Photoreceptors CRYTOCHROME2 and Phytochrome B Control Chromatin Compaction in Arabidopsis[1][W][OA]

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Development and acclimation processes to the environment are associated with large-scale changes in chromatin compaction in Arabidopsis (*Arabidopsis thaliana*). Here, we studied the effects of light signals on chromatin organization. A decrease in light intensity induces a large-scale reduction in chromatin compaction. This low light response is reversible and shows strong natural genetic variation. Moreover, the degree of chromatin compaction is affected by light quality signals relevant for natural canopy shade. The photoreceptor CRYPTOCHROME2 appears a general positive regulator of low light-induced chromatin decompaction. Phytochrome B also controls light-induced chromatin organization, but its effect appears to be dependent on the genetic background. We present a model in which chromatin compaction is regulated by the light environment via CRYPTOCHROME2 protein abundance, which is controlled by phytochrome B action.

Light plays a crucial role in numerous plant developmental processes (Sullivan and Deng, 2003; Chen et al., 2004). Consequently, variation in light conditions has an enormous impact on the life cycle of a plant. To deal with light intensity, spectral quality, light direction, and photoperiod, plants have developed signaling mechanisms that are based on light-sensitive receptors. Three major photoreceptor families are known in Arabidopsis (*Arabidopsis thaliana*): phytochromes, cryptochromes, and phototropins (Sullivan and Deng, 2003; Chen et al., 2004; Casal and Yanovsky, 2005; Franklin et al., 2005; Lorrain et al., 2006). Phytochromes (e.g. phyA–phyE) mainly mediate in red and far-red light perception, while cryptochromes (CRY1, CRY2) and phototropins (PHOT1, PHOT2) are involved in the perception of blue light and UV-A.

The functional activities of photoreceptors are reflected by their subcellular location. CRY2 is constitutively located in the nucleus (Guo et al., 1999; Kleiner et al., 1999). CRY1 is also present in the nucleus, but only during darkness. In response to light CRY1 is exported to the cytosol, where it exerts different activities (Yang et al., 2000; Wu and Spalding, 2007). In contrast to CRY1, phytochromes are located in the cytosol during darkness and translocate to the nucleus upon light activation (Chen et al., 2005; Fankhauser and Chen, 2008). Unlike the other photoreceptors, phototropins have not been detected in the nucleus, but reside in the plasma membrane (Sakamoto and Briggs, 2002). Corresponding to their nuclear localization cryptochromes and phytochromes are involved in the control of gene transcription. Specifically, these photoreceptors mediate light-stimulated degradation and stability of several transcription factors (Casal and Yanovsky, 2005; Lorrain et al., 2006; Castillon et al., 2009), leading to modifications in gene expression profiles (Ma et al., 2001). In addition, physical interactions have been demonstrated not only between phytochromes and cryptochromes (Ahmad et al., 1998b; Más et al., 2000), but also between these photoreceptors and other proteins. For example, CRY2 interacts with the E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), which plays a role in the degradation of transcription factors (Yi and Deng, 2005).

Accordingly, it is assumed that light-mediated transcriptional control involves chromatin remodeling

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(Casal and Yanovsky, 2005; Lorrain et al., 2006). CRY2 is associated with chromatin (Cutler et al., 2000) and it physically interacts with the transcription factor CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX1 (CIB1), which binds to G-box sequences in promoters (Liu et al., 2008). Moreover, blue light-activated CRY2 represses the COP1/DET/FUS complex via COP1 (Wang et al., 2001). The photomorphogenesis regulator DEETIOLATED1 (DET1) binds to the core histone H2B (Benvenuto et al., 2002). Previously, we demonstrated that large-scale reduction of chromatin compaction during floral transition is affected in the *cry2* mutant (Tessadori et al., 2007b). The phenomenon is photoperiod independent, since both long-day- and short-day-grown plants showed a reduction in chromatin compaction prior to flowering. Moreover, we recently demonstrated that *phyb* mutants display reduced chromatin compaction under standard light conditions (Tessadori et al., 2009). Together, these data indicate that light controls large-scale chromatin organization. A similar decrease in chromatin compaction has been reported in plants facing stress (protoplastization, *Pseudomonas syringae* infection; Pavet et al., 2006; Tessadori et al., 2007a) or progression of development (seedling establishment and leaf maturation; Mathieu et al., 2003; Tessadori et al., 2004). This led to the suggestion that changes in light perception, stress, and developmental changes trigger comparable responses in nuclear organization of chromatin. In this study we address the question on how light signaling leads to changes in chromatin compaction.

The Arabidopsis interphase nucleus provides an excellent system to monitor chromatin compaction. Epigenetic marks (Naumann et al., 2005), fluorescence in situ hybridization (FISH; Fransz et al., 2002), and heterochromatin quantification have been used to characterize the nuclear phenotype (Soppe et al., 2002; Fransz et al., 2003; Tessadori et al., 2004). Chromosomes display highly condensed heterochromatic domains (chromocenters) and less condensed gene-rich euchromatin loops (Fransz et al., 2002; Tessadori et al., 2004). Chromocenters are conspicuous heterochromatin regions that mainly consist of long, tandemly arranged repetitive DNA elements, which include pericentromeric and satellite repeats and ribosomal DNA (rDNA) genes. These regions are typically enriched in repressive markers such as histones H3 dimethylated at Lys-9 (H3K9Me2) and DNA methylation (5-methylcytosine; Soppe et al., 2002).

In this study, we demonstrate reversible changes in chromatin compaction induced by low light intensities. CRY2 plays a major role in the signaling process from light intensity to chromatin organization in the Columbia-0 (Col-0), Landsberg *erecta* (L*er*), and Wassilewskija-2 (Ws-2) accessions. This blue light photoreceptor is a positive regulator of low light-induced chromatin decompaction. PhyB also induces chromatin decondensation in low light-treated Col-0. Moreover, we show phyB control of CRY2 protein abundance in light-regulated chromatin compaction.

RESULTS

Induction of Chromatin Reorganization by Low Light Is Reversible

Interphase nuclei of Arabidopsis can be classified into three groups based on their appearance after 4′,6-diamidino-2-phenylindole (DAPI) staining (Fig. 1, A–C). We distinguish type 1, with six to 10 conspicuous round chromocenters; type 2 with an intermediate appearance, showing elongated or irregularly shaped chromocenters; and type 3 with reduced heterochromatin, showing one to three chromocenters. This classification is supported by FISH analysis with probes for centromere and pericentromere repeats (Fig. 1, D–F). Based on this classification the heterochromatin

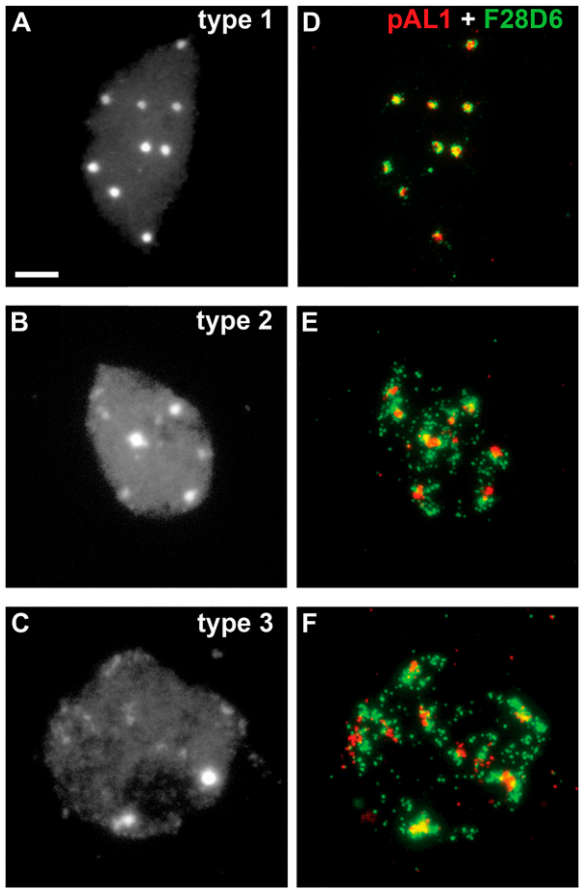

Figure 1. Cytogenetic analysis of Arabidopsis Col-0 mesophyll interphase nuclei. A to C, DAPI-stained nuclei. A, Type 1 nuclei have six to 10 conspicuous, round chromocenters. B, Type 2 displays an intermediate phenotype, consisting of elongated chromocenters and irregularly shaped regions of enhanced DAPI staining. C, Type 3 nuclei have few and small chromocenters. D to F, FISH signals for 180-bp centromeric tandem repeat (pAL1; red signal) and transposon-rich pericentromeric regions (BAC F28D6; green signal). D, In type 1 nuclei, both probes give a condensed signal localized at chromocenters. E, In type 2 nuclei, F28D6 is dispersed, while the 180-bp repeat is still condensed at the chromocenters, illustrating the intermediate state. F, In type 3, both centromeric and pericentric sequences are dispersed. Bar = 5 μm.

index (HX) is defined as the fraction of type 1 nuclei over the total number of nuclei and is utilized to monitor changes in chromatin compaction in response to low light intensity. In previous experiments plants were grown under constitutive light conditions (Tessadori et al., 2009). Here, we address the question if and how a period of low light stress by reducing photosynthetic active radiation (PAR) levels can lead to changes in chromatin compaction. Therefore we first grew plants under 200 μmol m^{-2} s^{-1} (control light) for 3 weeks and subsequently transferred them to 15 μmol m^{-2} s^{-1} (low light), without changing the spectral quality (Supplemental Fig. S1; Supplemental Table S1). Under these low light conditions we observed a sharp decrease in HX over time in the interphase mesophyll nuclei of accession Col-0, indicating a strong reduction in chromatin compaction (Supplemental Fig. S2). In plants grown under control light conditions the type 1 fraction remained constant (Fig. 2A). The data were confirmed by quantification of the relative heterochromatic fraction (RHF), which represents chromocenter density per individual nucleus (Fig. 2C). Continuation of low light treatment after 96 h did not further reduce the type 1 fraction in Col-0 (Fig. 2B). We observed similar HX changes in petioles and even in the roots (54% and 35%, respectively), suggesting that the low light signal is spread throughout the plant. Remarkably, reestablishment of control light conditions after 96 h resulted in an increase of the type 1 fraction to control levels (Fig. 2B). This indicates that large-scale decondensation of chromatin by low light is a reversible process.

No differences in visual chromatin were observed between plants kept in complete darkness for 96 h and plants kept in control light conditions (Supplemental Fig. S2). This suggests that the effects of low light intensity on chromatin compaction is not due to a general reduction for the plants' energy status and confirms that

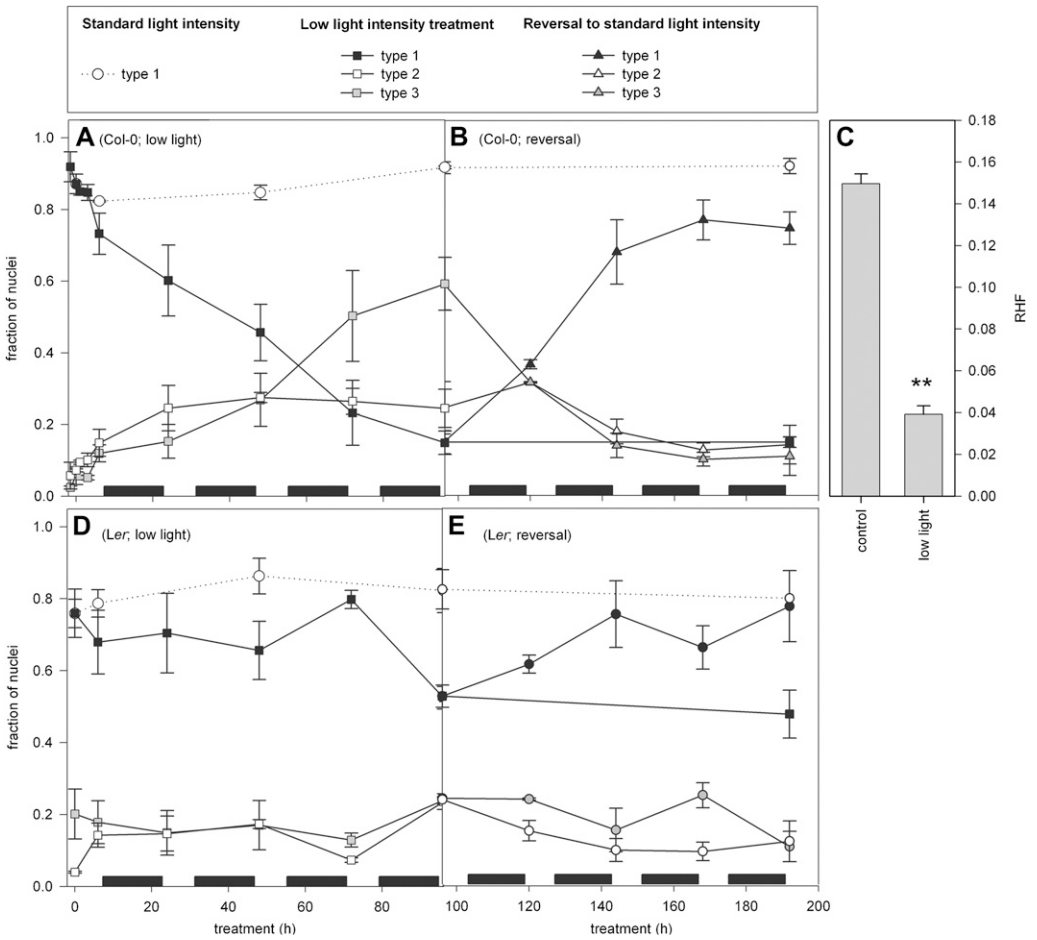

Figure 2. Changes of light intensity lead to changes of chromatin compaction. The fractions are of type 1, type 2, and type 3 nuclei from 3-week-old plants. Col-0 (A and B) and L*er* (D and E) plants were monitored after lowering the light intensity from 200 to 15 μmol m^{-2} s^{-1} (A and D; squares) and after return to standard (control) light conditions (B and E; triangles). Circles represent plants that were kept in control light conditions throughout the experiment. The RHF was measured in Col plants under normal light conditions and after 4 d of low light treatment (C). $n > 31$. Significance level: ** $P < 0.01$, two-tailed Student's *t* test, compared to control light. Error bars represent SE in all sections. Horizontal bars indicate the night (dark) period.

light signaling is causal for the observed changes. In agreement, no changes in HX were observed in plants grown in control light conditions, harvested at the beginning and the end of the dark period (night).

Since we showed a correlation between HX reduction and the floral transition (Tessadori et al., 2007b), we examined if the low light-induced reduction in HX affected flowering time. This, however, appeared not likely, since Col-0 plants showed delayed flowering even after 96 h of low light treatment ($P < 0.001$; appearance of the flower buds; 2.6 d and flower opening 3.1 d later). Moreover, the HX of plants grown in long-day conditions (16-h photoperiod) was not significantly different from the standard short-day-grown plants (HX; 0.88 ± 0.03 versus 0.92 ± 0.02) and after 96 h of low light treatment (HX; 0.15 ± 0.03 versus 0.26 ± 0.03). The latter indicates that chromatin compaction is not controlled by photoperiod.

Global chromatin decondensation during the floral transition occurred not only in heterochromatin regions, but also in gene-rich euchromatin (Tessadori et al., 2007b). To examine if this is also true for low light-treated plants, we applied bacterial artificial chromosome (BAC)-FISH to euchromatin regions. A clear decrease in compact FISH signals was observed (Supplemental Fig. S3). In addition, the euchromatin index (defined as the fraction of nuclei showing decondensation of euchromatin over the total number of nuclei) reduced significantly in response to the low light treatment (Supplemental Fig. S3). Together, this underscores the global nature of the chromatin decompaction response to low light treatment.

We observed similar low light responses in L*er*, but the HX reduction was less severe compared to Col-0 (Fig. 2, D and E; Supplemental Fig. S2), suggesting genetic variation in light-controlled chromatin compaction. We therefore tested a selected set of Arabidopsis accessions from different geographic origins. Interestingly, we found strong variation in the ability to respond to low light treatment (Fig. 3C). Even Cape Verde Islands-0 (Cvi-0), which already shows a typical low HX (fraction of type 1 nuclei: 0.19) in control light conditions, was able to reduce chromatin compaction during a prolonged low light period. The HX reduction under low light treatment did not correlate to any geographic or climatologic parameter (data not shown), which contrasts with the HX in control light conditions (Tessadori et al., 2009). This suggests differences in the genetic basis of light-controlled chromatin compaction at different light intensities. The question arose if accessions with high HX after 96 h of low light treatment, such as Shah and Stange, are unable to fully reduce chromatine compaction or if the accessions differ in the rate to reduce chromatin compaction. Considering that plants from accessions with similar fractions of type 1, 2, and 3, such as Be-0 and Pak-1, resemble Col-0 plants after 48 h of low light treatment, we suggest that the process of chromatin decondensation is still continuing in Be-0 and Pak-1. In fact, the distribution of type 1, 2, and 3 in each accession resembles the distribution of type 1, 2, and 3 fractions in Col-0 during low light treatment. To test this hypothesis we assayed the HX after a prolonged period of 192 h. All accessions indeed showed a further reduction, resulting in reduced HX comparable to Col-0 after 192 h (Fig. 3D). This indicates genetic variation in the rate to reduce chromatin compaction. The significance of the variation was confirmed by the negative linear correlation ($r^2 = -0.52$; $P < 0.02$) between type 1 nuclei and intermediate type 2 nuclei at this time point.

Spectral Light Controls Chromatin Compaction

The applied low light treatment is spectrally neutral and reduces the blue light component and the total PAR, but not the red-to-far red (R/Fr) ratio (Supplemental Fig. S1; Supplemental Table S1), which is an intrinsic component of several natural shade conditions. To test the functional importance of blue light wavelengths and the R/Fr signaling in light-dependent chromatin compaction, we dissected the light signal into different components using colored filters. Total PAR was kept between 90 and 115 μmol m^{-2} s^{-1}, which is sufficient to saturate the HX in Col-0 (Tessadori et al., 2009). Both low blue (yellow filter) and spectral shade (low blue + low R/Fr; green filter) induced a significant reduction of the HX in Col-0 and L*er* (Fig. 3, A and B). Since spectral shade led to a stronger reduction than low blue light shading only, we conclude that both blue light and the R/Fr ratio are important signals controlling chromatin compaction. None of these spectral treatments, however, resulted in a reduction in HX as strong as spectrally neutral low light, highlighting that perception of total PAR is an important factor in controlling chromatin compaction.

CRY2 and PhyB Are Major Factors in Light Signaling to Chromatin Compaction

Because multiple wavelengths influence HX (Fig. 3), we investigated which photoreceptor proteins are involved in the control of low light-mediated chromatin compaction. All but one (see below) of the tested mutants had a statistically similar HX as their respective wild types in control light conditions (Fig. 4A). We evaluated the HX after 96 h of low light treatment in a selection of photoreceptor mutants in the Col-0, L*er*, or Ws-2 genetic backgrounds. In agreement with the observation in Col-0 and L*er* (Fig. 3), Ws-2 wild type also responded to low blue and to spectral shade signals (Supplemental Fig. S4).

The HX after 96 h of low light treatment in *phot1* (L*er*), *phot2* (Ws-2) mutants, and *phot1 phot2* (L*er*/Ws-2) double mutant was not significantly different from their respective wild-type backgrounds (Fig. 4A). This indicates that phototropins are not involved in the response. In contrast, the cryptochrome mutants (*cry1*, *cry2*) in the Col-0 genetic background showed a significantly higher HX after low light treatment ($P < 0.05$

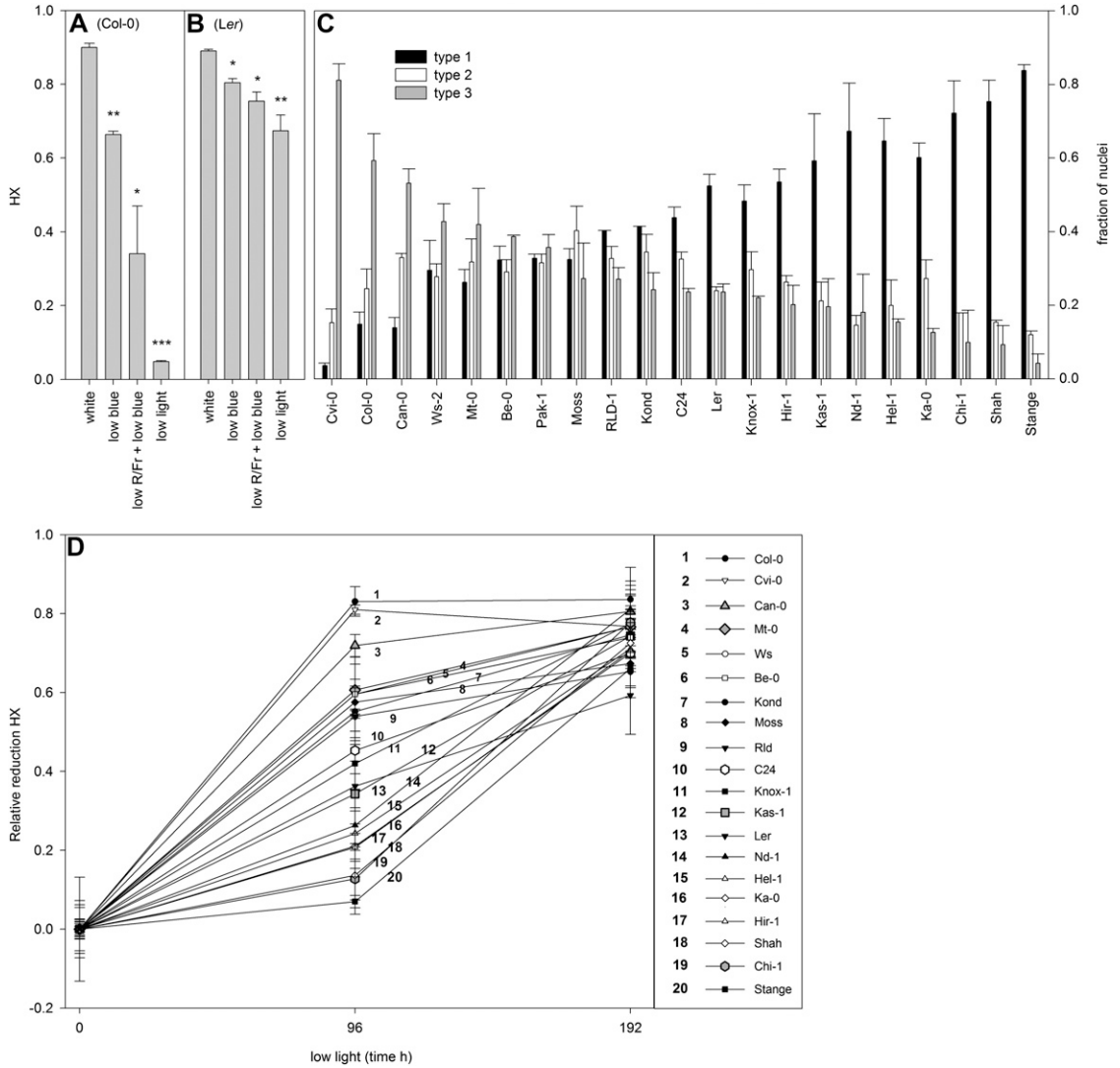

Figure 3. Light quality control and natural variation of chromatin compaction. A and B, Col-0 (A) and L*er* (B) plants after 96-h treatment with different light qualities. Conditions were as follows: control filter, indicated as white light (white; approximately 100 μmol m^{-2} s^{-1}); low blue light (yellow filter); and spectral shade (low R/Fr + low blue; green filter). Low light treatment consisted of spectral neutral shading by reducing PAR to 15 μmol m^{-2} s^{-1}. $n > 3$. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-tailed Student's *t* test, compared to white light. For light spectra and wavelength quantification, see Supplemental Figure S1 and Supplemental Table S1. C, The fraction of type 1, type 2, and type 3 nuclei after 4 d of low light treatment in a selected set of natural occurring Arabidopsis accessions. D, Relative reduction of HX of the accessions after 96- and 192-h exposure to low light (200 μmol m^{-2} s^{-1} to 15 μmol m^{-2} s^{-1}). Error bars represent SE in all sections, $n = 2$.

and $P < 0.001$, respectively) than the wild type (Fig. 4A; Supplemental Fig. S2). Moreover, ectopic expression of *CRY2* (driven by the 35S cauliflower mosaic virus promoter) in Ws-2 resulted in a slightly lower HX after low light treatment (Fig. 4A), although this difference was not significant. Apparently, cryptochromes are required for low light-controlled chromatin compaction in Col-0 and Ws-2. Surprisingly, we observed no difference in HX reduction in *cry* mutants in the L*er* background (*cry2* mutant, *cry1 cry2* double mutant; Fig. 4A). Moreover, the quadruple mutant lacking all dedicated blue light photoreceptors (*cry1 cry2 phot1 phot2*) was not significantly different from wild-type L*er* (Fig. 4A). These data point to a masking effect in L*er*, because L*er* is relatively insensitive for low light-mediated HX changes in chromatin compared to Col-0 (Fig. 2; Supplemental Fig. S2). Alternatively, cryptochromes may not be involved in light-mediated reduction of HX in the L*er* background.

The HX in the *phyb9* mutant in Col-0 was significantly higher ($P < 0.001$) after low light treatment than in the wild type (Fig. 4B). However, a mutation in the same gene in the L*er* background (*phyb5*) showed the

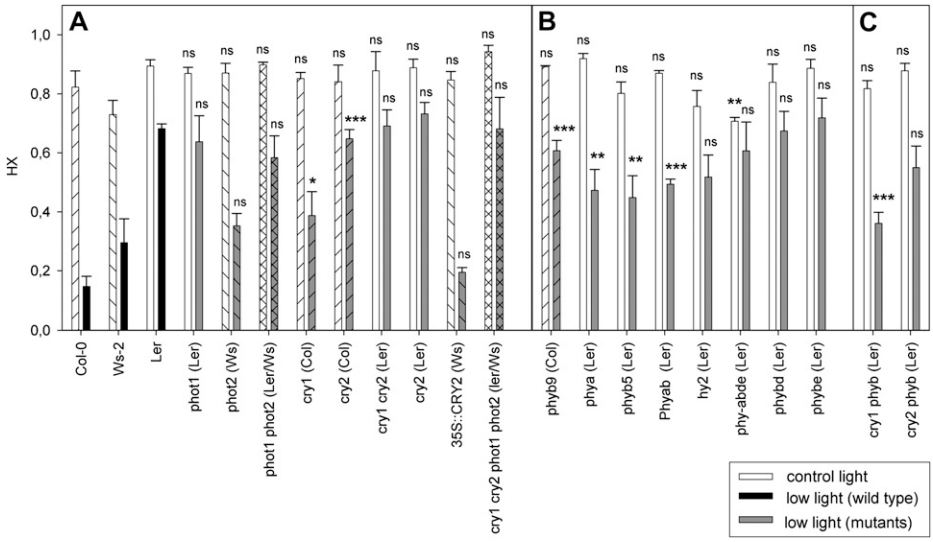

Figure 4. Involvement of photoreceptors in low light-induced reduction of chromatin compaction. HX in control light conditions (white bars; 200 μmol m^{-2} s^{-1}) and after 96 h of low light treatment (wild type in black and mutants in gray; 15 μmol m^{-2} s^{-1}) in *phot* and *cry* mutants (A), *phy* mutants (B), and *phyb cry* double mutants (C). Mutants are in the Col-0 (forward-dashed bars), Ws-2 (back-dashed bars), or L*er* (bars with no fill) genetic background. Mutants in a mixed Ws-2/L*er* genetic background have hived bars. The genetic background of each mutant is shown between brackets. Error bars represent SE in all sections. $n > 2$. Significance levels: ns = nonsignificant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-tailed Student's *t* test, compared to control or low light treatment in the wild types.

opposite effect: a significantly lower ($P < 0.01$) HX after low light treatment than wild type ($P < 0.05$; Fig. 4B). The same accounts for *phya* (Fig. 4B). This effect was confirmed in the *phya phyb* double mutant (Fig. 4B), suggesting that at least phyB has opposite effects in Col-0 and L*er*. To test for involvement of phyC–E, we examined the chromophore-deficient mutant *long hypocotyl2/genome uncoupled3* (*hy2/gun3*), which has reduced levels of active phytochromes (Kohchi et al., 2001). This line, and the quadruple *phy-abde* mutant, had a statistically similar HX than wild type after low light treatment (Fig. 4B). This suggests that phyD, phyE, or both, may act antagonistically to phyA and phyB. We confirmed the antagonistic action for phyB using the double mutants, *phyb phyd* and *phyb phye*, which complemented the low HX of the *phyb* mutant after low light treatment, to a level indistinguishable from wild type (Fig. 4B). Notably, the *phy-abde* quadruple mutant had a significantly lower HX under control light conditions (Fig. 4A). Apparently, loss of multiple phytochromes results in a low HX already under control light conditions.

The low HX in *phyb5* (L*er*) after low light treatment provides a tool to study whether cryptochromes in L*er* are involved in light-mediated chromatin compaction. The *cry1 phyb* double mutant showed a significantly lower HX ($P < 0.001$), compared to L*er* (Fig. 4C). In contrast, the *cry2 phyb* mutant had a HX that was statistically similar to wild type after low light treatment (Fig. 4C). This implies that also in L*er* *CRY2* is a positive regulator of HX reduction.

CRY2 Protein Abundance Correlates to Chromatin Compaction

Reduction of HX during floral transition was absent in *cry2* mutants (Tessadori et al., 2007b). Moreover, accession Cvi-0, which has stable CRY2 protein levels (El-Assal et al., 2001), typically shows a low HX (Tessadori et al., 2009). Similarly, *cry2* mutations and *CRY2* overexpression prevented and enhanced low light-induced reduction of HX, respectively (Fig. 4A). These data imply that CRY2 protein levels control the chromatin compaction state.

To examine this, we monitored CRY2 protein levels by western-blot analysis and observed consistently higher CRY2 levels throughout the photoperiod in plants exposed to low light (Fig. 5A). Plants kept for 96 h in low light conditions still showed a high CRY2 level, indicating that the CRY2 protein abundance persists under low light conditions (Fig. 5B). Together, this shows that low light enhances or stabilizes CRY2 protein levels over the photoperiod and confirms the existence of a negative correlation between chromatin decondensation and CRY2 abundance. The increase in CRY2 protein after 3-h matches with the observation that low light-induced reduction of chromatin compaction is detectable during the first hours of low light treatment (Fig. 2A).

To dissect if CRY2 protein concentration can explain the contrasting effects of low light on HX between *phyb* mutants in Col-0 and L*er* (Fig. 4B), we examined the CRY2 concentration in these mutants. *Phyb* mutants of both accessions showed reduced CRY2 levels in con-

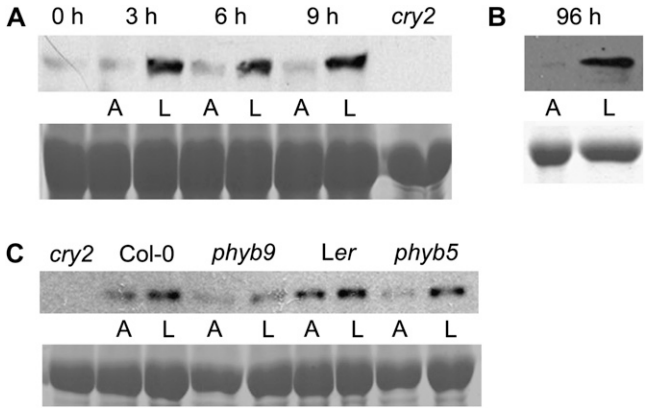

Figure 5. Low light treatment enhances CRY2 protein abundance. A to C, Western-blot analysis of plants in control light conditions (A: 200 μmol m^{-2} s^{-1}) and low light conditions (L: 200 μmol m^{-2} s^{-1} to 15 μmol m^{-2} s^{-1}; induced at the start of the photoperiod [$t = -1.5$ h] using CRY2 antibody [top lanes] and loading control [Coomassie Brilliant Blue; bottom lanes]). A, Col-0 plants sampled at different times during the photoperiod. B, Col-0 plants sampled after 96 h of low light conditions and control light conditions. C, *phyb* mutants in the Col-0 (*phyb9*) and L*er* (*phyb5*) genetic backgrounds, sampled at $t = 9$ h after low light treatment. Note that in A and C, the absence of a detectable product in the *cry2* (Col-0) mutant indicates correctness of the identified CRY2 protein.

trol light conditions (Fig. 4B). In contrast to Col-0, L*er* did not exhibit a pronounced difference in CRY2 protein levels between low and control light conditions. However, similarly to Col-0 and the *phyb9* mutant (Col-0 background), *phyb5* (L*er* background) was able to stabilize CRY2 protein levels in low light conditions (Fig. 5C). This indicates that phyB effects on CRY2 protein levels can account for the differential phenotypes observed in *phyb9* and *phyb5* mutants in Col-0 and L*er* and indicates that CRY2 and phyB are epistatic in the control of low light-induced chromatin decompaction.

DISCUSSION

CRY2 Controls Light-Mediated Chromatin Compaction

Many biological processes, such as biotic infestation and development entail major changes in chromatin organization (Mathieu et al., 2003; Tessadori et al., 2004, 2007a, 2007b; Pavet et al., 2006). This also includes the control of light-mediated processes (Casal and Yanovsky, 2005; Lorrain et al., 2006; Tessadori et al., 2007b, 2009). Interestingly, light intensity controls the abundance of some photoreceptor proteins, including CRY2 (El-Assal et al., 2001; Fig. 5). We provide evidence that CRY2 protein abundance controls chromatin compaction. CRY2 was demonstrated to decorate mitotic chromosomes (Cutler et al., 2000), indicating close association with chromatin. Moreover, cryptochrome-chromatin interaction has been reported by Lin and Shalitin (2003). Recently, CRY2 was shown to regulate transcription of the floral integrator FT via physical interaction with CIB1 (Liu et al., 2008). This transcription factor binds to the G box of the FT promoter, connecting CRY2 activity to chromatin. A second link between CRY2 and chromatin is provided by the interaction of CRY2 with the RING finger type E3 ubiquitine ligase (COP1). COP1 represses photomorphogenesis by targeting light-responsive transcription factors, such as HY5, for proteolysis (Wang et al., 2001; Gyula et al., 2003). CRY2 represses the COP/DET/FUS complex through a physical association with COP1. The interaction between CRY2 and the COP/DET/FUS complex is interesting because DET1 was shown to bind nonacetylated core histone H2B, thereby maintaining a condensed chromatin state (Benvenuto et al., 2002). Blue light-activated CRY2 releases the DET1 block permitting transcriptional activation, which might accompany the opening of the chromatin as observed under our low light conditions. In *cry2* null mutants the CRY2-mediated repression of the COP/DET/FUS complex is lost, which is a possible explanation for the absence of chromocenter decompaction during the low light treatment in this mutant. The role of CRY2 in chromatin decondensation may be the same as in photomorphogenesis, where CRY2 action is largely restricted to transduce light signals to repress HY5 down-regulation (Lin et al., 1998). Accordingly, we observed stabilization of the CRY2 protein under low light intensities in short-day conditions. In standard light conditions, CRY2 is prone to degradation under these photoperiod conditions (El-Assal et al., 2001). In addition, CRY2 in Cvi-0 was shown to be stabilized under short-day photoperiods, compared to L*er* (El-Assal et al., 2001). This indicates that CRY2 may also contribute to the reduced chromatin compaction phenotype in Cvi-0, as in Tessadori et al. (2009) we described that Cvi-0 has a constitutive low chromatin compaction in standard light conditions due to aberrant HISTONE DEACETYLASE6 (HDA6) and phyB action.

Phytochromes Control Light-Mediated Chromatin Compaction

Previously we showed that phyB is a positive regulator of chromatin compaction (Tessadori et al., 2009). Here, we demonstrate by mutant analysis that phyB also controls chromatin decompaction upon a decrease in light intensity. However, the control by phyB in Col-0 is opposite to L*er*, pointing to natural allelic variation in phyB signaling with respect to chromatin compaction. Yet, no sequence polymorphisms are present between the *phyb* alleles of Col-0 and L*er* (Filiault et al., 2008). Our study with the loss-of-function mutations in *phyb* in two independent backgrounds showed that CRY2 protein abundance was constitutively reduced in the *phyb* mutant background. This indicates that CRY2 protein levels are maintained by phyB. Active CRY2 interacts with phyB in specific nuclear speckles (Más et al., 2000), which may act as

transcriptosomes (Gyula et al., 2003). CRY2 is rapidly downregulated in blue light (Lin et al., 1998), probably in the same nuclear speckles (Yu et al., 2009). Additionally, COP1 can form nuclear bodies (Yang et al., 2000; Wang et al., 2001) and it has been suggested that cryptochromes, phytochromes, and COP1 interact in the nuclear bodies (Yu et al., 2009). This supports our observation that phyB modulates CRY2 stability, possibly by prohibiting CRY2 degradation. However, we cannot exclude that proteins other than phyB account for CRY2 stabilization, as low light consolidates the remaining CRY2 protein in *phyb* mutant backgrounds.

CONCLUSION

A Model for Light Control in Chromatin Compaction

Low light-induced global decondensation of chromatin concerns changes in the large-scale folding of chromatin, rather than changes at the nucleosome level. Specific local modifications of histones, however, are prone to occur. It underscores our previous reports where we found no alterations in global H3K9me2 and DNA methylation when chromatin drastically decondenses in cultured protoplasts (Tessadori et al., 2007a).

Both cryptochromes and phytochromes are involved in the global decompaction response of chromatin to low light conditions. Light induces activation of CRY2, but is also required to target the protein for degradation (Ahmad et al., 1998a; Shalitin et al., 2002; Yi and Deng, 2005; Yu et al., 2007, 2009). However, under low light conditions CRY2 accumulate to high levels, which may point to the significance of CRY2 under shade avoidance conditions (our data; Ahmad et al., 1998a). CRY1 and CRY2 have overlapping functions, but differ in stability depending on light intensity. At higher light intensities CRY2 is degraded, whereas CRY1 is not (Ahmad et al., 1998a). The data presented here indicate that low light triggers a reversible reduction of chromatin compaction via CRY2 protein stabilization. These data are in agreement with our previous work (Tessadori et al., 2007b), in which we showed that floral transition-associated reduction in chromatin compaction was absent in the *cry2* mutant background. In addition, phyB modulates CRY2 protein abundance. We therefore propose that phyB affects chromatin compaction by controlling CRY2 levels. However, CRY2 is also controlled directly by light, as CRY2 is stabilized by low light intensities independently of phyB activity. Alternatively, phyB may have a direct, CRY2-independent, role in controlling light-mediated chromatin compaction. Mutant analysis indicated that CRY2 is a negative regulator of chromatin compaction in both Col-0 and L*er* genetic backgrounds. On the contrary, the data points to natural variation between Col-0 and L*er* in phyB signaling toward chromatin compaction. Based on these data we propose a model on how light-mediated control of chromatin compaction via photoreceptor proteins may be operated (Fig. 6). We speculate that both CRY2 and phyB converge on a chromatin protein complex (CPC) that is functionally responsible for maintenance of chromatin compaction. Putative components of the CPC may be members of E3 ligase complexes, such as COP1, which is known to interact with cryptochromes and phytochromes (Yi and Deng, 2005) or HDA6, which was recently shown to control light-mediated chromatin compaction (Tessadori et al., 2009).

We conclude that different molecular mechanisms exist for shade-induced chromatin reduction and control of chromatin compaction under normal light conditions. Support for this conclusion comes from observations on mutant *hda6* (L*er* genetic background). This line showed a reduction of chromatin compaction under low light conditions similar to the wild type (relative reduction 0.54; data not shown), despite the fact that chromatin compaction is already low in this mutant under control light conditions (Probst et al., 2004; Fransz et al., 2006).

The data presented in this study support the concept that environmental stimuli and developmental changes trigger responses in the global organization of chromatin, i.e. low light, protoplast formation, bacterial infection, leaf maturation, floral transition, and seedling development (Mathieu et al., 2003; Tessadori et al., 2004, 2007a, 2007b, 2009; Pavet et al., 2006). The biological relevance of large-scale chromatin compaction, how-

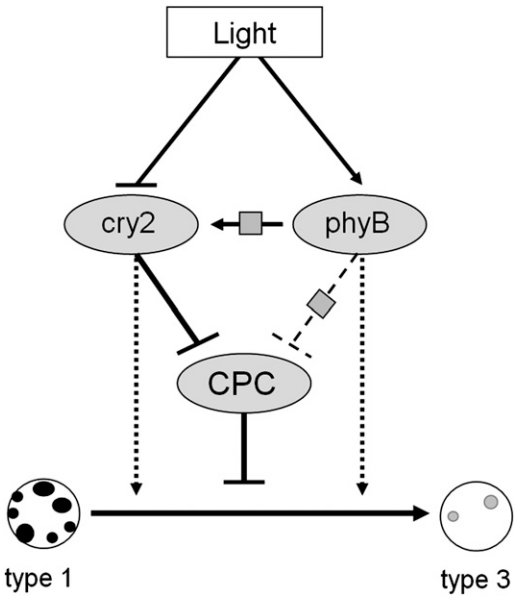

Figure 6. A model for light control of chromatin compaction. Light triggers CRY2 degradation, thereby maintaining a condensed chromatin compaction state (type 1 nuclei) via control of a CPC or directly (dashed arrow). PhyB stabilizes CRY2 protein levels and may have a direct (dashed arrows) effect on chromatin decompaction (type 1–type 3) or, alternatively, via the same CPC as CRY2 (dashed inhibitory sign). Natural variation in the phyB signaling, however, affects the role of this photoreceptor in controlling chromatin compaction (possible points of natural genetic variation are indicated by square boxes).

ever, is not yet clear. We speculate that global decondensation of chromatin facilitates the accessibility of target genes controlling a rapid response to changing environmental conditions or developmental stimuli. Without global decondensation this response may be delayed. This hypothesis is supported by the observation that *cry2* mutants, which lack global chromatin decondensation (Tessadori et al., 2007b; this study), are delayed in floral transition (Guo et al., 1998) and low light-induced hyponastic leaf movement (Millenaar et al., 2009).

MATERIALS AND METHODS

Plant Material and Growth Conditions

The origins of the Arabidopsis (*Arabidopsis thaliana*) accessions used are described in Supplemental Table S2. Mutants were either from the Nottingham Arabidopsis Stock Center (NASC) or were a kind gift of authors who described these mutants: *phot1-101* (Liscum and Briggs, 1995), *phot2-5* (Jarillo et al., 2001; Kagawa et al., 2001), *phot1-101 phot2-5* (Ws-2/L*er*; Sakai et al., 2001), *cry1 cry2 phot1 phot2* (in Ws-2/L*er*; Ohgishi et al., 2004), *cry1* (Col; Mockler et al., 1999), *cry2-1* (in Col; Guo et al., 1998), *cry2* (*fha1* in L*er*; Koornneef et al., 1991; Guo et al., 1998), *cry1 cry2* (*hy4 fha1* in L*er*; Yanovsky et al., 2000), *35S:CRY2* (Lin et al., 1998), *phyb9* (in Col; Reed et al., 1993), *phya201* (N6219; Nagatani et al., 1993), *phyb5* (in L*er*; N69; Koornneef et al., 1980), *phya-201 phyb5* (N6224; Reed et al., 1994), *hy2* (N68; Koornneef et al., 1980; Kohchi et al., 2001), *phy-abde* (Franklin et al., 2003), *phyb-1 phyd-2* (Devlin et al., 1999), *phyb phye-1* (Devlin et al., 1998), *phyb5 cry1-1* (Casal and Mazzella, 1998), and *phyb5 cry2* (Mazzella et al., 2001).

Plants were grown on a fertilized mixture of potting soil and perlite (1:2 [v/v]) as described by Millenaar et al. (2005), at 20°C, 70% (v/v) relative humidity, 9-h photoperiod of 200 μmol m^{-2} s^{-1} PAR. Pots were daily saturated automatically with tap water at the start of the photoperiod. The long-day photoperiod used was 16 h. Twenty-two-day-old plants, at developmental stage 1.05 to 1.07 (Boyes et al., 2001), which is well before the floral transition, were used for all experiments. Plants used for scoring flowering time were transferred to low light conditions (15 μmol m^{-2} s^{-1}) or long-day regime (16-h photoperiod) 18 d after sowing. After the treatment plants were returned to control light conditions (200 μmol m^{-2} s^{-1}). Flowering time (number of days) was scored at bolting (appearance of flower buds) and at the time of opening of the first flower.

Light Treatment

Plants were transferred to the experimental setups 1 d before the start of the treatment to allow acclimatization. Treatments always started (t = 0 h) 1.5 h after photoperiod start, to minimize diurnal and/or circadian effects, except for CRY2 protein level analysis where low light was induced at the start of the photoperiod (t = −1.5 h). Reduction of light intensities consisted of a 90% reduction of the PAR (400–700 nm); from 200 μmol m^{-2} s^{-1} to 15 μmol m^{-2} s^{-1} and was accomplished by shading the plants with shade cloth. This did not influence the spectral quality (Supplemental Fig. S1; Supplemental Table S1) as checked with a LI-COR 1800 spectro-radiometer (LI-COR). Complete darkness was accomplished by transferring the plants in large, light-tight boxes in the same growth room.

For colored-filter (Lee Filter) treatments, plants were kept in flow-through aerated boxes, only allowing the filtered top light to reach the plants. Spectral shade treatment and low blue were induced by filtering white light through a double layer of fern green filter (Lee filter no. 122) and a double layer of yellow filters (Lee filter no. 200 double CT blue), respectively. Both filter treatments also reduced total PAR. Therefore, control plants were shaded with shade cloth to 90 and 115 μmol m^{-2} s^{-1}. For spectra and quantification of wavelength regions of all light treatments used, see Supplemental Figure S1 and Supplemental Table S1.

Sample Preparation

Young rosette leaves were harvested, fixed in Carnoy's solution (ethanol/acetic acid 3:1), and stored at −20°C. Each sample consisted of two plants. Spread preparations were made essentially as described by Schubert et al. (2001), with a modified enzymatic cell wall-degrading mixture: 0.6% (Yakult Pharmaceuticals), 0.25% Macerozyme R10 (Duchefa) in 10 mM citrate buffer, pH 4.5. Slides were mounted in Vectashield (Vector Laboratories) with DAPI (2 μg mL^{-1}) before observation. For HX calculation, 100 to 130 nuclei of at least two samples (four plants) were analyzed.

Measure of HX and RHF

HX (Tessadori et al., 2007b) was defined as the percentage of nuclei showing high content of compact chromatin (type 1; Fig. 1A), represented by conspicuous chromocenters, as opposed to nuclei with less compact chromatin (type 2 or type 3; Fig. 1, B and C). For RHF quantification, automated digital analysis of gray-scale images was carried out with in house developed macros in Image-Pro-Plus (Media Cybernetics). RHF, defined as the fluorescence intensity of all DAPI-stained chromocenters relative to the fluorescence of the entire nucleus, was calculated for each sample as described earlier (Soppe et al., 2002; Tessadori et al., 2004). Statistical analysis was two-tailed Student's t test.

FISH

Plasmid pAL1 (Martinez-Zapater et al., 1986) was used to detect the 180-bp centromeric tandem repeat. BAC F28D6 (GenBank accession no. AF147262) obtained from NASC in pBeloBAC-Kan vector was used for the detection of pericentromeric repeats. 5S rDNA was from Campell et al. (1992); 45S rDNA probe was from Gerlach and Bedbrook (1979). The euchromatin region was detected with the BACs F13C5, T18B16, and F28A21 (DDBJ/EMBL/GenBank accessions no. AL021711, 119.1 kb; no. AL021687, 96.5 kb; and no. AL025526, 94.3 kb, respectively), which are mapped in the middle of chromosome 4.

FISH experiments were carried out essentially as described by Schubert et al. (2001). The nuclei were counterstained with DAPI (2 μg mL^{-1} in Vectashield, Vector Laboratories) prior to observation. Slides were examined with an Olympus BX6000 epifluorescence microscope (Olympus) coupled to a CCD camera (Coolsnap FX Photometrics). After acquisition the images were processed, pseudocolored, and merged using Adobe Photoshop software (Adobe).

Western-Blot Analysis

Young Arabidopsis rosette leaves were used. Rosette leaves were snap frozen in liquid nitrogen and stored at −80°C. The plant material was ground in liquid nitrogen and protein isolation buffer was added (50 mM Tris, pH 7.5, 5 mM EGTA, 5 mM EDTA, 2 mM dithiothreitol, 25 mM NaF, 1 mM Na3VO4, 50 mM β-glycerophosphate, complete protease inhibitors [Roche]). The extract was centrifuged for 10 min at 10,000*g*. Proteins in the supernatant were separated using a 10% acrylamide gel. Goat anti-rabbit conjugated with horseradish peroxidase (Amersham Biosciences) was used to detect the anti-CRY2 and detected by enhanced chemiluminescence (Pierce). Anti-CRY2 polyclonal antibody serum was kindly provided by Margret Ahmad. Loading control was performed using colloidal Coomassie Brilliant Blue (Sigma-Aldrich).

Accession numbers: BAC F28D6 (GenBank accession no. AF147262); BACs F13C5, T18B16, and F28A21 (DDBJ/EMBL/GenBank accessions no. AL021711, 119.1 kb; no. AL021687, 96.5 kb; and no. AL025526, 94.3 kb, respectively). NASC provided several Arabidopsis lines: *phya201* (N6219), *phyb5* (in L*er*; N69), *phya-201 phyb5* (N6224), *hy2* (N68).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Light spectra of the treatments used in this study.

Supplemental Figure S2. Effect of 96 h of low light and 96 h of complete darkness on visual chromatin.

Supplemental Figure S3. Cytogenetic analysis of euchromatin in standard and low light conditions.

Supplemental Figure S4. HX of Ws-2 after 96-h treatment with different light qualities.

Supplemental Table S1. Quantification of light intensities in specified wavelength regions, and the R/Fr ratio of the used light treatments.

Supplemental Table S2. Accessions used in this study and quantification of nuclei types.

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LITERATURE CITED

Ahmad M, Jarillo JA, Cashmore AR (1998a) Chimeric proteins between cry1 and cry2 *Arabidopsis* blue light photoreceptors indicate overlapping functions and varying protein stability. Plant Cell **10:** 197–207

Ahmad M, Jarillo JA, Smirnova O, Cashmore AR (1998b) The CRY1 blue light photoreceptor of Arabidopsis interacts with phytochrome A *in vitro*. Mol Cell **1:** 939–948

Benvenuto G, Formiggini F, Laflamme P, Malakhov M, Bowler C (2002) The photomorphogenesis regulator DET1 binds the amino-terminal tail of histone H2B in a nucleosome context. Curr Biol **12:** 1529–1534

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Görlach J (2001) Growth stage-based phenotypic analysis of *Arabidopsis*: a model for high throughput functional genomics in plants. Plant Cell **13:** 1499–1510

Campell BR, Song YG, Posch TE, Cullis CA, Town CD (1992) Sequence and organization of 5S ribosomal RNA-encoding genes of *Arabidopsis thaliana.* Gene **112:** 225–228

Casal JJ, Mazzella MA (1998) Conditional synergism between cryptochrome 1 and phytochrome B is shown by the analysis of *phyA, phyB*, and *hy4* simple, double, and triple mutants in Arabidopsis. Plant Physiol **118:** 19–25

Casal JJ, Yanovsky MJ (2005) Regulation of gene expression by light. Int J Dev Biol **49:** 501–511

Castillon A, Shen H, Huq E (2009) Blue light induces degradation of the negative regulator phytochrome interacting factor 1 to promote photomorphogenic development of Arabidopsis seedlings. Genetics **182:** 161–171

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

Chen M, Tao Y, Lim J, Shaw A, Chory J (2005) Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. Curr Biol **15:** 637–642

Cutler SR, Ehrhardt DW, Griffitts JS, Somerville CR (2000) Random GFP: cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. Proc Natl Acad Sci USA **97:** 3718–3723

Devlin PF, Patel SR, Whitelam GC (1998) Phytochrome E influences internode elongation and flowering time in *Arabidopsis*. Plant Cell **10:** 1479–1487

Devlin PF, Robson PRH, Patel SR, Goosey L, Sharrock RA, Whitelam GC (1999) Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. Plant Physiol **119:** 909–915

El-Assal S, Alonso-Blanco C, Peeters AJM, Raz V, Koornneef M (2001) A QTL for flowering time in Arabidopsis reveals a novel allele of *CRY2*. Nat Genet **29:** 435–440

Fankhauser C, Chen M (2008) Transposing phytochrome into the nucleus. Trends Plant Sci **13:** 596–601

Filiault DL, Wessinger CA, Dinneny JR, Lutes J, Borevitz JO, Weigel D, Chory J, Maloof JN (2008) Amino acid polymorphisms in Arabidopsis phytochrome B cause differential responses to light. Proc Natl Acad Sci USA **105:** 3157–3162

Franklin KA, Larner VS, Whitelam GC (2005) The signal transducing photoreceptors of plants. Int J Dev Biol **49:** 653–664

Franklin KA, Praekelt U, Stoddart WM, Billingham OE, Halliday KJ, Whitelam GC (2003) Phytochromes B, D, and E act redundantly to control multiple physiological responses in Arabidopsis. Plant Physiol **131:** 1340–1346

Fransz P, De Jong JH, Lysak M, Castiglione MR, Schubert I (2002) Interphase chromosomes in Arabidopsis are organized as well defined chromocenters from which euchromatin loops emanate. Proc Natl Acad Sci USA **99:** 14584–14589

Fransz P, Soppe W, Schubert I (2003) Heterochromatin in interphase nuclei of *Arabidopsis thaliana*. Chromosome Res **11:** 227–240

Fransz P, ten Hoopen R, Tessadori F (2006) Composition and formation of heterochromatin in *Arabidopsis thaliana*. Chromosome Res **14:** 71–82

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res **7:** 1869–1885

Guo H, Duong H, Ma N, Lin C (1999) The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J **19:** 279–287

Guo HW, Yang H, Mockler TC, Lin CT (1998) Regulation of flowering time by Arabidopsis photoreceptors. Science **279:** 1360–1363

Gyula P, Schäfer E, Nagy F (2003) Light perception and signalling in higher plants. Curr Opin Plant Biol **6:** 446–452

Jarillo JA, Gabrys H, Capel J, Alonso JM, Ecker JR, Cashmore AR (2001) Phototropin-related NPL1 controls chloroplast relocation induced by blue light. Nature **410:** 952–954

Kagawa T, Sakai T, Suetsugu N, Oikawa K, Ishiguro S, Kato T, Tabata S, Okada K, Wada M (2001) Arabidopsis *NPL1*: a phototropin homolog controlling the chloroplast high-light avoidance response. Science **291:** 2138–2141

Kleiner O, Kircher S, Harter K, Batschauer A (1999) Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. Plant J **19:** 289–296

Kohchi T, Mukougawa K, Frankenberg N, Masuda M, Yokota A, Lagarias JC (2001) The *Arabidopsis HY2* gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase. Plant Cell **13:** 425–436

Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana.* Mol Gen Genet **229:** 57–66

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenphysiol **100:** 147–160

Lin C, Shalitin D (2003) Cryptochrome structure and signal transduction. Annu Rev Plant Biol **54:** 469–496

Lin C, Yang H, Guo H, Mockler T, Chen J, Cashmore AR (1998) Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proc Natl Acad Sci USA **95:** 2686–2690

Liscum E, Briggs WR (1995) Mutations in the *NPH1* locus of *Arabidopsis* disrupt the perception of phototropic stimuli. Plant Cell **7:** 473–485

Liu H, Yu X, Li K, Klejnot J, Yang H, Lisiero D, Lin C (2008) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science **322:** 1535–1539

Lorrain S, Genoud T, Fankhauser C (2006) Let there be light in the nucleus! Curr Opin Plant Biol **9:** 509–514

Ma L, Li J, Qu L, Hager J, Chen Z, Zhao H, Deng XW (2001) Light control of *Arabidopsis* development entails coordinated regulation of genome expression and cellular pathways. Plant Cell **13:** 2589–2607

Martinez-Zapater JM, Estelle MA, Somerville CR (1986) A high repeated DNA sequence in *Arabidopsis thaliana*. Mol Gen Genet **204:** 417–423

Más P, Devlin PF, Panda S, Kay SA (2000) Functional interaction of phytochrome B and cryptochrome 2. Nature **408:** 207–211

Mathieu O, Jasencakova Z, Vaillant I, Gendrel AV, Colot V, Schubert I, Tourmente S (2003) Changes in 5S rDNA chromatin organization and transcription during heterochromatin establishment in *Arabidopsis*. Plant Cell **15:** 2929–2939

Mazzella MA, Cerdán PD, Staneloni RJ, Casal JJ (2001) Hierarchical coupling of phytochromes and cryptochromes reconciles stability and light modulation of Arabidopsis development. Development **128:** 2291–2299

Millenaar FF, Cox MC, van Berkel YE, Welschen RA, Pierik R, Voesenek LA, Peeters AJM (2005) Ethylene-induced differential growth of petioles in Arabidopsis: analyzing natural variation, response kinetics, and regulation. Plant Physiol **137:** 998–1008

Millenaar FF, van Zanten M, Cox MCH, Pierik R, Voesenek LACJ, Peeters AJM (2009) Differential petiole growth in *Arabidopsis thaliana*: photocontrol and hormonal regulation. New Phytol **184:** 141–152

Mockler TC, Guo H, Yang H, Duong H, Lin C (1999) Antagonistic actions of Arabidopsis cryptochromes and phytochrome B in the regulation of floral induction. Development **126:** 2073–2082

Nagatani A, Reed JW, Chory J (1993) Isolation and initial characterization

of Arabidopsis mutants that are deficient in phytochrome A. Plant Physiol **102:** 269–277

Naumann K, Fischer A, Hofmann I, Krauss V, Phalke S, Irmler K, Hause G, Aurich AC, Dorn R, Jenuwein T, et al (2005) Pivotal role of AtSUVH2 in heterochromatic histone methylation and gene silencing in Arabidopsis. EMBO J **24:** 1418–1429

Ohgishi M, Saji K, Okada K, Sakai T (2004) Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in Arabidopsis. Proc Natl Acad Sci USA **101:** 2223–2228

Pavet V, Quintero C, Cecchini NM, Rosa AL, Alvarez ME (2006) Arabidopsis displays centromeric DNA hypomethylation and cytological alterations of heterochromatin upon attack by pseudomonas syringae. Mol Plant Microbe Interact **19:** 577–587

Probst AV, Fagard M, Proux F, Mourrain P, Boutet S, Earley K, Lawrence RJ, Pikaard CS, Murfett J, Furner I, et al (2004) *Arabidopsis* histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. Plant Cell **16:** 1021–1034

Reed JW, Nagatani A, Elich TD, Fagan M, Chory J (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol **104:** 1139–1149

Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell **5:** 147–157

Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, Briggs WR, Wada M, Okada K (2001) *Arabidopsis* nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation. Proc Natl Acad Sci USA **98:** 6969–6974

Sakamoto K, Briggs WR (2002) Cellular and subcellular localization of phototropin 1. Plant Cell **14:** 1723–1735

Schubert I, Fransz PF, Fuchs J, de Jong JH (2001) Chromosome painting in plants. Methods Cell Sci **23:** 57–69

Shalitin D, Yang H, Mockler TC, Maymon M, Guo H, Whitelam GC, Lin C (2002) Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent phosphorylation. Nature **417:** 763–767

Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, Huang MS, Jacobsen SE, Schubert I, Fransz PF (2002) DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis. EMBO J **21:** 6549–6559

Sullivan JA, Deng XW (2003) From seed to seed: the role of photoreceptors in Arabidopsis development. Dev Biol **260:** 289–297

Tessadori F, Chupeau MC, Chupeau Y, Knip M, Germann S, van Driel R, Fransz P, Gaudin V (2007a) Large-scale dissociation and sequential reassembly of pericentric heterochromatin in dedifferentiated Arabidopsis cells. J Cell Sci **120:** 1200–1208

Tessadori F, Schulkes RK, van Driel R, Fransz P (2007b) Light-regulated large-scale reorganization of chromatin during the floral transition in Arabidopsis. Plant J **50:** 848–857

Tessadori F, van Driel R, Fransz P (2004) Cytogenetics as a tool to study gene regulation. Trends Plant Sci **9:** 147–153

Tessadori F, van Zanten M, Pavlova P, Clifton R, Pontvianne F, Snoek LB, Millenaar FF, Schulkes R-K, van Driel R, Voesenek LACJ, et al (2009) Phytochrome B and histone deacetylase 6 control light-induced chromatin compaction in *Arabidopsis thaliana*. PLoS Genet **5:** e1000638

Wang H, Ma LG, Li JM, Zhao HY, Deng XW (2001) Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. Science **294:** 154–158

Wu G, Spalding EP (2007) Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. Proc Natl Acad Sci USA **104:** 18813–18818

Yang HQ, Wu YJ, Tang RH, Liu D, Liu Y, Cashmore AR (2000) The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell **103:** 815–827

Yanovsky MJ, Mazzella MA, Casal JJ (2000) A quadruple photoreceptor mutant still keeps track of time. Curr Biol **10:** 1013–1015

Yi C, Deng XW (2005) COP1—from plant photomorphogenesis to mammalian tumorigenesis. Trends Cell Biol **15:** 618–625

Yu X, Klejnot J, Zhao X, Shalitin D, Maymon M, Yang H, Lee J, Liu X, Lopez J, Lin C (2007) *Arabidopsis* cryptochrome 2 completes its post-translational life cycle in the nucleus. Plant Cell **19:** 3146–3156

Yu X, Sayegh R, Maymon M, Warpeha K, Klejnot J, Yang H, Huang J, Lee J, Kaufman L, Lin C (2009) Formation of nuclear bodies of *Arabidopsis* CRY2 in response to blue light is associated with its blue light-dependent degradation. Plant Cell **21:** 118–130