3 K14 (acK14H3 IP). Right, histograms derived from three independent amplifications with two independent immunoprecipitations.

IP to those precipitated with antibodies directed against H3 acetylated at K14 (acK14H3 IP) in the dark or 20 min after the light pulse (Figure 2B), which corresponds to the time of maximal H3 acetylation found at the *al-3* LRR in the first experiment (Figure 2A). We found that the change in H3 acetylation at K14 in the light mirrored that found with K9/K14 acetylated H3. Although we were not able to test K9 separately, our results suggest that H3 K14 is the main, and perhaps exclusive, target of light-inducible histone H3. These findings represent the first evidence that *Neurospora* light signal transduction involves chromatin modifications.

WC-1, the Blue Light Photoreceptor of *Neurospora*, Is Required for Light-inducible Acetylation of H3 K14

To determine whether the increase in acetylation at H3 K14 depends on the blue light photoreceptor WC-1, we repeated our ChIP experiments with chromatin isolated from a *wc-1* null mutant (Figure 3). In stark contrast to our findings with wild type (Figure 2B, right), we detected no changes in the levels of H3 K14 acetylation at the *al-3* LRR in this mutant (Figure 3, right). The same result was obtained with a *wc-2* mutant allele (our unpublished data), as expected because an intact WCC is known to be required for appropriate light response in *N. crassa* (Harding and Turner, 1981). WC-2 was not further investigated in this work, because it does not directly respond to light. This result demonstrates that the H3 K14 modification requires the photoreceptor and establishes an epistatic relationship between WC-1 and the unidentified H3 K14 histone acetyltransferase.

An *hH3^{K14Q}* Mutant Has *wc-1*-like Phenotypes and Exhibits a Loss of Photoinducibility

Our ChIP experiments revealed that a transient light-inducible increase of acetylation at K14 of histone H3 occurs in chromatin associated with the *al-3* promoter and that this acetylation depends on the presence of WC-1. The relevance of this chromatin modification was directly evaluated in vivo by construction of a *Neurospora* strain that carries an ectopic mutant copy of the histone H3 gene (*hH3^{K14Q}*) engineered to give a lysine to glutamine substitution at residue 14. The lysine was replaced with glutamine because this amino acid is nonacetylatable but resembles lysine in size. Additional work with this mutant showed that the mutant histone gene is dominant in another assay (Berge and Selker, unpublished data), and we reasoned that if acetylation of K14 is important for induction by light, this strain might show impaired induction because of having a mixture of

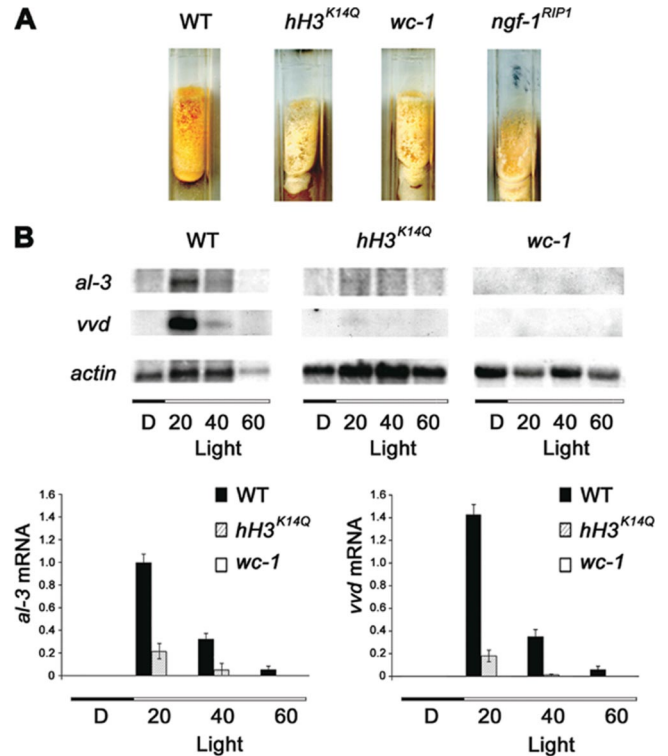


Figure 4. A histone H3 K14Q mutation reduces the light response. (A) Phenotypes of *Neurospora* strains with wild-type histone H3 (WT; N644) and a dominant histone H3 K14Q substitution (*hH3^{K14Q}*; N3095). The *hH3^{K14Q}* mutant phenocopied *wc-1* (FGSC3081) and *ngf-1^{RIP1}* (N2842). Strains were grown on minimal medium for 1 wk in the dark, induced under saturating light, and the production of carotenoids was observed after 6 h at 4°C. (B) Northern analysis of *al-3*, *vvd*, and *actin* (control) revealed light-induced expression in WT. This regulation was abolished in *wc-1* and reduced in *hH3^{K14Q}* mutants. Densitometric analysis for *al-3* (left) and *vvd* (right) revealed a five- or sevenfold reduction, respectively, in the *hH3^{K14Q}* strain compared with wild type (below).

wild-type and mutant histone H3, rendering fewer H3 tails available for light-dependent acetylation. Indeed, compared with the isogenic wild type, light-induced carotenogenesis was strongly impaired in the *hH3^{K14Q}* strain (Figure 4A). This light-induced phenotype resembles that of the *wc-1* mutant.

To investigate the possibility that the morphological phenotype was related to transcriptional activation of carotenogenesis genes, we measured *al-3* transcript levels in wild-type, *wc-1*, and the *hH3^{K14Q}* strains (Figure 4). Induction of *al-3* was normal in the strain with the wild-type H3 gene but was absent in the *wc-1* mutant, as expected from a previous study (Baima *et al.*, 1992). The *hH3^{K14Q}* strain showed a fivefold reduction of light inducible *al-3* mRNA compared with its parental strain (Figure 4B). Light induction in the *hH3^{K14Q}* mutant was reduced sevenfold for a second light-inducible gene, *vvd* (Figure 4B). These results support the suggestion that acetylation of K14 is a requirement for the transcriptional response to light.

The Histone Acetyltransferase NGF-1 Controls Light-induced Acetylation of H3 K14

We wanted to identify the HAT, or HATs, responsible for acetylation of K14 of histone H3 in *N. crassa*. In the yeast *S. cerevisiae*, histone H3 is preferentially acetylated by the co-activator Gcn5p (Georgakopoulos and Thireos, 1992). We

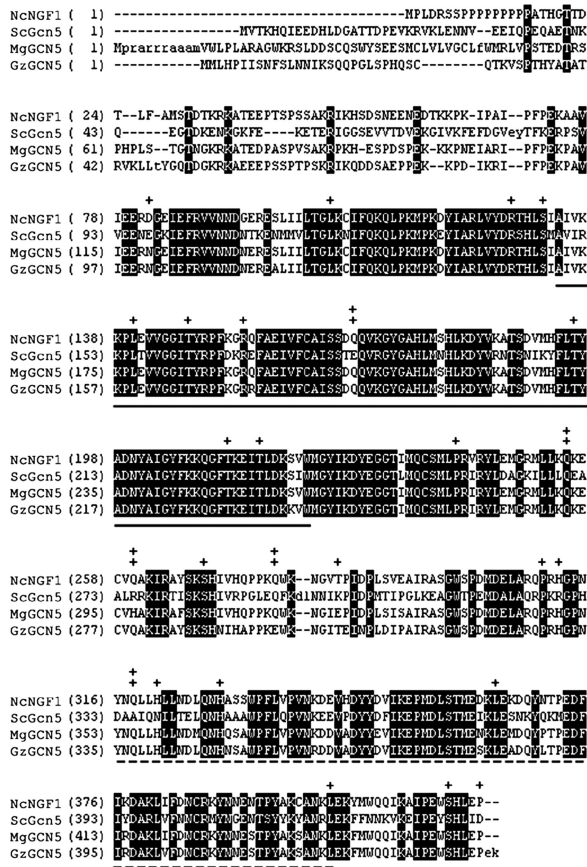


Figure 5. NGF-1 is the *Neurospora* homologue of the GCN5 histone acetyltransferase. Alignment of *Neurospora* NGF-1 (NcNGF1) with homologues from *S. cerevisiae* (ScGcn5), *M. grisea* (MgGcn5), and *G. zeae* (GzGcn5). Bold letters indicate identity among proteins. Mutations resulting in substitutions between wild-type NGF-1 and the predicted amino acid sequence of the mutant allele, *ngf-1^{RIP1}*, are indicated (+). Predicted nonsense codons are also shown (§). Solid and dashed underlines indicate the catalytic HAT domain and bromodomain, respectively.

amplified portions of the *Neurospora* GCN5 homologue by PCR with degenerate primers based on the conserved HAT domain, screened a *Neurospora* cosmid library, and sequenced the identified gene, which we called *ngf-1*. After the *N. crassa* genome became available, we found this sequence with the program tblastn and *S. cerevisiae* Gcn5p as bait (Borkovich *et al.*, 2004); its locus number in the most recent genome annotation is NCU10847.2. Overall, the predicted NGF-1 sequence aligns well with known and predicted GCN5 homologues from other fungi (Figure 5). In particular, the catalytic HAT domain (residues 107–219; 78% identity) and the bromodomain (residues 316–401; 63% identity) are conserved between *S. cerevisiae* Gcn5p and *Neurospora* NGF-1. Long stretches of C nucleotides are found in the 5' portion of *ngf-1*, and this region is not conserved among filamentous fungi (Figure 5), suggesting the existence of an intron. We have attempted, but failed, to isolate complete cDNA. Thus, although most of the gene structure is defined, the DNA sequence upstream of the conserved HAT domain remains uncertain, precluding attempts to generate recombinant NGF-1 protein.

We took advantage of *Neurospora*'s premeiotic mutagenic genome defense system RIP (for review, see Galagan and Selker, 2004) to generate *ngf-1* mutants. One mutant, *ngf-1^{RIP1}*,

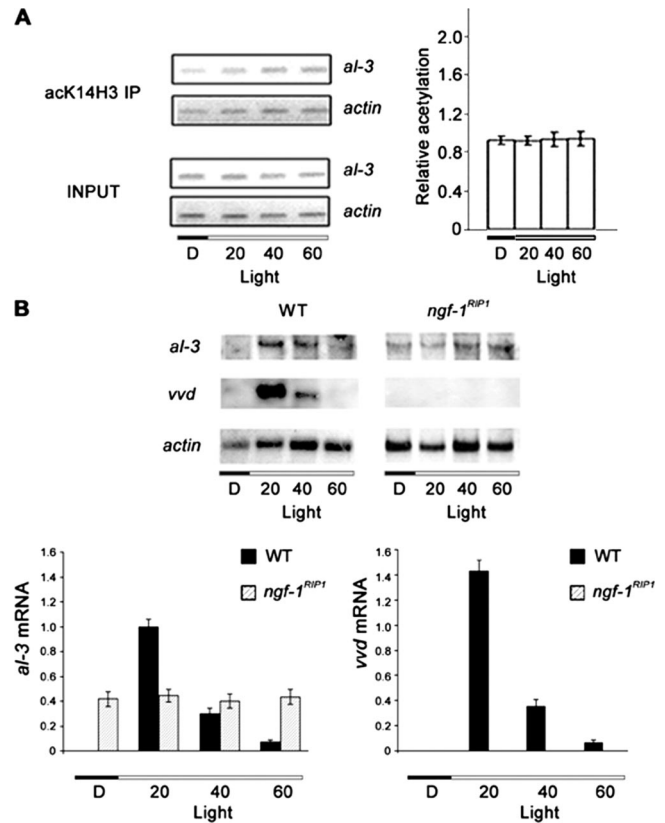


Figure 6. The *ngf-1^{RIP1}* mutant is defective in light inducibility. (A) Loss of light-inducible H3 K14 acetylation in the *ngf-1^{RIP1}* mutant. Left, representative PCR coamplifications of *al-3* and *actin* promoter regions after immunoprecipitation of chromatin from the *ngf-1^{RIP1}* mutant with antibodies directed against histone H3 K14 (acK14H3 IP). Right, histograms derived from three independent amplifications with two independent immunoprecipitations. (B) Loss of mRNA light inducibility in the *ngf-1^{RIP1}* mutant. Northern analyses (blots shown on top; densitometric results on bottom) revealed loss of response to light in the *ngf-1^{RIP1}* mutant for *al-3* and *vvd*; *vvd* expression was absent, whereas *al-3* mRNA was constitutively expressed in both dark and light conditions.

showed numerous predicted amino acid substitutions and nonsense mutations (Figure 5 and Supplemental Figure 1). The first predicted stop codon (residue 141) should interrupt the catalytic HAT domain. The *ngf-1^{RIP1}* mutant grows very poorly, produces few conidia, exhibits colonial growth on sucrose plates, and produces only pale pink mycelia after light induction, all defects that involve the *Neurospora* light-dependent response. The last phenotype is strikingly similar to that of *wc-1* and *hH3^{K14Q}* mutants (Figure 4A).

We next addressed whether NGF-1 is involved in light-inducible acetylation of H3 K14 (Figure 6A). In contrast to the situation in wild-type strains, no increase of H3 K14 acetylation was observed in the *ngf-1^{RIP1}* mutant after light induction (compare Figure 6A with 2). We conclude that NGF-1 is responsible for acetylation of K14 in histone H3 in response to light.

As a separate measure of the involvement of NGF-1 in light regulation, we measured *al-3* and *vvd* transcript levels in the *ngf-1^{RIP1}* mutant (Figure 6B). Light inducible expression was lost for both *al-3* and *vvd*, confirming the role of NGF-1 as a coactivator. Unexpectedly, *al-3* expression was found to be constitutive in the *ngf-1^{RIP1}* strain and its transcript was detected both in the dark and the light, in contrast

to the situation in wild type or the semidominant *hH3^{K14Q}* mutant (compare Figure 6B with 4B). This suggests that the normal light-induced expression of *al-3* requires both activation and release of repression.

DISCUSSION

The eukaryotic genome is organized on nucleosomes, the basic units of chromatin. The simple, widely accepted idea that the chromatin constitutes a barrier to the expression of the genes has been refined in the last decade. We now know that nucleosomes can be moved by ATP-dependent remodeling complexes and that histones are substrates for enzymatic modifications (acetylation, methylation, phosphorylation, ubiquitination, ADP-ribosylation, and sumoylation) that can influence gene expression and are in many cases reversible. Histone modifications have been proposed to form an epigenetic code (Strahl and Allis, 2000; Turner, 2000). Modification of histone tails by acetylation of specific lysine residues is regarded as one important mechanism for the regulation of chromatin accessibility to transcription factors and ancillary proteins responsible for global gene expression (Cheung *et al.*, 2000). Here, we report the first evidence for the importance of chromatin acetylation in the well-studied light transduction pathway of *Neurospora*, a classical organism for photobiology studies. Our findings complement evidence for a relationship between H3 acetylation and rhythmic transcription in a mammalian circadian system (Etchegaray *et al.*, 2003; Curtis *et al.*, 2004). Although a transient burst of histone H3 S10 phosphorylation has been reported in the mammalian hypothalamic suprachiasmatic nuclei after a light pulse (Crosio *et al.*, 2000), we have not observed a statistically significant change in H3 S10 phosphorylation after a light pulse in our system (our unpublished data).

Light-induced Acetylation of K14 of Histone H3

To focus our studies on the critical regions of a light-inducible gene, we first determined that a short region within the photoinducible *al-3* promoter, the LRR, is sufficient for light-inducible regulation in vivo (Figure 1). We then discovered that histone H3 associated with this region becomes transiently acetylated after photoinduction (Figure 2A), with kinetics similar to that described for *al-3* mRNA induction (Baima *et al.*, 1991). A similar coupling of histone acetylation/transcription induction timing has been also observed for the rhythmic expression of circadian genes (Etchegaray *et al.*, 2003). Lysine 14 of the H3 N-terminal tail is the specific target of the light-dependent modification (Figure 2B), and, as with all other described *Neurospora* light responses, this modification depends strictly on the presence of the photoreceptor WC-1 (Figure 3). We demonstrated the relevance of the acetyltable H3 K14 residue by genetic manipulation. A strain with a K14Q substitution in histone H3 (*hH3^{K14Q}*) exhibited a pale pink phenotype strongly resembling that of a *wc-1* null mutant (Figure 4A). The *hH3^{K14Q}* mutant showed a fivefold reduction of induced *al-3* mRNA compared with an otherwise isogenic strain bearing only the wild-type *hH3* gene (Figure 4B). This marked molecular phenotype was observed in a strain containing both a wild-type and a mutated copy of *hH3* gene, indicating that the H3 K14Q mutation is semidominant under these conditions. This result indicates the importance of H3 K14 acetylation in *al-3* photoinduction. The requirement of an acetyltable K14 was also demonstrated for another photoinducible gene, *vvd* (Figure 3B). These findings indicate that chromatin acetylation is a general regulatory step operating in the *Neurospora* transcriptional photoresponse.

The *Neurospora* GCN5 homologue NGF-1 Is Responsible for Acetylation of K14 in Response to Light

We wanted to identify the HAT responsible for acetylation of K14 in response to light. Seven putative histone acetyltransferases have been computationally identified in the *Neurospora* genome (Galagan *et al.*, 2003). Because yeast Gcn5p is known to directly acetylate H3 K14 (Kuo *et al.*, 1996), we tested the possibility that a *Neurospora* homologue of this HAT could be responsible for the observed chromatin modification. We isolated the *Neurospora* GCN5 homologue *ngf-1* by homology-based PCR amplification with degenerate primers, sequenced most of the gene, and later also found it in the *Neurospora* genome sequence, at the terminus of what is now contig 7.2 (http://www.broad.mit.edu/annotation/fungi/neurospora_crassa_7/index.html; Borkovich *et al.*, 2004).

Based on our sequence information, we amplified a large fragment of the *ngf-1* gene and used it to generate mutants by RIP (Selker, 1990). Tellingly, the morphological phenotype of the *ngf-1^{RIP1}* mutant is similar to the *wc* phenotype, white mycelium and pale pink conidia (Figure 4A). Moreover, the *ngf-1^{RIP1}* showed additional defects, many of which are related to light induction and/or circadian rhythms (e.g., slow growth, reduced number of conidia, and colonial growth) and that may be explained by the general importance of GCN5-type HATs in gene activation (Dyda *et al.*, 2000).

We suggest that NGF-1 is required for light-induced H3 K14 acetylation on the *al-3* promoter (Figure 6A). NGF-1 activity depends on the presence of WC-1, because both *wc-1* and *ngf-1^{RIP1}* mutants are impaired in H3 K14 acetylation (Figures 3 and 6A). Northern analysis of *al-3* and *vvd* mRNA in the *ngf-1^{RIP1}* mutant showed loss of photoinduction (Figure 6A), consistent with a role of NGF-1 as coactivator in light-inducible transcription. As expected, we did not observe *vvd* transcripts either in the dark or upon a light pulse but constitutive *al-3* expression occurred in the *ngf-1^{RIP1}* mutant. This finding suggests that a NGF-1-mediated repression mechanism may operate on *al-3* transcriptional regulation. Together, our results identify NGF-1 as a new element of the *Neurospora* light transduction system.

Previous studies on transcriptional activation by coactivators such as CBP/p300 have revealed that locus- and time-specific histone modifications are achieved by direct interaction between histone-modifying enzymes and transcriptional regulators (Roth *et al.*, 2001). For example, CBP/p300 interacts with activated nuclear receptors, and this interaction targets HAT activity to specific promoter regions (Tsai and Fondell, 2004). Anafi and colleagues used yeast genetics and in vitro heterologous protein interaction to demonstrate that yeast Gcn5p can regulate a human nuclear receptor (Anafi *et al.*, 2000). In preliminary work to investigate the possibility of direct interactions between WC-1 and a HAT, we carried out a pull-down assay with WC-1 and labeled yeast Gcn5p, reasoning that its high conservation might allow it to mimic its *Neurospora* homologue that we were unable to express. We found that WC-1 is able to interact in vitro with Gcn5p and that the interaction depends on the region of WC-1 between amino acids 838-1000 (our unpublished data). This region contains a DNA-binding zinc finger domain that has been suggested to be involved in protein-protein interactions (Scazzocchio, 2000). Notably, the WC-1 region required for Gcn5p interaction also contains an AF2 LXXLL motif, which has been shown to be important for the interaction of steroid hormone nuclear receptors with HAT-containing coactivator complexes

(Anafi *et al.*, 2000; Savkur and Burris, 2004). The LXXLL motif is also present in a PAS-containing protein that controls the mammalian circadian clock, NPAS2, and its deletion prevents HAT-mediated transcription of clock-regulated genes (Curtis *et al.*, 2004). Our observations are further supported by recent findings from a related system, the time-keeping transcription factor CLOCK is itself a HAT and controls mammalian circadian rhythm by alternative interactions with corepressors and coactivators (Doi *et al.*, 2006).

WCC transiently binds to the promoters of *al-3* and other light-inducible genes after illumination (He and Liu, 2005). Notably, this binding follows kinetics similar to what we observed for WC-1-dependent acetylation of H3 at the *al-3* promoter. It has also been reported that the form of WCC present in the light (L-WCC) is larger than that observed in the dark (D-WCC) (Froehlich *et al.*, 2002; He and Liu, 2005). L-WCC is thought to owe its increased size to multimerization of WC proteins and/or the presence of unidentified additional factors. In light of our results, we propose that NGF-1 may be recruited in the light by the WCC to form an L-WCC that acts to target the histone acetyltransferase activity on light regulated genes. This could lead to an "open" chromatin structure. Together, our findings reveal a direct link between histone modifications and light transcriptional control and contribute to the developing understanding of the molecular mechanisms operating in light-inducible gene activation.

ACKNOWLEDGMENTS

We thank L. Corrochiano for the gift of the plasmid pCH102 and J. C. Dunlap for the *wc-1* null strain. We thank Z. Lewis for comments. This work was partially supported by Fondazione Pasteur Cenci Bolognietti, Ministero dell'Istruzione, dell'Università e della Ricerca (Fondo per gli Investimenti della Ricerca di Base RBNE01KMT9_009), Progetti Ricerca Interesse Nazionale 2006, and Consiglio Nazionale delle Ricerche RTL (Ricerca a Tema Libero). B.G. was supported by a contract of the University of Rome "La Sapienza." Work in the laboratory of E.U.S. was supported by U.S. Public Health Service Grant GM-35690 from the National Institutes of Health.

REFERENCES

Anafi, M., Yang, Y. F., Barlev, N. A., Govindan, M. V., Berger, S. L., Butt, T. R., and Walfish, P. G. (2000). GCN5 and ADA adaptor proteins regulate triiodothyronine/GRIPI1 and SRC-1 coactivator-dependent gene activation by the human thyroid hormone receptor. *Mol. Endocrinol.* *14*, 718–732.

Avendano, A., *et al.* (2005). Swi/SNF-GCN5-dependent chromatin remodeling determines induced expression of GDH3, one of the paralogous genes responsible for ammonium assimilation and glutamate biosynthesis in *Saccharomyces cerevisiae*. *Mol. Microbiol.* *57*, 291–305.

Baima, S., Carattoli, A., Macino, G., and Morelli, G. (1992). Photoinduction of *albino-3* gene expression in *Neurospora crassa* conidiation. *J. Photochem. Photobiol. B15*, 233–238.

Baima, S., Macino, G., and Morelli, G. (1991). Photoregulation of the *albino-3* gene in *Neurospora crassa*. *J. Photochem. Photobiol.* *11*, 107–115.

Ballario, P., Vittorioso, P., Magrelli, A., Talora, C., Cabibbo, A., and Macino, G. (1996). White collar 1, a central regulator of the blue light responses in *Neurospora*, is a zinc-finger protein. *EMBO J.* *15*, 1650–1657.

Borkovich, K. A., *et al.* (2004). Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. *Microbiol. Mol. Biol. Rev.* *68*, 1–108.

Carattoli, A., Cogoni, C., Morelli, G., and Macino, G. (1994). Molecular characterization of upstream regulatory sequences controlling the photoinduced expression of the *albino-3* gene of *Neurospora crassa*. *Mol. Microbiol.* *13*, 787–795.

Carattoli, A., Kato, E., Rodriguez-Franco, M., Stuart, W. D., and Macino, G. (1995). A chimeric light-regulated amino acid transport system allows the isolation of blue light regulator (*blr*) mutants of *Neurospora crassa*. *J. Biol. Chem.* *270*, 6612–6616.

Carattoli, A., Romano, N., Ballario, P., Morelli, G., and Macino, G. (1991). The *Neurospora crassa* carotenoid biosynthetic gene (*albino 3*) reveals highly conserved regions among prenyltransferases. *J. Biol. Chem.* *266*, 5854–5859.

Carrozza, M. J., Uteley, R. T., Workman, J. L., and Cote, J. (2003). The diverse functions of histone acetyltransferase complexes. *Trends Genet.* *19*, 321–329.

Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher plants. *Annu. Rev. Genet.* *38*, 87–117.

Cheng, P., Yang, Y., Gardner, K. H., and Liu, Y. (2002). PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. *Mol. Cell. Biol.* *22*, 517–524.

Cheung, P., Allis, C. D., and Sassone-Corsi, P. (2000). Signaling to chromatin through histone modifications. *Cell* *103*, 263–271.

Crosio, C., Cermakian, N., Allis, C. D., and Sassone-Corsi, P. (2000). Light induced chromatin modification in cells of the circadian clock. *Nat. Neurosci.* *3*, 1241–1247.

Curtis, A. M., Seo, S. B., Westgate, E. J., Rudic, R. D., Smyth, E. M., Chakravarti, D., FitzGerald, G. A., and McNamara, P. (2004). Histone acetyltransferase-dependent chromatin remodeling and the vascular clock. *J. Biol. Chem.* *279*, 7091–7097.

DeFabo, E. C., Harding, R. W., and Shropshire, W. (1976). Action spectrum between 260 and 800 nanometers for the photoinduction of carotenoid biosynthesis in *Neurospora crassa*. *Plant Physiol.* *57*, 440–445.

Doi, M., Hirayama, J., and Sassone Corsi, P. (2006). Circadian regulator CLOCK is a histone acetyltransferase. *Cell* *127*, 497–508.

Dunlap, J. C., and Loros, J. J. (2004). The *Neurospora* circadian system. *J. Biol. Rhythms* *19*, 414–424.

Dyda, F., Klein, D. C., and Hickman, A. B. (2000). GCN5-related N-acetyltransferases: a structural overview. *Annu. Rev. Biophys. Biomol. Struct.* *29*, 81–103.

Etchegaray, J. P., Lee, C., Wade, P. A., and Reppert, S. M. (2003). Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. *Nature* *421*, 177–182.

Feng, B., and Marzluf, A. (1998). Interaction between major nitrogen regulatory protein NIT2 and pathways-specific regulatory factor NIT4 is required for their synergistic activation of gene expression in *Neurospora crassa*. *Mol. Cell. Biol.* *18*, 3983–3990.

Franchi, L., Fulci, V., and Macino, G. (2005). Protein kinase C modulates light responses in *Neurospora* by regulating the blue light photoreceptor WC-1. *Mol. Microbiol.* *56*, 334–345.

Froehlich, A. C., Liu, Y., Loros, J. J., and Dunlap, J. C. (2002). White collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. *Science* *297*, 815–819.

Froehlich, A. C., Noh, B., Vierstra, R. D., Loros, J. J., and Dunlap, J. C. (2005). Genetic and molecular analysis of Phytochromes from the filamentous fungus *Neurospora crassa*. *Eukaryot. Cell* *4*, 2140–2152.

Galagan, J. E., *et al.* (2003). The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* *422*, 859–868.

Galagan, J. E., and Selker, E. U. (2004). RIP: the evolutionary cost of genome defense. *Trends Genet.* *20*, 417–423.

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.

Harding, R. W., and Turner, R. V. (1981). Photoregulation of the carotenoid biosynthetic pathway in albino and white collar mutants of *Neurospora crassa*. *Plant Physiol.* *68*, 745–749.

Hastings, M. H., and Herzog, E. D. (2004). Clock genes, oscillators and cellular networks in the suprachiasmatic nuclei. *J. Biol. Rhythms* *19*, 400–413.

He, Q., Cheng, P., Yang, Y., Wang, L., Gardner, K. H., and Liu, Y. (2002). White collar-1, a DNA binding transcription factor and a light sensor. *Science* *297*, 840–843.

He, Q., and Liu, Y. (2005). Molecular mechanism of light responses in *Neurospora*: from light-induced transcription to photoadaptation. *Genes Dev.* *19*, 2888–2899.

He, Q., Shu, H., Cheng, P., Chen, S., Wang, L., and Liu, Y. (2005). Light-independent phosphorylation of WHITE COLLAR-1 regulates its function in the *Neurospora* circadian negative feedback loop. *J. Biol. Chem.* *280*, 17526–17532.

Hebbar, P. B., and Archer, T. K. (2003). Chromatin remodeling by nuclear receptors. *Chromosoma* *111*, 495–504.

- Huala, E., Oeller, P. W., Liscum, E., Han, I. S., Larsen, E., and Briggs, W. R. (1997). *Arabidopsis* NPH 1, a protein kinase with a putative redox-sensing domain. *Science* 278, 2120–2123.
- Irelan, J. T., and Selker, E. U. (1997). Cytosine methylation associated with repeat-induced point mutation causes epigenetic gene silencing in *Neurospora crassa*. *Genetics* 146, 509–523.
- Jenuwein, T., and Allis, C. D. (2001). Translating the histone code. *Science* 293, 1074–1080.
- Kaldi, K., Gonzales, B. H., and Brunner, M. (2006). Transcriptional regulation of the *Neurospora* circadian clock gene *wc-1* affects the phase of circadian output. *EMBO Rep.* 7, 119–204.
- 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* 381, 269–272.
- Linden, H., and Macino, G. (1997). White collar 2, a partner in blue light signal transduction, controlling expression of light regulated genes in *Neurospora crassa*. *EMBO J.* 16, 98–109.
- Liu, Y., He, Q., and Cheng, P. (2003). Photoreception in *Neurospora*: a tale of two White Collar proteins. *Cell. Mol. Life. Sci.* 60, 2131–2138.
- Margolin, B. S., Freitag, M., and Selker, E. U. (1997). Improved plasmids for gene targeting at the *his-3* locus of *Neurospora crassa* by electroporation. *Fungal Genet. Newsl.* 44, 34–36.
- Naruse, Y., Oh-hashi, K., Iijima, N., Naruse, M., Yoshioka, H., and Tanaka, M. (2004). Circadian and light induced transcription of Clock gene *Per-1* depends on histone acetylation and deacetylation. *Mol. Cell. Biol.* 24, 6278–6287.
- Orbach, M. J., Porro, E. B., and Yanofsky, C. (1986). Cloning and characterization of the gene for α -tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. *Mol. Cell. Biol.* 6, 2453–2461.
- Roth, S. Y., Denu, J. M., and Allis, C. D. (2001). Histone acetyltransferases. *Annu. Rev. Biochem.* 70, 81–120.
- Sargent, M. L., and Briggs, W. R. (1967). The effects of light on a circadian rhythm of conidiation in *Neurospora*. *Plant Physiol.* 42, 1504–1510.
- Savkur, R. S., and Burris, T. P. (2004). The coactivator LXXLL nuclear receptor recognition motif. *J. Pept. Res.* 63, 207–212.
- Scazzocchio, C. (2000). The fungal GATA factors. *Curr. Opin. Microbiol.* 3, 126–131.
- Schwerdtfeger, C., and Linden, H. (2001). Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. *Mol. Microbiol.* 39, 1080–1087.
- Selker, E. U. (1990). Premeiotic instability of repeated sequences in *Neurospora crassa*. *Annu. Rev. Genet.* 24, 579–613.
- Strahl, B. D., and Allis, C. D. (2000). The language of covalent histone modifications. *Nature* 403, 41–45.
- Talora, C., Franchi, L., Linden, H., Ballario, P., and Macino, G. (1999). Rule of a white collar-1-white collar-2 complex in blue-light signal transduction. *EMBO J.* 18, 4961–4968.
- Tamaru, H., and Selker, E. U. (2001). A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* 414, 277–283.
- Turner, B. M. (2000). Histone acetylation and an epigenetic code. *Bioessays* 22, 836–845.
- Tsai, C. C., and Fondell, J. D. (2004). Nuclear receptor recruitment of histone-modifying enzymes to target gene promoters. *Vitam. Horm.* 68, 93–122.
- Urnov, F. D. (2002). A feel for the template: zinc finger protein transcription factors and chromatin. *Biochem. Cell Biol.* 80, 321–333.