**RNA Extraction, cDNA Preparation and Dye Labeling**

Total RNA was extracted using TRIZOL reagent (Invitrogen) with a homogenizer (Tissuelyzer, Qiagen) containing stainless beads for pulverizing. Total RNA was then subjected to an additional purification step using RNeasy spin columns (Qiagen). After quantification by a spectrophotometer, 20 μg RNA from each time point was used for cDNA synthesis. 5 μg anchored oligo(dT) 20mer (2.5μg/μl, Invitrogen) was mixed with the RNA sample in a total of 18.4 μl. After incubation at 70 ˚C for 10 min, the mixture was cooled on ice for another 10 min. Reverse transcription was performed using 400 U Superscript II Reverse Transcriptase (200 U/μl, Invitrogen) with amino-allyl-dUPT/dNTP (aadUPT: 15 mM, dTTP: 10 mM, dATP: 25 mM, dCTP: 25 mM, dGTP: 25 mM) at 42 ˚C for 2 h in a 30 μl reaction volume. The RNA was then hydrolyzed by adding 10 μl 1 M NaOH and 10 μl 0.5 M EDTA. After incubation at 65 ˚C for 15 min, 25 μl 1 M Tris pH 7.4 buffer was added to neutralize the reaction. The cDNA was then purified with a YM 30 column (Millipore). The eluted cDNA was dried in a speed vacuum without heat for 1 to 1.5 h and then resuspend in 5 μl $H_2O$ at RT for 15 min, where upon 3 μl 25 mg/ml sodium bicarbonate buffer (Sigma) and 2 μl Alexa dye (Invitrogen) was added. The coupling reaction was incubated for 1 h in the dark at RT and then inactivated by adding 4.5 μl 4 M hydroxylamine. After15 min in the dark at RT, the labeled cDNA was purified using the CyScribe GFX Purification Kit (Amersham) following the manufacturer's protocol. cDNA was dried in the speed vacuum without heat for another 1-1.5 h. Alexa Fluor 555 was used to label cDNA synthesized from the reference RNA, which is a mixture containing equal amounts of RNA samples harvested from different circadian time points and light treatment durations. The same batch of pooled RNA was used as a reference for each array experiment (Novoradovskaya *et al.*, 2004). Alexa Fluor 647 was used exclusively to label cDNA representing sample RNA.

**Microarray Hybridization**

Before prehybridization, microarray slides from the Fungal Genetics Stock Center (Tian *et al.*, 2007) were pre-treated with 600 mJ UV and Pronto Background Reduction kit (Corning) according to the manufacturer's protocol. Slides were prehybridized at 42°C for 45 min with prehybridization buffer (5X SSC, 0.1% SDS and 1% BSA) and then spun dry for one min at 1000 RPM. The dried cDNA sample was rehydrated in 31.5 μl hybridization buffer (40% formamide, 5X SSC, 0.1 SDS) for several minutes before adding 3.5 μl 10X blocker (1μg/μl

ssDNA and 2µg/µl tRNA in water, Sigma). The mixture was heated at 95 °C for 3 min, cooled on ice for 30 seconds and quickly spun before pipetting onto the slide under a Lifter slip (VWR). The slide was placed in the oven (Boekel InSlide Out Model 241000 hybridization oven) for hybridization overnight at 42°C. To maintain humidity in the hybridization cassette, 2 ml of hybridization buffer containing 40% formamide were pipetted onto 4 Whatman 42.5 mm filter paper circles within the cassette. 2X SSC/0.1% SDS was used for the first wash at 42°C for 5 min and then followed by 0.1X SSC/0.1% SDS at RT for 10 min. The final wash was performed twice using 0.1X SSC only at RT for 5 min. Slides were spun dry before scanning.

**Quantitative RT-PCR and Semi-Quantitative RT-PCR**

To validate the microarray data, independent biological samples were collected and used for RNA extraction as described above. 2 µg of RNA was treated with 1 U DNase (Invitrogen) for 10 min. After inactivation of DNase with 1 µl 25 mM EDTA, the reverse transcription reaction was carried out using Superscript III first-strand synthesis system for RT-PCR (Invitrogen) according to the manufacturer's protocol. Analysis of cDNA was performed using the SYBR green-based method (ABI) with the primer sets summarized in Supplemental Table V. RT-QPCR data were measured with ABI 7500 detection system (Applied Biosystems) and analyzed using ABI instrument software SDS 1.3.1. The expression of individual genes was compared and normalized using the relative delta-Ct method against the level of *actin* mRNA (Pfaffl, 2001), which was found to be constant and not light-responsive across the different time points evaluated here. Meanwhile, results of semi-quantitative RT-PCR were compared after 25 cycles of standard PCR condition.

**Luciferase Constructs and Site-Directed Mutagenesis**

The entire upstream promoter regions, which include the sequence covering the 3'UTR of the previous gene to the start codon of NCU01107.2 and NCU06597.2, were amplified by PCR from genomic DNA and cloned into the pTOPO-TA vector (Invitrogen) for site-directed mutagenesis (Stratagene, QuickChange II XL). After confirming the mutation sites by sequencing, primers containing homologous recombination sites for the *his-3* targeting plasmid pLL07, a derivative of pHK40-2 (Colot *et al.*, 2006), which contains only the optimized luciferase coding sequence (Gooch *et al.*, 2008), were used to amplify the promoter sequences again from the modified

pTOPO-TA vector. The fragments were co-transformed into yeast for assembly (Colot *et al.*, 2006), and the resulting constructs, the *his-3* targeting plasmid pLL07-NCUO01107-luciferase, pLL07-NCU01107-m-luciferase, pLL07-NCU06597-luciferase and pLL07-NCU06597-m-luciferase, were transformed into FGSC# 9014 strain (*his-3, rid, A*). Histidine prototrophs displaying similar luciferase activity, which may indicate they have close number of integration, were selected for experiments. At least two individual transformants were tested for each transgene.

**Chromatin-immunoprecipitation (ChIP) assay**

The sequence of primer pairs used in the ChIP assays is included in Supplemental Table V. ChIP assays were performed as previously described (Belden et al., 2007b).

**Bootstrapping analysis**

In order to identify statistically significant expression clusters, the same group of genes used for the unsupervised hierarchical clustering was further analyzed by a scale-free bootstrapping analysis (Shimodaira, 2004). Data from strains with wild-type light responses were included and shown here (Supplementary Figure 2). Sampling was 500 fold with replacement and the significant clusters were selected to fall within a 95% confidence interval containing ten or more genes using the pvclust R package (Suzuki & Shimodaira, 2006).

**Significance Analysis of Microarrays (SAM)**

Microarray data from strains with wild-type light responses were categorized as class l (Figure 1, lanes 1 to 5) in contrast to class ll, which included strains either with an impaired light response (e.g. *wc* knock-outs) or in constant darkness (Figure 1, lanes 6 to 9). A two class unpaired time course analysis was performed with a T-statistic and signed area method. Missing data were imputed via a K-Nearest Neighbor algorithm (k =10) before permutations (n=100) for estimating the false discovery rate (FDR).

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