Supporting information

Seaton et al, 2018, Dawn and photoperiod sensing by phytochrome A

Materials and Methods

Coexpression clustering

The subset of 10,297 transcripts chosen for clustering were selected on the basis of their identification as being rhythmically expressed in diurnal conditions in the microarray experiments of (1). Clustering was performed in Python using the scikit-learn affinity propagation clustering algorithm (parameters: damping = 0.5, preference = -14) (2). Similarities between genes were defined by calculating Pearson's correlation coefficient across each dataset listed above, and then summing these coefficients. This measure of similarity allows genes to cluster by similarity of their dynamics in a particular experiment, rather than by changes in average expression across experiments in different growth conditions, developmental stages etc., processed by different labs.

Data mining of functional genomics datasets

140 gene lists were consolidated from 47 publications, and are listed and described in Table S2. The gene lists and gene list descriptions are provided as text files in Datafile S3. These do not include the 387 transcription factor DAP-seq datasets described in (3), and available therein.

RNA isolation and transcript levels analysis by qPCR

In brief, seedlings were grown for 2 weeks at 22°C in SD or LD photoperiods (white light 100 µmol m−2 s−1) . Samples were collected in liquid nitrogen and RNA was extracted with RNeasy Plant Mini kit (Qiagen). cDNA synthesis was performed using the SuperScript VILO cDNA synthesis kit (Invitrogen). The qPCR was performed with a LightCycler 480 (Roche). All samples were processed in biological triplicates. Primers used for qPCR gene expression analyses are listed in Supplementary Table S5.

phyA Immunoblots

Total proteins were extracted from 100 mg of tissue from plants grown under short or long days for two weeks (see plant material and growth conditions) and harvested at the indicated times on day 14. Two separate experiments

were performed for Supplementary Fig S5A and B.

For Supplementary Fig S5A (phyA quantification in short and long days): Total protein was extracted from seedlings grown under short days or long days for 2 weeks, and harvested at each time point on day 14. Whole protein extract was extracted using a buffer containing 50 mM Na-phosphate (pH 7.4), 100 mM NaCl, 10% (v/v) glycerol, 5 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50 µM MG-132, 2mM Na3VO4, 2 mM NaF, and Pierce Protease Inhibitor Tablets, EDTA-free (Thermo Fisher Scientific). All extraction procedure was performed under dim white light in 4°C. 30 µg protein in each sample was run in 8% SDS-PAGE gels, and transferred to Nitrocellulose membranes (Bio-rad). phyA protein was detected by using anti-PHYA antibody (a kind gift from Dr. Akira Nagatani, Kyoto Univ.) at a dilution of 1:3000, whereas actin protein was detected by anti-actin antibody (MA1-744, Life Technologies) at a dilution of 1:5000, followed by a HRP-condugated goat anti-mouse antibody (Thermo Fisher Scientific) at a dilution of 1:10,000. Immunoreactive proteins were visualized with SuperSignal West Pico Chesmiluminescent Substrate (Thermo Fisher Scientific) and Amersham ECL Select Western Blotting Detection Reagent (GE healthcare). For protein quantification, signals from immunoblottted membranes incubated in chemiluminescent detection reagents were imaged and quantified by a high sensitivity cooled CCD camera system (NightOWL, Berthold) and the IndiGo program (Berthold). Actin was used for normalization of a protein in whole extract.

For Supplementary Fig S5B (phyA quantification in PIF mutants): Protein was extracted in a buffer containing 100 mM Tris-HCl pH 8, 50 mM EDTA, 150 mM NaCl, 10% glycerol, 5,M MgCl2, 1 mM DTT, 1mM PMSF, 50 uM MC132 and 1% protease complete inhibitor cocktail with no EDTA (Roche). Extraction was conducted under green safe light at 4°C. Samples were ground in liquid nitrogen and powder resuspended by vortexing. Samples were incubated at 4°C with rotation in darkness for 15 min, centrifuged in darkness at 4°C for 5 min at maximum speed. Supernatant was recovered and used to measure total protein by Bradford. 30 µg of total protein were loaded. Samples were run in a 10% PAGE-SDS gel, followed by wet transfer to nitrocellulose. phyA protein was detected using the AA01antibody (from Akira Nagatani, Kyoto University) at a dilution of 1:1000 (working solution 1 mg/ml) and probing with mouse-anti horseradish peroxidase antibody (1:5000 dilution). Loading was confirmed by reprobing the membranes with an anti-goat UGP-ase antibody (Agrisera) at 1:1000 dilution followed by a HRP-conjugated sheep anti-goat antibody (Biorad) at a 1:5000 dilution. Signal was detected with Amersham ECL kit (GE Health care) according to the manufacturer's protocol. Quantification was performed using Image-J software.

Simulation of natural dawn

Natural dawns in short photoperiods were simulated based on weather data from Edinburgh, UK. Light intensity data were obtained for 15-30th November and 15-30th January, from 2006 to 2015. These dates correspond to when the photoperiod is ~8h i.e. the short photoperiod condition which is being simulated. We calculated the distribution of times taken to reach 100µmol m^{-2} s^{-1}. This showed a clear peak around 50min (Fig S9A), which was taken as the 'fast dawn' condition. A 'slow dawn' (90 min duration) was chosen to represent the tail of the distribution of dawn durations (Fig S9A). These dawns were simulated in the growth chamber by increasing the light intensity in 10µmol $m^{-2}s^{-1}$ increments, to reach 100 µmol $m^{-2}s^{-1}$ at the specified time.