Check for updates

New Phytologist

Research

Insights into cryptochrome modulation of ABA signaling to mediate dormancy regulation in *Marchantia polymorpha*

Jiakai Liao[1,2,3]*, Ban Deng[4]*, Qixin Yang[2,3,5], Yu Li[6], Yuxiang Zhang[1], Jiajing Cong[1], Xiaqin Wang[3,5,7], Markus V. Kohnen[1,3], Zhong-Jian Liu[5,8], Meng-Zhu Lu[7], Deshu Lin[1], Lianfeng Gu[3] and Bobin Liu[2,3,5]

[1]Basic Forestry and Proteomics Research Center, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China; [2]Jiangsu Key Laboratory for Bioresources of Saline Soils, Jiangsu Synthetic Innovation Center for Coastal Bio-agriculture, School of Wetlands, Yancheng Teachers University, Yancheng 224007, China; [3]Basic Forestry and Proteomics Research Center, College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China; [4]Center for Genomics and Biotechnology, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China; [5]Fujian Colleges and Universities Engineering Research Institute of Conservation and Utilization of Natural Bioresources, College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China; [6]Key Laboratory of Plant Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China; [7]State Key Laboratory of Subtropical Silviculture, School of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou, Zhejiang 311300, China; [8]Key Laboratory of National Forestry and Grassland Administration for Orchid Conservation and Utilization at College of Landscape Architecture, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China

Authors for correspondence:
Bobin Liu
Email: liubb@yctu.edu.cn

Lianfeng Gu
Email: lfgu@fafu.edu.cn

Deshu Lin
Email: deshu.lin@fafu.edu.cn

Received: *18 December 2022*
Accepted: *7 February 2023*

New Phytologist (2023)
doi: 10.1111/nph.18815

Key words: abscisic acid signaling, blue light, cryptochrome, gemma dormancy, *Marchantia*.

Summary

- The acquisition of dormancy capabilities has enabled plants to survive in adverse terrestrial environmental conditions. Dormancy accumulation and release is coupled with light signaling, which is well studied in *Arabidopsis*, but it is unclear in the distant nonvascular relative. We study the characteristics and function on dormancy regulation of a blue light receptor cryptochrome in *Marchantia polymorpha* (MpCRY).
- Here, we identified MpCRY via bioinformatics and mutant complement analysis. The biochemical characteristics were assessed by multiple protein-binding assays. The function of MpCRY in gemma dormancy was clarified by overexpression and mutation of MpCRY, and its mechanism was analyzed via RNA sequencing and quantitative PCR analyses associated with hormone treatment.
- We found that the unique MpCRY protein in *M. polymorpha* undergoes both blue light-promoted interaction with itself (self-interaction) and blue light-dependent phosphorylation. MpCRY has the specific characteristics of blue light-induced nuclear localization and degradation. We further demonstrated that MpCRY transcriptionally represses abscisic acid (ABA) signaling-related gene expression to suppress gemma dormancy, which is dependent on blue light signaling.
- Our findings indicate that MpCRY possesses specific biochemical and molecular characteristics, and modulates ABA signaling under blue light conditions to regulate gemma dormancy in *M. polymorpha*.

Introduction

Dormancy is a temporary growth suspension in any meristem-containing plant structure responding to environmental or developmental signaling (Graeber *et al.*, 2012) that facilitates plant survival under adverse environmental conditions, including high irradiance, drought, and temperature stress, and regrowth is then resumed when conditions are favorable (de Vries & Archibald, 2018). For example, seed dormancy delays seed germination until a more suitable season arrives, and bud dormancy prevents bud growth in winter (Graeber *et al.*, 2012). In addition, seed dormancy promotes seed dispersal, which further decreases the competition between progeny and parents of the same species and enables the colonization of new habitats (Finkelstein *et al.*, 2008; Willis *et al.*, 2014; Penfield, 2017). Therefore, the acquisition of dormancy has helped land plants overcome unfavorable habitat conditions, regrow in optimal environments, and thrive on land (Finch-Savage & Leubner-Metzger, 2006; Eklund *et al.*, 2015, 2018; Martin-Fontecha *et al.*, 2018).

Dormancy has developed independently throughout the evolution of different plant lineages, from cyanophytes to spermatophytes. Cyanobacteria, pioneer organisms on Earth, enter a dormant state under nitrogen starvation (Sawers, 2016). Cyanobacteria also generate spores called akinetes, which appear to be

*These authors contributed equally to this work.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

the ancestral form of dormancy for all plants (Agrawal, 2009; Xie, 2013). However, the dormancy status of spores varies among species: The thick-walled spores of *Chlamydomonas*, which is a single-cell green alga, are capable of achieving dormancy (Agrawal, 2009); by contrast, the spores of *Physcomitrium patens* lack dormancy capabilities (Vesty *et al.*, 2016). Another example occurs in the bryophyte *Marchantia polymorpha*, a species of liverwort, which can generate asexual propagules known as gemmae. As a gemma becomes mature, its apical notch and specialized cells, such as rhizoidal initial cells and oil cells, are developed (Shimamura, 2016; Kato *et al.*, 2020). The mature gemmae remaining in the gemma cup maintain high dormancy levels until the gemmae depart from the gemma cup or until their parental plant dies (Eklund *et al.*, 2015, 2018). As rhizoid emergence is a sign of dormancy release, gemmae without rhizoids are considered dormant, while nondormant gemmae generate visible rhizoids, at which point gemma dormancy is released, and germination commences under suitable conditions when the gemmae depart from the gemma cup (Molisch, 1922; Eklund *et al.*, 2015, 2018). Gemma dormancy in *M. polymorpha* is another kind of dormancy that may play a significant role in dispersal, in decreasing competition with the parental plant, and in adaptation to the variable terrestrial environment.

In spermatophytes, seeds are highly specialized and evolved dormant bodies in adverse environments that are produced through sexual reproduction (Linkies *et al.*, 2010). The unprecedented evolutionary success of seed plants is closely related to this survival advantage conferred by seeds, and the acquisition of dormancy has been critical for plant adaptation to land (Xie, 2013; Carol & Jerry, 2014). In *Arabidopsis*, seed dormancy is induced in the seed maturation stage, and seed dormancy reaches a certain level and gradually decreases in the desiccation stage. After that, the seed geminates under appropriate conditions. Generally, dormancy induction, maintenance, and release and germination is the process that occurs from seed dormancy to germination (Finkelstein *et al.*, 2008; Liao *et al.*, 2022). Abscisic acid (ABA) is one of the critical phytohormones involved in seed dormancy establishment, maintenance, and release (Finkelstein *et al.*, 2008; Finkelstein, 2013; Yan & Chen, 2017). In *M. polymorpha*, it has been reported that when gemmae are in the gemma cup, ABA signaling functions inside the gemmae to induce and/or maintain gemma dormancy, indicating that the ABA function in dormancy regulation has been conserved in land plants (Eklund *et al.*, 2018). In addition, thallus apex-generated auxin, which is synthesized by the IPyA pathway, can promote dormancy of the gemmae inside the gemma cups (Eklund *et al.*, 2015; Kato *et al.*, 2017). This function is similar to what occurs in *Arabidopsis* seeds in that the dormancy of the seed is regulated by auxin, and exogenous auxin delays germination initiation (Liu *et al.*, 2013). However, it remains unclear how gemma dormancy regulation responds to environmental changes in *M. polymorpha*.

Environmental cues, such as light, moisture, temperature change, and nutrients, are vital regulatory factors for dormancy (Agrawal, 2009). Light is one of the critical environmental signals regulating plant dormancy from algae to Angiospermae (Dring, 1988; Serrano-Bueno *et al.*, 2017). Changes in light conditions can break the dormancy of *Chlamydomonas* spores and can induce germination (Agrawal, 2009), and phytochrome-mediated red/far-red light signaling can modulate the gibberellic acid (GA) content to regulate seed dormancy in *Arabidopsis* (Jiang *et al.*, 2016). However, the earliest algae likely lived in the abyssal ocean, where red light and UV-B are unable to penetrate; consequently, the light signal and major energy source for plant development were supported by blue light (Singh *et al.*, 2015). The blue light receptor cryptochrome (CRY) was the first photoreceptor to evolve in plants (Han *et al.*, 2019). It has been reported that the genomes of green algal species encode one CRY protein, such as VcCRYp (from *Volvox carteri*) and CrCRYp (from *Chlamydomonas reinhardtii*) (Kianianmomeni & Hallmann, 2014); notably, CrCRYp is the central regulator of the life cycle and circadian clock (Forbes-Stovall *et al.*, 2014; Muller *et al.*, 2017). In *P. patens*, *PpCRY1a* and *PpCRY1b* are the two cryptochrome genes that redundantly regulate the differentiation and growth of gametophores and induce side branching on protonema in blue light by changing auxin sensitivities (Imaizumi *et al.*, 2002). In *Chlamydomonas* and *Adiantum capillus-veneris*, spore germination is regulated by the CRY-mediated blue light signaling pathway (Imaizumi *et al.*, 2000; Kianianmomeni & Hallmann, 2014). Recently, opposing results in seed plants have been reported: blue light was shown to alleviate dormancy in *Arabidopsis* but promoted dormancy in barley (Barrero *et al.*, 2014; Stawska & Oracz, 2019). However, how nonvascular plants respond to blue light to adjust dormancy mechanisms is unclear.

In this study, we used one of the distant relatives of vascular plants, the liverwort *M. polymorpha* as material. To determine whether blue light can regulate the dormancy of gemmae, we focused on MpCRY, which is the homolog of *Arabidopsis* blue light receptor CRYs in *M. polymorpha* and investigated the biochemical properties and physiological functions of this protein. Then, we elucidated elements of a proposed MpCRY-mediated signaling pathway of gemma dormancy, providing insight into the dormancy regulation that allows plants to adapt to new habitats and to terrestrial blue light environment changes.

Materials and Methods

Detailed methods for the RNA extraction and RT–qPCR assays, RNA sequencing (RNA-seq) and data analysis, human cell culture and transfection, immunoblot and coimmunoprecipitation (co-IP) assays, bimolecular fluorescence complementation assays (BiFC), and yeast two-hybrid assays are provided in Supporting Information Methods S1.

Plant materials and growth conditions

The *M. polymorpha* L. subsp. *ruderalis* Japanese strain Takaragaike-1 (Tak-1; male) is the wild-type (WT) plant used in this study (Ishizaki *et al.*, 2008; Bowman *et al.*, 2017). All the *M. polymorpha* gemmae and subcultivated or maintenance plants were grown aseptically on ½ B5 medium (Gamborg *et al.*, 1968) containing 1% sucrose, 1% agar, and 0.05% MES (pH = 5.5) and were cultured in a growth chamber (HiPoint 740F-LED;

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Taiwan HiPoint Co. Ltd, Taiwan, China) with continuous white light (60 μmol m^{-2} s^{-1}) at 22°C.

Light sources

We grew the *M. polymorpha* gemmae on a ½ B5 agar medium plate and handled the materials under dim green safelight. Monochromatic blue (peak 450 nm; half-bandwidth of 20 nm), red (peak 660 nm; half-bandwidth of 20 nm), or far-red (peak 730 nm; half-bandwidth of 20 nm) light was generated by LED panels. The light intensity was measured by an LI-250A light meter equipped with an LI-190R Quantum sensor (Li-Cor Biosciences, Li-Cor Inc., Lincoln, NE, USA).

Gene identification and phylogenetic analysis

Protein or transcript sequences of CRYs were collected from the MarpolBase (https://marchantia.info), Phytozome, and NCBI GenBank databases. The Multiple Sequence Comparison by Log-Expectation (Muscle) program was used to perform multiple amino acid sequence alignments (Edgar, 2004). Based on these alignments, phylogenetic trees were built with IQ-Tree software (http://iqtree.cibiv.univie.ac.at/) using the default settings (Trifinopoulos *et al.*, 2016). The trees were edited with the PhyML tool. The Muscle and PhyML v.3.1 programs (Guindon *et al.*, 2010) are available in SeaView v.4.7 (Gouy *et al.*, 2010).

Generation of transgenic lines

To generate the Mp*CRY* overexpression transgenic lines, the coding sequence (CDS) of Mp*CRY* (Mp2g17590) was amplified from Tak-1 cDNA by PCR using PrimeSTAR® GXL (TaKaRa Bio Inc., Otsu, Japan) with specific primers (Table S2) and cloned into the pDONR207 vector through the BP recombination reaction (Invitrogen, Thermo Fisher Scientific (China) Co. Ltd, Shanghai, China). The LR recombination reaction was used to clone Mp*CRY* into the pMpGWB136 vector, with further transformation performed by following the regenerating thallus transformation protocol (Kubota *et al.*, 2013). A total of 15 independent positive transformants were confirmed by an RT–qPCR assay to check the mRNA expression level of Mp*CRY*. After two cycles of gemma transplantation from the approved overexpression lines, four isogenic G2 lines (*proEF*:Mp*CRY-2/-4/-6/-11*) were used for the experiments.

CRISPR/Cas9-mediated mutagenesis was used to generate Mp*cry* mutants. The guide RNA (gRNA) was designed in the second exon of Mp*CRY* and cloned into the PstI- and SacI-digested pMpGE_En01 vector (Sugano *et al.*, 2018). The gRNA cassette was cloned into the pMpGE010 binary vector carrying Atco-Cas9 (Sugano *et al.*, 2018) by the LR recombination reaction. The final construct pMpGE010 was brought into *Agrobacterium tumefaciens* strain GV3101. Regenerating thalli (Tak-1) were used for *Agrobacterium*-mediated transformation, and the transformants were screened on hygromycin containing ½ B5 agar medium as described previously (Kubota *et al.*, 2013). A total of 33 independent positive transformants were sequenced and genotyped to identify the mutations to obtain monoclonal genome-edited lines as described previously (Sugano & Nishihama, 2018). Four independent genome-edited Mp*cry* mutant lines (Mp*cry-3*^{ge}*/-20*^{ge}*/-26*^{ge}*/-33*^{ge}) (loss-of-function) were generated for further experiments.

Gemma dormancy release assays

For the gemma dormancy assay, all gemmae were obtained from the dormant mature gemmae in the top of the gemma cups of white light-grown 3-wk-old plants. The gemmae were plated on ½ B5 agar medium under dim green light and then immediately exposed to continuous blue light (50 μmol m^{-2} s^{-1}), red light (30 μmol m^{-2} s^{-1}), far-red light (5 μmol m^{-2} s^{-1}), or white light (60 μmol m^{-2} s^{-1}) following the light intensity protocols used in previously published papers (Xu *et al.*, 2018; Wang *et al.*, 2019; Mao *et al.*, 2020). The gemmae were observed, and the images were taken by a Leica M205FA stereomicroscope (Leica Microsystems (Shanghai) Trading Co. Ltd, Shanghai, China). The mature gemmae either lacked visible rhizoids (scored as dormant) or had visible rhizoids (scored as nondormant; Eklund *et al.*, 2018), so we used the rhizoid emergence rate, which was calculated by the percentage of gemmae with visible rhizoids, to assess the gemma dormancy level. At least 30 gemmae of each genotype were used, and statistical analysis was performed for three replicates in each experiment. All experiments were repeated at least three independent times.

Phenotypic analysis of gemmae in gemma cups

Mature gemmae obtained from 3- to 4-wk-old plants were planted on ½ B5 agar medium under white light and then were grown under continuous white light (60 μmol m^{-2} s^{-1}) for 3 wk, after which they were transferred to blue light (50 μmol m^{-2} s^{-1}) conditions for an additional 2 wk of growth. The gemmae inside the gemma cup were observed, and the images were taken by a Leica M205FA stereomicroscope and a Hitachi TM3030 Plus scanning electron microscope (Hitachi High-Tech (Shanghai) Co. Ltd, Shanghai, China). If the gemmae in the gemma cup itself did not have rhizoids, the cup was scored as dormant and as germinating if rhizoids were detectable (Eklund *et al.*, 2015), so we used the percentage of gemma cups containing visibly germinating gemmae to assess the dormancy level of gemmae inside the gemma cups. At least 30 gemma cups of each genotype were used, and statistical analysis was performed for three replicates in each experiment. All experiments were repeated independently at least three times.

Confocal microscopy

Gemmae of *proEF*:Mp*CRY* plants were cultured under continuous white light for 1 d, transferred to darkness for 2 d and then exposed to continuous blue light (50 μmol m^{-2} s^{-1}), red light (30 μmol m^{-2} s^{-1}), and far-red light (5 μmol m^{-2} s^{-1}). Fluorescence derived from red fluorescent protein (RFP) was analyzed by a Carl Zeiss LSM 880 (Carl Zeiss (Shanghai) Management Co. Ltd, Shanghai, China) and Leica TCS SP8X confocal laser

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

scanning microscope, and the wavelength range of the RFP absorption spectrum was offset to 571–615 nm to eliminate the spontaneous fluorescence of chloroplasts.

Quantification and statistical analysis

Significant differences were determined using Student's *t*-test or the recommended multiple comparisons test, which was conducted in one- or two-way ANOVA using GraphPad Prism v.7.0a for Mac (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). The expression levels for RT–qPCR were normalized to those of the reference gene Mp*APT* (Saint-Marcoux *et al.*, 2015) using the $2^{-\Delta\Delta C_T}$ method. Then, the relative expression unit (REU) value was calculated by normalization to the gene mRNA level in the WT or mock treatment.

Results

MpCRY is the only cryptochrome in *M. polymorpha*

There was only one protein sequence (Mp2g17590) that shared significant similarity with both CRY1 and CRY2 (Fig. S1); this protein was previously named MpCRY (Bowman *et al.*, 2017; Li *et al.*, 2021). Alignment showed that MpCRY contains the typical CRY domains: the conserved N-terminal photolyase homology region (PHR) domain and the highly variable CRY C-terminal extension (CCE) domain (Fig. S1a–c). To determine whether MpCRY was the functional cryptochrome in *M. polymorpha*, we constitutively overexpressed WT Mp*CRY* (*pro35S*::Mp*CRY*) in *Arabidopsis cry1cry2* double mutants (Fig. S1d). Then, we planted WT (Col-4), *pro35S*::Mp*CRY/cry1cry2*, and *cry1cry2 Arabidopsis* seedlings in darkness, while a matching set of seedlings was simultaneously placed under 15 μmol m^{-2} s^{-1} blue light for 5 d. As shown in Fig. S1(d), MpCRY partly complemented the short hypocotyl phenotype of the *cry1cry2* mutant under blue light. We also used long-day photoperiod light conditions (16 h : 8 h, light : dark) to grow *pro35S*::Mp*CRY/cry1cry2* and *cry1cry2* adult *Arabidopsis* plants and found that MpCRY could complement the delayed flowering phenotype of the *cry1cry2* mutant (Fig. S1e). Thus, we confirmed that MpCRY in *M. polymorpha* had conserved functions in hypocotyl inhibition and photoperiodic control of floral origination in *Arabidopsis* CRYs to a certain extent.

Next, we performed a phylogenetic analysis by using the entire MpCRY protein sequence along with the other 63 CRY protein sequences, which were obtained from 24 representative diverse species from algae groups (Charophyta and Chlorophyta), Bryophyta, Lycophyta, Monilophyta, and Spermatophyta (Gymnospermae and Angiospermae; Fig. 1; Table S1). CRY from the alga *Ostreococcus tauri* was used as an outgroup. There is only one CRY present in all algal species and in *M. polymorpha* (Fig. 1). Two CRYs are encoded in *P. patens* and in the hornwort *Anthoceros angustus*, which are species from the other two members of Bryophyta: AaCRY1 is closely related to algal CRY, while AaCRY is grouped with MpCRY, PpCRY1a, and PpCRY1b (Fig. 1). It is clear that CRYs further expanded in *Selaginella moellendorffii* and that the CRY1 and CRY2 subfamilies formed from Monilophyta (Fig. 1), which may have come from the large-scale whole-genome duplication events that occurred during evolution from mosses (McGrath *et al.*, 2014; Clark & Donoghue, 2018). Given the evolutionary history of the liverwort *M. polymorpha* (Mishler & Churchill, 1984; Kenrick & Crane, 1997; Qiu *et al.*, 2006; Chang *et al.*, 2016; McDaniel, 2021), in addition to the complementary results we found in this study and our phylogenetic analysis, we inferred that MpCRY is a distant nonvascular plant relative of vascular plant CRYs.

The oligomerization of MpCRY is promoted by blue light

In *Arabidopsis*, the physiologically active forms of both CRY1 and CRY2 are oligomers, and the formation of these oligomers is necessary for CRY function (Sang *et al.*, 2005; Yu *et al.*, 2007b; Wang *et al.*, 2016). The PHR domain is critical for the formation of oligomers, of which MpCRY shares 63% and 64% sequence similarity with *Arabidopsis* CRY1 and CRY2, respectively (Fig. S1a,b). The PHR domain of MpCRY contains two chromophore-binding motifs, the N-terminal MTHF-binding α/β motif and the C-terminal FAD-binding α motif (Fig. S1a,b), which are responsible for blue light absorption (Lin & Todo, 2005). These results suggested that the PHR domain of MpCRY might be functionally conserved, and we further tested whether self-interaction was mediated by the PHR domain.

To investigate the interaction between MpCRYs, we generated full-length and truncated versions of the MpCRY protein and then fused them with GAL4 DNA-BD or GAL4 DNA-AD for subsequent yeast two-hybrid assays (Figs 2a, S2a). The yeast cells containing BD-MpCRY and AD-MpCRY grew on double dropout synthetically defined (DDO, SD/−L/−W) medium under both blue light and darkness (Fig. 2b). In addition, only the yeast cells containing BD-MpCRY and AD-MpCRY showed blue colonies on quadruple dropout (QDO/X, SD/−L/−W/−H/−A/X-α-Gal) medium under blue light rather than darkness (Fig. 2b). Further analysis showed that the β-galactosidase activity of the yeast cells containing BD-MpCRY and AD-MpCRY grown under blue light was significantly higher than that of the yeast grown under darkness (Fig. 2c). These results indicated that blue light could promote MpCRY self-interaction. On the other hand, the yeast cells containing BD-MpCRY and AD-MpCRYPHR grew on QDO (SD/−L/−W/−H/−A) medium under both blue light and darkness (Fig. S2b). However, the yeast cells cotransformed with BD-MpCRYCCE and AD-MpCRY or BD-MpCRYCCE and AD-MpCRYCCE could not grow on QDO medium under either darkness or blue light (Fig. S2c). Based on the results of the β-galactosidase activity assay for each yeast cell (Fig. S2d,e), we hypothesized that the interaction between MpCRYs was mediated by the PHR domain under darkness and blue light conditions but that the interaction was enhanced under blue light in *M. polymorpha*.

We further analyzed the interaction between MpCRYs by BiFC assays in *Nicotiana benthamiana* leaf epidermal cells under both blue light and darkness. We found that the reconstituted yellow fluorescent protein (YFP) signal intensity of cYFP-

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

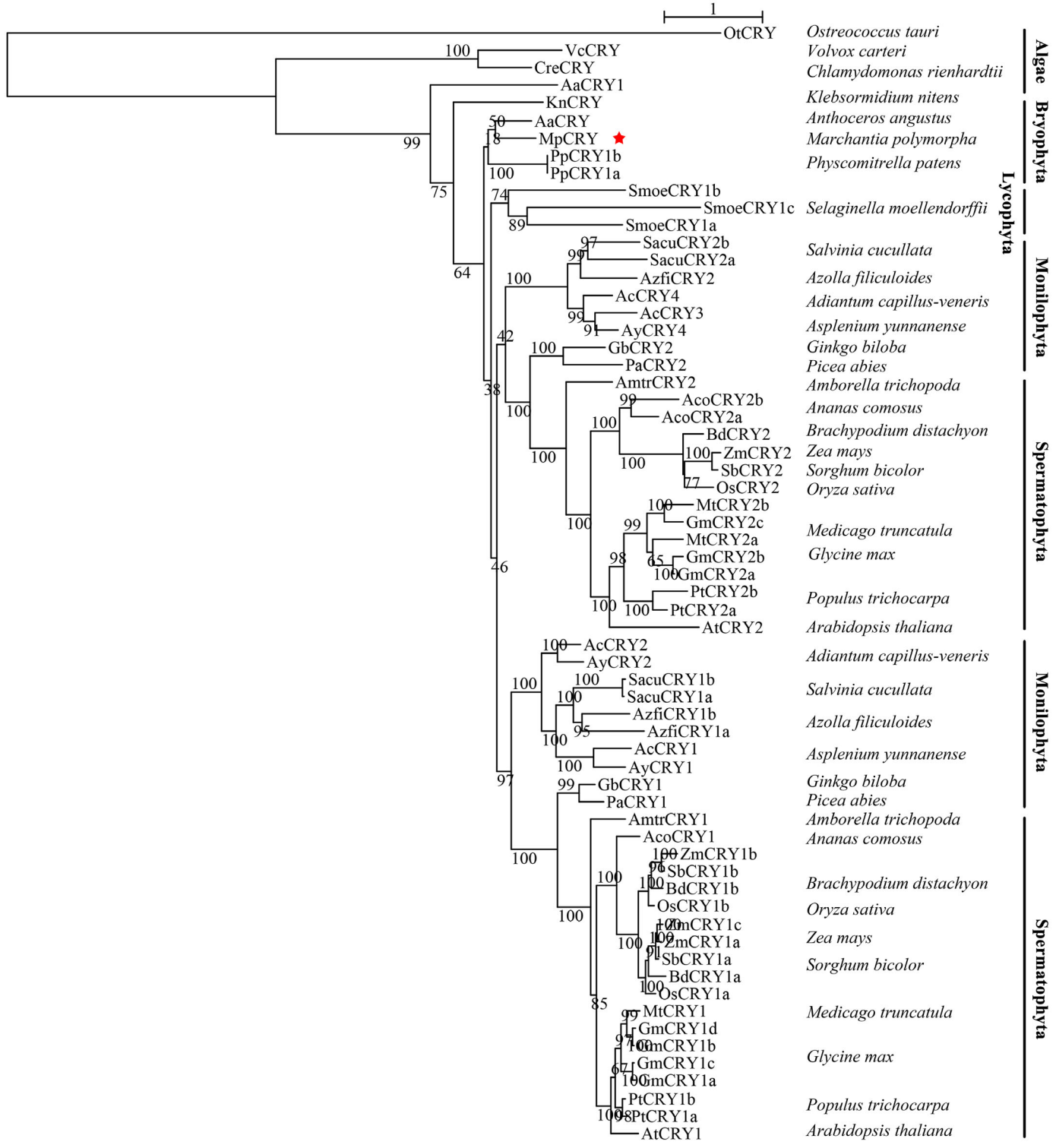


Fig. 1 MpCRY in *Marchantia polymorpha* represents a related divergent lineage to extant land plant cryptochromes. Protein sequences of all the plant CRYs in the phylogenetic tree were collected from the MarpolBase (https://marchantia.info), Phytozome, and NCBI GenBank databases. CRYs are divided into two clades, namely CRY1 and CRY2, starting from Monilophyta during the evolution of plants. Information on the plants used for the phylogenetic analysis is listed in Supporting Information Table S1. MpCRY is marked with a red star. The numbers close to the branch nodes represent bootstrap value and bar represents 1 substitutions in each site.

MpCRY and nYFP-MpCRY under blue light was significantly higher than that in darkness (Fig. 2d,e). By contrast, no YFP signals could be observed when cYFP-MpCRY was co-expressed with nYFP and cYFP was co-expressed with nYFP-MpCRY or nYFP (Fig. S3). Furthermore, we observed that MpCRY localized in the nucleus and formed typical nuclear bodies (Fig. 2d),

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

New Phytologist

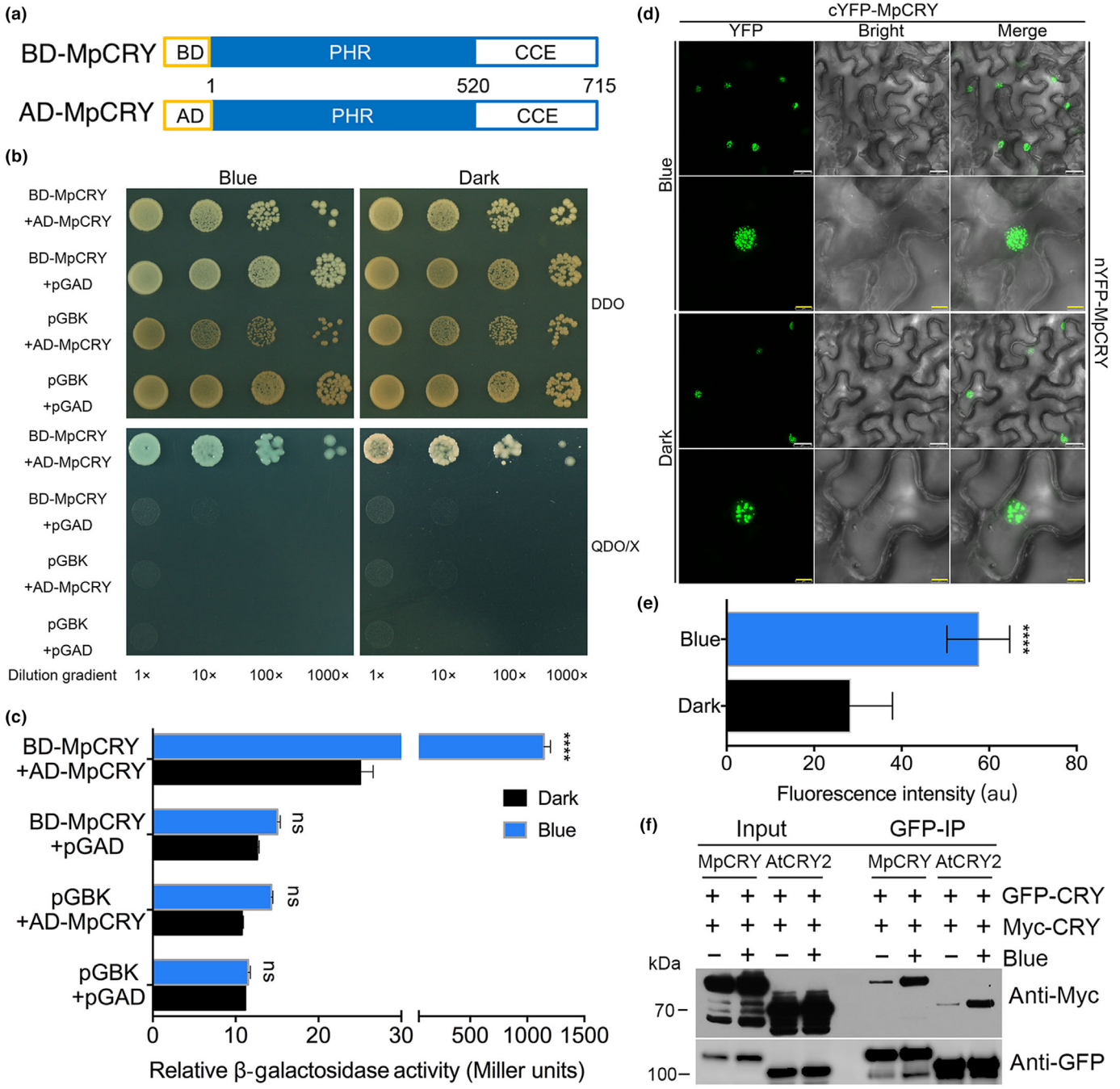


Fig. 2 Blue light can promote the self-interaction of MpCRY. (a) The diagrams display the full-length MpCRY protein. Full-length MpCRY was fused with the GAL4 DNA-binding domain (BD) or GAL4 DNA-activation domain (AD). (b) Yeast two-hybrid growth analysis of the interaction between the MpCRY protein in darkness or blue light conditions (50 μmol m^{-2} s^{-1}) on DDO (SD/−L/−W) or QDO/X (SD/−L/−W/−H/−A/X-α-Gal) medium agar plates. The various combinations of mated yeast cell cultures were dropped on the medium with the indicated concentration gradient. (c) Quantitative analysis of the yeast two-hybrid assay used to determine the MpCRY interaction strength of the yeast cells from (b). The data are shown as the mean ± SD, and the asterisks indicate a significant difference between darkness and blue light: ****, $P < 0.0001$; ns, not significant (Student's *t*-test). (d) Bimolecular fluorescence complementation (BiFC) assay showing the interactions of MpCRY with itself in *Nicotiana benthamiana* leaf epidermal cells in both darkness and blue light. The cYFP-MpCRY, nYFP, and cYFP, nYFP-MpCRY combinations served as an internal control, and the nYFP, cYFP combination served as an empty vector control, as shown in Supporting Information Fig. S3. The second row of blue and dark treatments, where only one nucleus is visible, shows higher magnification. The YFP channel is false-coloured green. Bars: (white) 25 μm; (yellow) 8 μm. (e) Quantitative analysis of the fluorescence signal from (d). The fluorescence intensity is the gray value of the signal, which was calculated by the ImageJ program. At least 15 signal areas were used for calculations, and the data are shown as the mean ± SD. The asterisks indicate a significant difference between darkness and blue light: ****, $P < 0.0001$ (Student's *t*-test). (f) Coimmunoprecipitation (Co-IP) assay to confirm the self-interaction of MpCRY by using the HEK293T cell transfection system. The indicated proteins were co-expressed in HEK293T cells, kept in darkness or exposed to blue light (100 μmol m^{-2} s^{-1}) for 2 h, and immunoprecipitated by GFP-trap beads. The IP signal (GFP-MpCRY) or the co-IP signals (Myc-MpCRY) were analyzed by immunoblots probed with antibodies against GFP or Myc, respectively. *Arabidopsis* CRY2 was used as a positive control.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

which has also been observed for *Arabidopsis* CRY2 (Yu *et al.*, 2009). These results suggested that MpCRY could interact with itself in *N. benthamiana* cells in darkness and blue light conditions and that blue light promoted its self-interaction.

For further validation of this finding, we analyzed blue light-enhanced MpCRY interaction by a previously described *in vivo* co-IP assay (Wang *et al.*, 2016). HEK293T cells that were cotransfected with two differentially tagged MpCRYs, namely GFP-MpCRY and Myc-MpCRY, were used for the co-IP assay, with blue light-dependent *Arabidopsis* CRY2 interaction as the positive control. Immunoblot analysis revealed that both green fluorescent protein (GFP) and Myc antibodies could detect the corresponding MpCRY protein from both darkness- and blue light-grown HEK293T cells co-expressing similar amounts of GFP-MpCRY and Myc-MpCRY proteins before coprecipitation with GFP-labeled magnetic beads (input) (Fig. 2f). After the proteins were co-expressed in darkness-grown cells and then coprecipitated with GFP-labeled magnetic beads (GFP-IP), a faint Myc-MpCRY band was detected by the Myc antibody (Fig. 2f). However, after exposure to blue light, more Myc-MpCRY protein coprecipitated with GFP-MpCRY (Fig. 2f). These results indicated that MpCRY could undergo self-interaction when expressed in HEK293 cells under both darkness and blue light but that blue light could promote this interaction reaction.

Evolutionarily conserved blue light-triggered phosphorylation and specific degradation of MpCRY

Blue light-dependent phosphorylation has been found in *Arabidopsis* CRY1 and CRY2, and this reaction is critical for CRY1 and CRY2 physiological activity enhancement and polyubiquitination with subsequent degradation of nuclear CRY1 and CRY2 (Shalitin *et al.*, 2002, 2003; Wang *et al.*, 2015; Liu *et al.*, 2017, 2022; Wang & Lin, 2020). In our alignment comparison, the CCE domain of MpCRY showed 20% sequence similarity to AtCRY1 and only 8.4% sequence similarity to AtCRY2 (Fig. S1a,c). However, the CCE domain of MpCRY contains a DQXVP-acidic-STAES (DAS) motif consisting of the N-terminal DQXVP sequence, an acidic amino acid (E or D) sequence, and a C-terminal STAES sequence (Fig. S1c), which are responsible for CRY1 and CRY2 phosphorylation (Lin & Shalitin, 2003); the CCE domain of MpCRY is also similar to that of CRY1 and CRY2 (Fig. S1c; Yu *et al.*, 2010).

To analyze whether the phosphorylation of MpCRY is evolutionarily conserved, we used transgenic plants that constitutively expressed Mp*CRY* (*proEF*:Mp*CRY-2*) to analyze the protein abundance and phosphorylation modification of MpCRY (Fig. 3). Immunoblot analysis revealed that some of the MpCRY protein migrated more slowly and significantly degraded only under blue light but not under dark, red, or far-red light conditions (Fig. 3a–c). In addition, the blue light-induced phosphorylated MpCRY protein was eliminated by lambda protein phosphatase (λ-PPase; Fig. S4). This result indicated that MpCRY phosphorylation and degradation were dependent on a specific blue light wavelength. We further found that the abundance of MpCRY decreased as the blue light intensity increased, and relatively more MpCRY phosphorylation was detected at higher blue light fluence rates (Fig. 3d–f). The blue light treatment time course showed that MpCRY started to degrade after a 1-h exposure time and that the protein abundance decreased with prolonged blue light treatment (Fig. 3g,h). The blue light-triggered phosphorylation of MpCRY was analyzed soon after gemmalings were exposed to blue light, and the relative abundance of phosphorylated MpCRY increased with exposure time (Fig. 3g,i). These results revealed that the characteristics of MpCRY phosphorylation and degradation also depended on the fluence rate and exposure duration of blue light. Compared with phosphorylation and degradation in *Arabidopsis*, MpCRY phosphorylation was similar to that of AtCRYs (Shalitin *et al.*, 2002, 2003), but the MpCRY blue light-induced degradation took longer than that of CRY2, which degraded rapidly, while CRY1 was stable under blue light (Liu *et al.*, 2016). These results suggested that MpCRY phosphorylation has been conserved but that the protein degradation mechanism was different from that in *Arabidopsis*.

Blue light-induced nuclear localization of activated MpCRY to modulate gene expression

To investigate the subcellular localization of MpCRY, we used *proEF*:Mp*CRY-2* transgenic plants for experiments, as the fluorescence signal was too weak for endogenous MpCRY promoter-driven *RFP*-Mp*CRY*, and MpCRY could degrade under blue light. In darkness-grown gemmalings, the RFP-MpCRY fusion protein was located mainly in the cytosol and plasma membrane (Figs 4a, S5). After blue light exposure, RFP-MpCRY accumulation occurred almost entirely in a single large cell organelle – the nucleus – and typical nuclear bodies were formed (Figs 4a, S5), similar to what occurs with the nuclear bodies or photobodies that are the activated state of *Arabidopsis* CRY2 (Yu *et al.*, 2009; Ozkan-Dagliyan *et al.*, 2013; Wang *et al.*, 2016), although levels of cytosol-localized RFP-MpCRY were low (Figs 4a, S5). However, red and far-red light did not affect the localization of RFP-MpCRY (Fig. S5). This result suggested that only blue light can induce MpCRY localization and activation in the nucleus. Interestingly, as the exposure time increased to 8 h, the red fluorescent signal intensity became weaker and continuously decreased (Fig. 4a). These results were consistent with the previously observed exposure time-dependent blue light-triggered degradation of MpCRY (Fig. 3g,h). Taken together, our results indicated that nuclear localization of activated MpCRY was induced in a blue light-dependent manner and was followed by degradation under prolonged blue light treatment.

To further explore the change in gene expression after blue light-induced nuclear localization of activated MpCRY, we generated MpCRY knockout mutants (Mpcry^{ge}; Fig. S6a,b) using conventional clustered, regularly interspaced, short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) (CRISPR/Cas9) technology (Sugano *et al.*, 2014; Sugano & Nishihama, 2018). We performed transcriptome analysis by RNA-Seq of the dormant gemmae of WT and Mpcry^{ge} plants at three time points: 0 (8 h dark condition treatment, still maintained dormancy), 8 and 72 h of 50 μmol m^{-2} s^{-1} blue light treatment (Fig. 4b) with

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

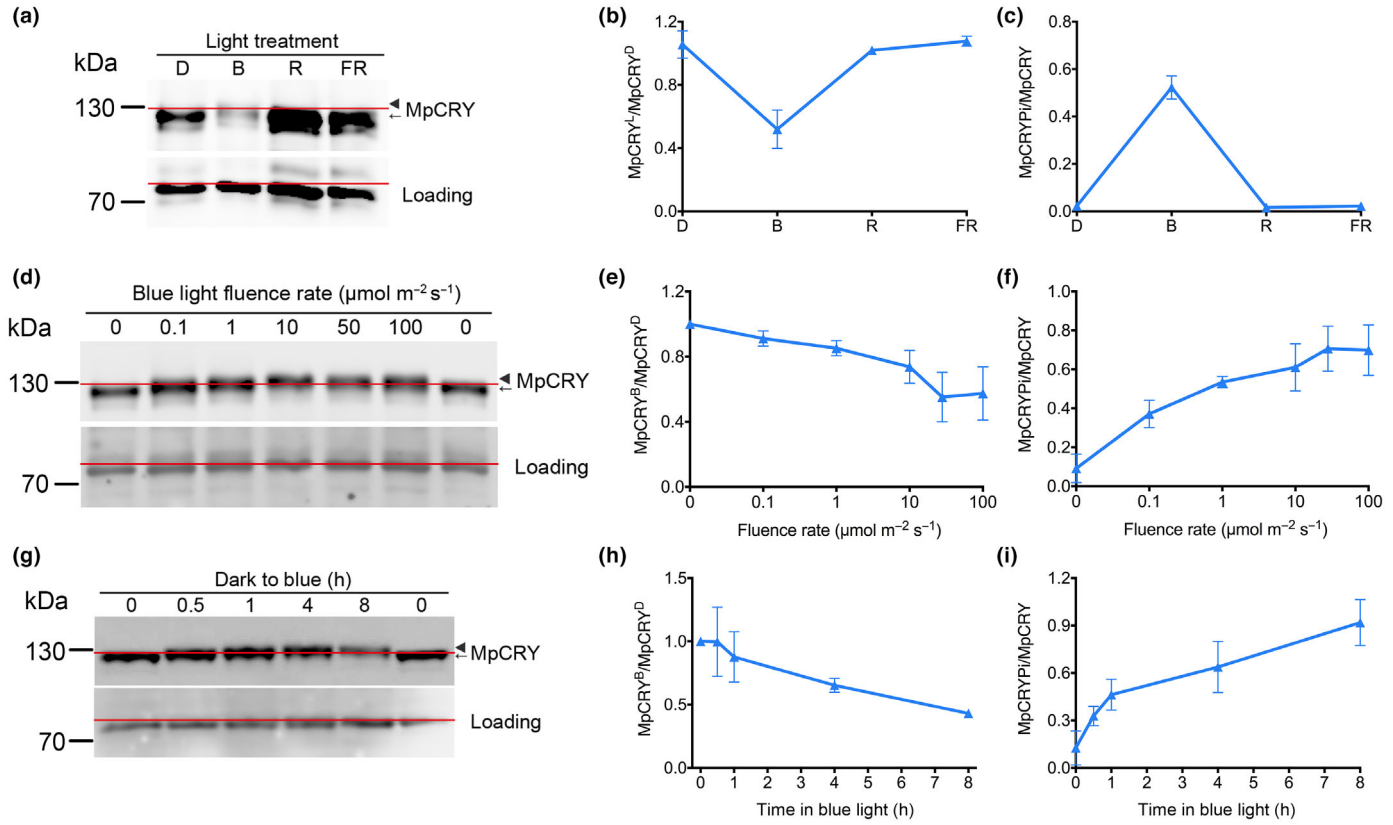


Fig. 3 Blue light-dependent degradation and phosphorylation of the MpCRY protein in *Marchantia polymorpha*. (a) Immunoblot assay of continuous white light-grown 10-d-old gemmalings of the $_{pro}$*EF*:Mp*CRY-2* plants transferred to darkness for 3 d and then kept in darkness or exposed to continuous blue light (50 μmol m^{-2} s^{-1}), red light (30 μmol m^{-2} s^{-1}), or far-red light (5 μmol m^{-2} s^{-1}) for 3 d. D, B, R, and FR represent dark, continuous blue, red, and far-red light, respectively. (b, c) The relative band intensities of MpCRY from the immunoblot of (a) were digitized and quantified by choosing the corresponding band to calculate their gray value with the PHOTOSHOP program, showing MpCRY degradation ($MpCRY^L/MpCRY^D$) or phosphorylation (MpCRYPi/MpCRY). $MpCRY^L$ represents the amount of MpCRY protein under light conditions, $MpCRY^D$ represents the amount of MpCRY protein in the dark, MpCRYPi represents the amount of phosphorylated MpCRY protein, and MpCRY represents the amount of total MpCRY protein. All the band intensities of the MpCRY protein were normalized to the loading control. The data are shown as the mean ± SD from three independent experimental gels. (d) Immunoblot assay of continuous white light-grown 10-d-old gemmalings of the $_{pro}$*EF*:Mp*CRY-2* plants transferred to darkness for 3 d and then exposed to different blue light fluence rates (0–100 μmol m^{-2} s^{-1}) for 8 h. (e, f) The relative band intensities of MpCRY from the immunoblot of (d) were digitized and quantified as in (b, c). $MpCRY^B$ represents the amount of MpCRY protein in blue light. (g) Immunoblot assay of continuous white light-grown 10-d-old gemmalings of the $_{pro}$*EF*:Mp*CRY-2* plants transferred to darkness for 3 d and then exposed to blue light (50 μmol m^{-2} s^{-1}) for the indicated time. (h, i) The relative band intensities of MpCRY from the immunoblot of (g) were digitized and quantified as in (e, f). Based on the protein bands under dark conditions, we added a red line to serve as the horizontal line, the red line separates the phosphorylated MpCRY on the top and the unphosphorylated MpCRY on the bottom. Arrowheads indicate phosphorylated MpCRY, and arrows indicate unphosphorylated MpCRY. Loading is the nonspecific band to indicate the amount of protein in the immunoblot assays.

three biological replicates. Among these time points, we found that there were almost no differences in gene expression under dark conditions, with only six genes being upregulated and six genes being downregulated in Mp*cry*^{ge} plants compared with the WT plants (FDR < 0.05; $\log_2$(FC) > 1; FC, fold change) (Fig. 4c). Differences in transcription were analyzed at 8 h of light treatment, including for 78 upregulated genes and 87 downregulated genes (Fig. 4c). After 72 h of blue light exposure, 385 upregulated genes and 98 downregulated genes were analyzed (Fig. 4c). These results indicated that blue light-activated MpCRY localization changes regulate gene expression changes in *M. polymorpha*. Gene Ontology (GO) enrichment analysis showed that genes downregulated in Mp*cry*^{ge} plants compared with the WT plants after 8 h of blue light illumination were associated with light signaling pathways, such as 'photosynthetic electron transport in photosystem I', 'response to high light intensity', 'cellular response to light intensity', and 'response to gibberellin' (Fig. 4d); all the GO terms are listed in Table S3. Among the upregulated genes in Mp*cry*^{ge}, we found that those associated with dormancy regulation were strongly represented. Gene Ontology categories such as 'seed dormancy process', 'response to abscisic acid', and 'response to water deprivation' were highly represented (Fig. 4d), implying that dormancy status in Mp*cry*^{ge} may be enhanced. Interestingly, the down- and upregulated genes in Mp*cry*^{ge} after 72 h of blue light treatment showed similar GO enrichment terms, that is, light signaling and dormancy regulation (Fig. 4e). These results suggested that blue light-activated MpCRY transcriptionally regulated the genes involved in blue light signaling in gemmae and might have a function in dormancy regulation via ABA signaling.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

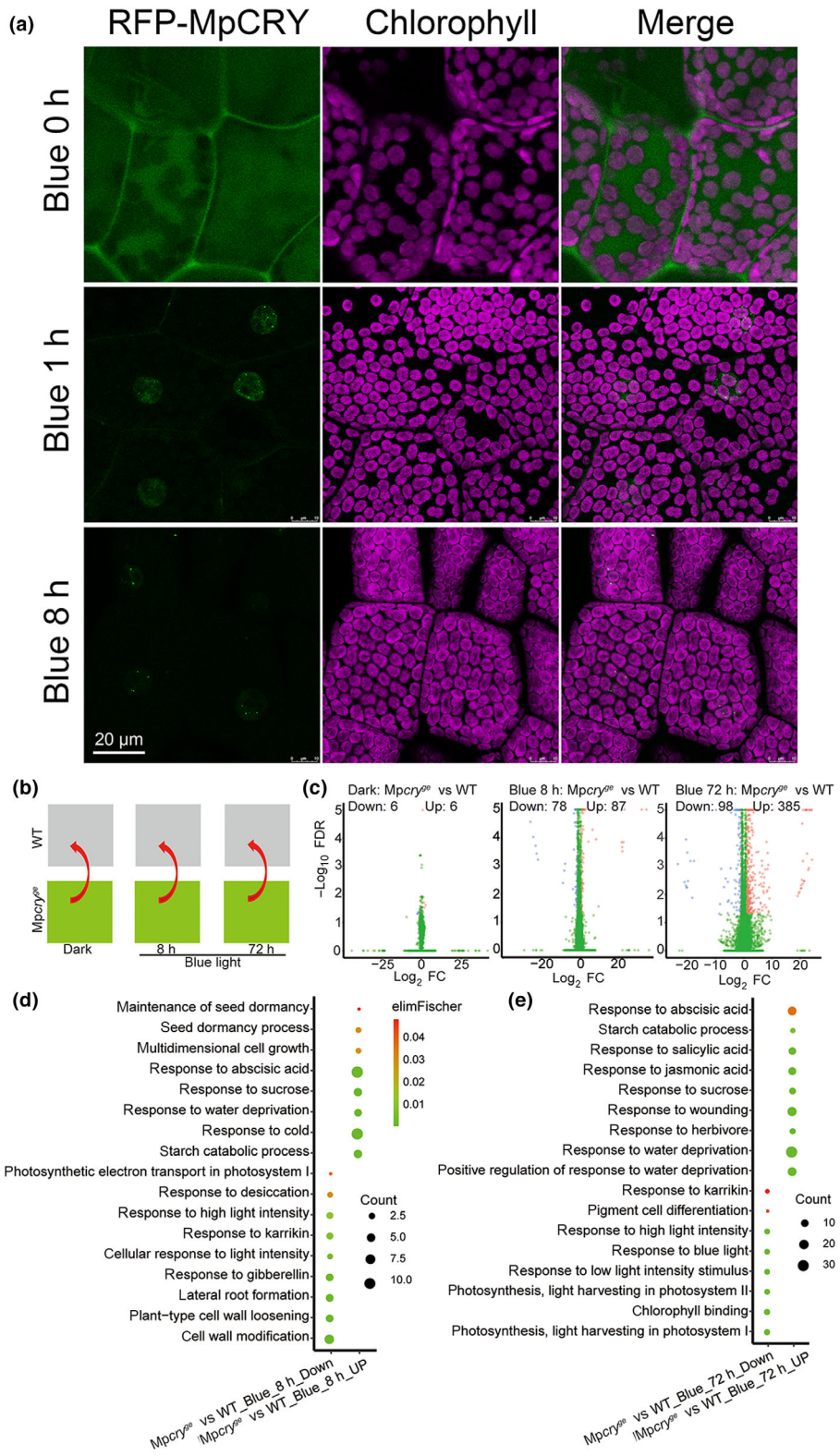


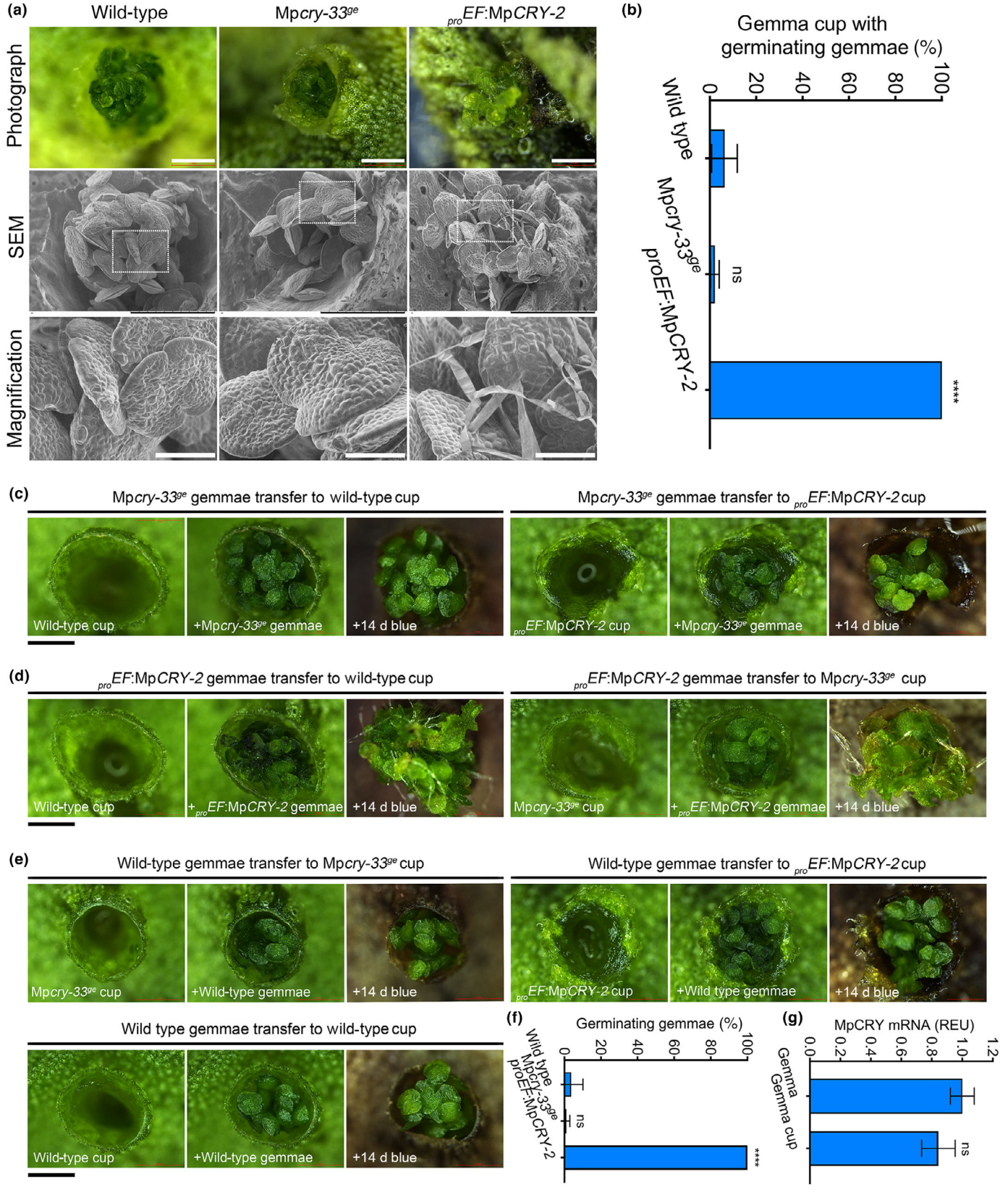
Fig. 4 Blue light-activated MpCRY localization changes to regulate the expression of various genes in *Marchantia polymorpha*. (a) The gemmae of the $_{pro}$*EF*:Mp*CRY-2* plants were grown under continuous white light for 1 d, transferred to darkness for 2 d, and then exposed to continuous blue light (50 μmol m^{-2} s^{-1}) for the indicated time. The RFP channel is false-coloured green and the Chlorophyll autofluorescence is false-coloured magenta. Bar, 20 μm. (b) Schematic diagram of RNA-Seq comparison analysis. Dormant gemmae of wild-type (WT) and Mp*cry*^{ge} plants harvested from gemma cups were grown in darkness and under 50 μmol m^{-2} s^{-1} blue light (8 and 72 h). RNA samples from dark-grown gemmae and gemmae exposed to blue light for 8 and 72 h were subjected to RNA-seq analysis. The DEGs were calculated by comparing Mp*cry*^{ge} to the WT indicated by red arrows at three time points. (c) Volcano plot showing differentially expressed genes (log_2|FC| > 1; FDR < 0.05) between Mp*cry*^{ge} and WT gemmae grown in darkness or under 8 or 72 h of blue light illumination. The upregulated or downregulated genes in Mp*cry*^{ge} are depicted in orange or sky blue, respectively. (d, e) GO enrichment analysis for differentially expressed genes in Mp*cry*^{ge} under 8 and 72 h of blue light exposure.

MpCRY overexpression inhibits gemma dormancy in gemma cups

Since we had already determined the conserved and specific characteristics of MpCRY, we were eager to investigate its physiological functions. We grew WT, Mp*cry-33*^{ge}, and $_{pro}$*EF*:Mp*CRY-2* plants under white light (60 μmol m^{-2} s^{-1}) for 3 wk and then transferred the plants to blue light (50 μmol m^{-2} s^{-1}) with an additional 2 wk of treatment to analyze the dormancy phenotype. We found that an average of 8% of the gemma cups from WT plants containing rhizoids produced gemmae (Fig. 5a,b), which is indicative of nondormancy (Eklund *et al.*, 2015).

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



Meanwhile, 5% of the Mp*cry*^{ge} mutant gemma cups containing rhizoids produced gemmae, with no significant difference compared with the WT (Fig. 5a,b). Because the gemmae in the gemma cups maintained a high dormancy level, it appeared it would not be easy to analyze the dormancy function. However, almost all the gemma cups of the $_{pro}$*EF*:Mp*CRY* plants contained

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Fig. 5 MpCRY overexpression functions inside gemmae to suppress dormancy under blue light in *Marchantia polymorpha*. (a) White light-grown 3-wk-old wild-type (WT), Mp*cry-33*^{ge}, and $_{pro}$*EF*:Mp*CRY-2* plants were placed under blue light for an additional 2 wk of treatment. The gemmae in the gemma cup were observed under a stereomicroscope and scanning electron microscope (SEM). The third row of (a) shows higher magnification for the white rectangle marked areas of the second-row pictures. Bars: (top, middle rows) 1 mm; (bottom row) 200 μm. (b) Statistical analysis of the percentage of gemma cups containing visibly germinating gemmae shown in (a). At least 30 gemma cups of each genotype were used for calculations, and three replicates were performed. (c) White light-grown 3-wk-old Mp*cry-33*^{ge} gemmae were transferred into the same 3-wk-old empty WT or $_{pro}$*EF*:Mp*CRY-2* gemma cups and then placed under blue light for 2 wk of treatment. (d) White light-grown 3-wk-old $_{pro}$*EF*:Mp*CRY-2* gemmae were transferred into the same 3-wk-old empty WT or Mp*cry-33*^{ge} gemma cups and then placed under blue light for 2 wk of treatment. (e) White light-grown 3-wk-old WT gemmae were transferred into the same 3-wk-old empty Mp*cry-33*^{ge}, $_{pro}$*EF*:Mp*CRY-2*, or WT gemma cups and then placed under blue light for 2 wk of treatment. (f) Statistical analysis of the percentage of each genotype containing visibly germinating gemmae shown in (c–e). At least 10 gemma cups of each genotype were used for calculations, and three replicates were performed. (g) Relative Mp*CRY* mRNA level of blue light-grown gemmae and gemma cup. For (c–e), in one set of transfer experiments, the left panel indicates the empty gemma cup with all the gemmae removed, the middle panel indicates the empty gemma cup with gemmae of other plants, and the right panel indicates plants that were placed under blue light with an additional 2 wk of treatment after transfer. Bars, 1 mm. For (b, f, g), the data are shown as the mean ± SD, and the asterisks indicate a significant difference between transgenic and WT lines for (b, f) or gemma and gemma cup for (g): ****, $P < 0.0001$; ns, not significant (Student's *t*-test).

large numbers of gemmae with visible rhizoids (Fig. 5a,b). In addition, the number of gemmae with rhizoids in each gemma cup was consistent with the MpCRY mRNA expression level in different $_{pro}$*EF*:Mp*CRY* lines (Fig. S6c–e). These results suggested that MpCRY overexpression might inhibit gemma dormancy in gemma cups under blue light.

To determine whether MpCRY directly functions in gemmae to regulate dormancy or acts on the gemma cup indirectly to affect gemma dormancy under blue light, we transferred dormant mature gemmae within gemma cups of white light-grown 3-wk-old WT plants into the same 3-wk-old empty Mp*cry-33*^{ge} or $_{pro}$*EF*:Mp*CRY-2* gemma cups where the gemmae had just been removed, and then, the gemmae in the gemma cups were grown under blue light for 2 wk. A similar manipulation was used to transfer Mp*cry*^{ge} gemmae to WT or $_{pro}$*EF*:Mp*CRY* gemma cups and $_{pro}$*EF*:Mp*CRY* gemmae to WT or Mp*cry*^{ge} gemma cups, and the transfer of WT gemmae to WT gemma cups was set as a negative control (Fig. 5c–e). The WT gemmae remained dormant after transfer into WT, Mp*cry*^{ge}, and $_{pro}$*EF*:Mp*CRY* gemma cups for 2 wk of blue light treatment (Fig. 5e,f). A similar result was also observed in Mp*cry*^{ge}, and the ratio of gemma cups with rhizoid-produced gemmae was not significantly different from that in the WT (Fig. 5c,f). However, $_{pro}$*EF*:Mp*CRY* gemmae produced rhizoids when transferred into WT or Mp*cry*^{ge} gemma cups for 2 wk of blue light treatment (Fig. 5d,f). In addition, we also found that there was no difference in the gemma dormancy phenotype among the WT, Mp*cry*^{ge}, and $_{pro}$*EF*:Mp*CRY* lines under red light conditions (Fig. S7). These results indicated that MpCRY overexpression functioned inside the gemmae itself rather than in the gemma cup to regulate dormancy under blue light, although the MpCRY mRNA level was almost the same in blue light-grown gemmae and gemma cups (Fig. 5g).

MpCRY is involved in gemma dormancy release regulation after its dispersion

To study the function of MpCRY in gemma dormancy release, we grew dormant mature gemmae from white light-grown 3-wk-old WT, Mp*cry-33*^{ge}, and $_{pro}$*EF*:Mp*CRY-2* plants on ½ B5 agar medium to observe the speed of gemmae rhizoid emergence over 80 h of blue light treatment. The rhizoid emergence rate was used to assess the gemma dormancy level, which is like seed dormancy release marked by radicle emergence. We first checked the gemma dormancy level from the top (old) and bottom (young) of the gemma cup and found that their dormancy level was almost the same (Fig. 6a,b); thus, we only removed the top position gemmae for this experiment for the sake of convenience. The WT gemmae rhizoids emerged after 16 h of plating and gradually increased to a 100% rhizoid emergence rate at 72 h, and Mp*cry*^{ge} gemmae had a similar tendency (Fig. 6c,d). However, the rhizoid emergence rate in the Mp*cry*^{ge} gemmae was much slower than that in the WT (Fig. 6c,d). For example, Mp*cry*^{ge} gemmae needed > 48 h to reach a 50% rhizoid emergence rate, whereas it took *c.* 36 h for the WT (Fig. 6d). The Mp*cry*^{ge} gemmae all exhibited rhizoids after 72 h of growth on agar, similar to the gemmae of the WT (Fig. 6c,d), suggesting that there was no developmental defect regulated by MpCRY. The Mp*cry*^{ge} gemmae rhizoid emergence rate was consistent with the MpCRY mutations caused by genome editing (Figs 6d, S6a,b,f). The gemmae from $_{pro}$*EF*:Mp*CRY* plants exhibited the fastest rhizoid emergence rate, which reached 50% after *c.* 9 h of growth under blue light and reached 100% after only 16 h (Fig. 6d). Moreover, the rhizoid emergence rate of gemmae from $_{pro}$*EF*:Mp*CRY* was consistent with the MpCRY mRNA expression level in these lines (Figs 6d, S6c,f). To eliminate the possible function of MpCRY in gemma dormancy regulation in the gemma cup, we harvested the gemmae from red light-grown 3-wk-old plants to carry out the same dormancy assay, and a similar result was observed (Fig. S8a,b).

We next found that the gemmae of both WT and Mp*cry*^{ge} plants could not grow and that no gemmae produced rhizoids in darkness or under far-red light (Fig. S8c,d), which was similar to the findings of a previous report (Inoue *et al.*, 2016). In addition, the gemmae rhizoid emergence rates of the WT and Mp*cry*^{ge} plants were consistently the same under red light (Fig. S8e). However, the MpCRY-overexpressing gemmae exhibited 20% and 35% rhizoid emergence rates in darkness and under far-red light, respectively (Fig. S8c,d), and the rhizoid emergence rate under red light was similar to that under the previous blue light conditions (Fig. S8e), probably because blue light-activated MpCRY degraded slowly (Fig. 3g). Furthermore, we investigated whether MpCRY-mediated blue light signaling affects gemma dormancy release depending on the blue light fluence rate. We found that as the blue light intensity increased, the gemmae rhizoid emergence rate of these two

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

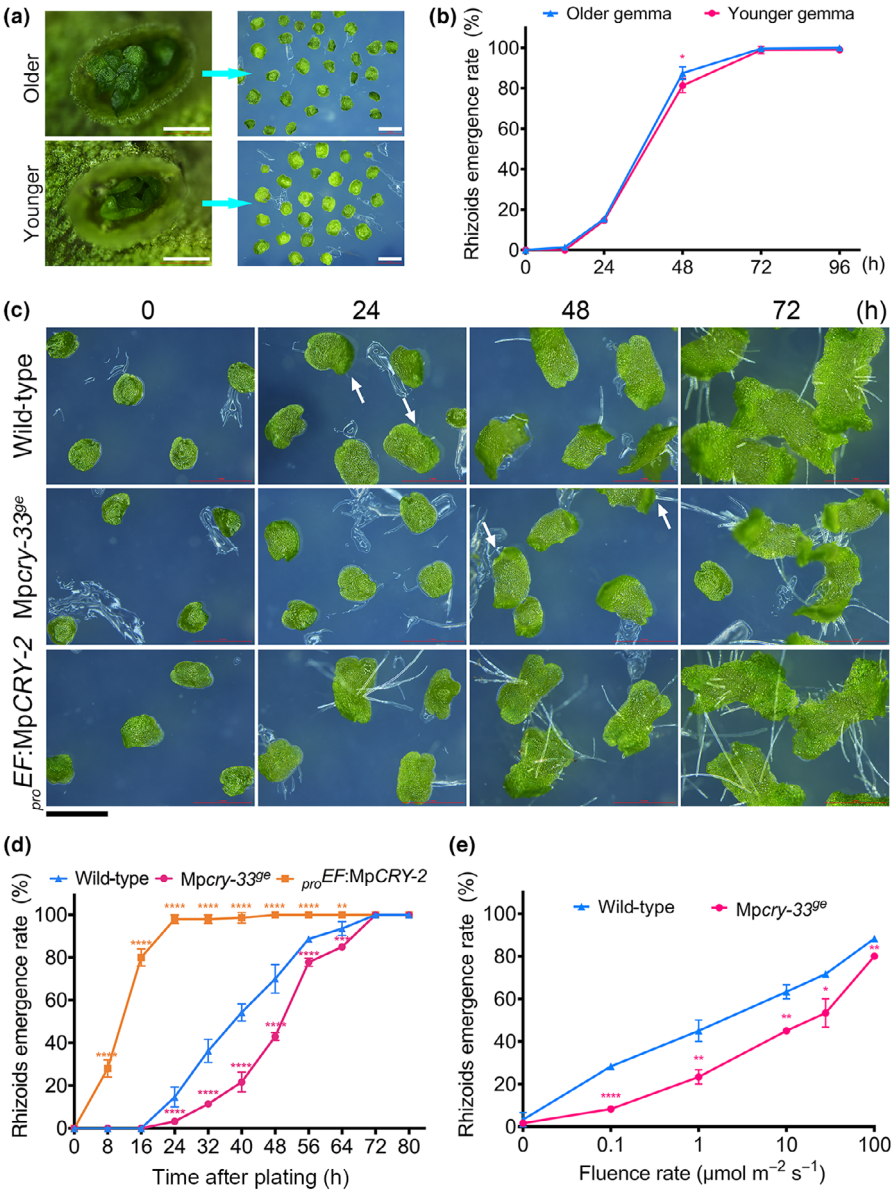


Fig. 6 MpCRY accelerates gemma dormancy release under blue light in *Marchantia polymorpha*. (a) Pictures show the position of the gemmae in the gemma cups of white light-grown 3-wk-old wild-type (WT) plants, and the gemmae plated on agar medium, the older gemmae from the top of the cup, the younger gemmae from the bottom of the cup. Bars, 1 mm. (b) Statistical analysis of the rhizoid emergence rate from the gemma dormancy assay of dormant mature older and younger gemmae, which were obtained from the gemma cups of white light-grown 3-wk-old WT plants and were plated in continuous blue light (50 μmol m^{-2} s^{-1}) for the indicated time. At least 30 gemmae were used for the calculations, and three replicates were performed. (c) The dormant mature gemmae of all the genotypes were obtained from the top of the gemma cups of white light-grown 3-wk-old plants and were plated in continuous blue light (50 μmol m^{-2} s^{-1}) for the indicated time. The gemmae were observed under a stereomicroscope, and representative pictures are shown. The white arrows indicate the emerged rhizoids. Bar, 1 mm. (d) Statistical analysis of the rhizoid emergence rate shown in (c). At least 30 gemmae of each genotype were used for calculations, and three replicates were performed. (e) Statistical analysis of the rhizoid emergence rate of dormant mature gemmae were obtained from the gemma cups of white light-grown 3-wk-old WT and Mp*cry-33*^{ge} plants and were plated in different blue light fluence rates (0–100 μmol m^{-2} s^{-1}) for 48 h. At least 30 gemmae of each genotype were used for calculations, and three replicates were performed. For (b, d, e), the data are shown as the mean ± SD, and the asterisks indicate a significant difference between younger and older gemmae for (b) or the transgenic and WT lines for (d, e): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ (Student's *t*-test).

genotypes gradually increased (Fig. 6e). In addition, the rhizoid emergence rate of Mp*cry*^{ge} gemmae was consistently significantly lower than that of the WT gemmae at the same blue light intensity (Fig. 6e). Overall, these results suggested that MpCRY specifically regulates the dormancy release of gemmae after its dispersion from the gemma cup under blue light.

MpCRY transcriptionally represses ABA signaling genes to promote gemma dormancy release

It is widely known that the phytohormone ABA is the major endogenous signal controlling seed dormancy (Kucera *et al.*, 2005; Finkelstein *et al.*, 2008; Graeber *et al.*, 2012; Shu *et al.*,

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2016). We found that the GO term 'response to abscisic acid' was enriched in upregulated genes in Mp*cry*^{ge} after 8 and 72 h of blue light treatment (Fig. 4d,e). Thus, we further investigated whether MpCRY may be involved in ABA signaling-regulated gemma dormancy. To investigate this possibility, we first confirmed that gemmae rhizoid formation was delayed by ABA treatment in a dose-dependent manner and that the gemmaling size was reduced (Fig. S9). These observations were consistent with the findings of a previous report (Eklund *et al.*, 2018). Then, we performed dormancy experiments by using dormant mature gemmae of the WT, Mp*cry-33*^{ge}, and $_{pro}$*EF*:Mp*CRY-2* lines under blue light. As the results show, the gemmae of all the plants were able to generate rhizoids when they were growing; however, gemma dormancy release was delayed by ABA in a dose-dependent manner, and the Mp*cry*^{ge} mutant line was hypersensitive to ABA, while the overexpression line $_{pro}$*EF*:Mp*CRY* was hyposensitive (Figs 7a–c, S10). Regardless of ABA concentration, the rhizoid emergence rate of the gemmae was always the highest in $_{pro}$*EF*:Mp*CRY* plants and the lowest in Mp*cry*^{ge} plants, and that of the WT plants exhibited an intermediate value (Fig. 7a–c). These findings implied that MpCRY might inhibit ABA-delayed rhizoid formation in gemmae.

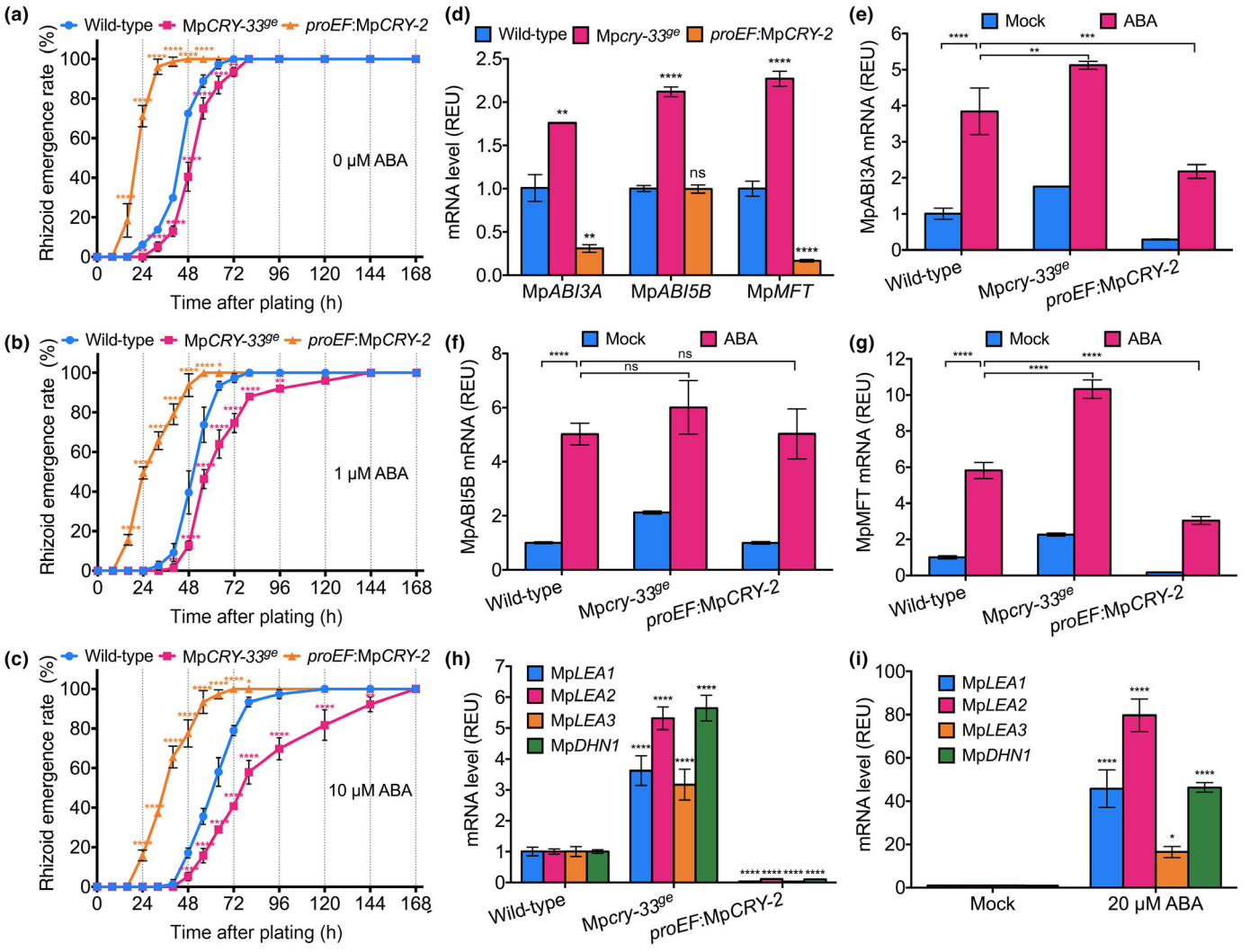


Fig. 7 MpCRY accelerates gemma dormancy release under blue light by regulating abscisic acid (ABA)-related genes in *Marchantia polymorpha*. (a–c) Statistical analysis of the rhizoid emergence rate of the dormant mature gemmae of the wild-type (WT), Mp*cry-33*^{ge}, and *proEF*:Mp*CRY-2* plants that were plated on various concentrations of ABA-containing agar medium, as indicated in the figure, under dim green light and then exposed to continuous blue light (50 μmol m^{-2} s^{-1}) for the indicated time. At least 30 gemmae of each genotype were used for calculations, and three replicates were performed. (d) RT–qPCR analysis of ABA signaling-related gene (Mp*ABI3A*, Mp*ABI5B*, and Mp*MFT*) mRNA levels in WT, Mp*cry-33*^{ge}, and *proEF*:Mp*CRY-2* gemmae grown on mock medium under blue light for 8 h, as shown in (a). (e–g) RT–qPCR analysis of ABA signaling-regulated gene (Mp*ABI3A*, Mp*ABI5B*, and Mp*MFT*) mRNA levels in WT, Mp*cry-33*^{ge}, and *proEF*:Mp*CRY-2* gemmae grown on mock or 20 μm ABA medium under blue light for 8 h. (h) RT–qPCR analysis of ABA-responsive gene (Mp*LEA1/2/3* and Mp*DHN1*) mRNA levels in WT, Mp*cry-33*^{ge}, and *proEF*:Mp*CRY-2* gemmae grown on mock medium under blue light for 8 h, as shown in (a). (i) RT–qPCR analysis of ABA-responsive gene (Mp*LEA1/2/3* and Mp*DHN1*) mRNA levels in WT gemmae grown on mock or 20 μm ABA medium under blue light for 8 h. For (a–i), the data are shown as the mean ± SD, and the asterisks indicate a significant difference between the transgenic and WT lines for (a–h) or mock and ABA for (e–g, i): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant (Student's *t*-test).

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

To further analyze how MpCRY is involved in ABA signaling, we analyzed three ABA signaling-regulated genes, namely Mp*ABI3A*, Mp*ABI5B*, and Mp*MFT*, which are orthologs of the *Arabidopsis* genes At*ABI3*, At*ABI5*, and At*MFT* (Eklund *et al.*, 2018). The mRNA expression levels were analyzed in the gemmae of the WT, Mp*cry-33*^{ge}, and $_{pro}$*EF*:Mp*CRY-2* lines grown on agar medium for 8 h under blue light. RT–qPCR analysis showed that the mRNA expression levels of these three genes were all upregulated in the Mp*cry*^{ge} gemmae compared with the WT gemmae (Fig. 7d). Correspondingly, the Mp*ABI3A* and Mp*MFT* mRNA expression levels were downregulated in the $_{pro}$*EF*:Mp*CRY* gemmae compared with the WT gemmae, even though there was no change in Mp*ABI5B* gene expression (Fig. 7d). The same RT–qPCR assay was performed using gemmae cultured for 8 h under red light conditions, and there was no ABA signaling gene expression change except that Mp*ABI3A* expression was lower in $_{pro}$*EF*:Mp*CRY* (Fig. S11a). These analyses revealed that MpCRY could transcriptionally regulate ABA signaling-regulated genes when the gemmae dispersed on the medium under blue light.

To establish whether mutation of MpCRY could mimic exogenous ABA-induced ABA signaling-regulated gene expression, we tested the mRNA expression levels of these ABA signaling-regulated genes in the gemmae of the WT, Mp*cry-33*^{ge}, and $_{pro}$*EF*:Mp*CRY-2* lines grown on 20 μM ABA or mock medium under blue light for 8 h. RT–qPCR analysis showed that the Mp*ABI3A* mRNA expression level was increased in both ABA-treated WT gemmae compared with the mock-treated gemmae and in the Mp*cry*^{ge} gemmae compared with the WT gemmae (Fig. 7e). Correspondingly, the Mp*ABI3A* mRNA expression level was decreased in both ABA- and mock-treated $_{pro}$*EF*:Mp*CRY* gemmae compared with the WT gemmae (Fig. 7e). The expression patterns of Mp*ABI5B* and Mp*MFT* under these conditions were similar to that of Mp*ABI3A* (Fig. 7f,g). These results indicated that MpCRY could transcriptionally repress ABA-induced ABA signaling-regulated genes when the gemmae dispersed on medium with or without ABA under blue light.

In addition, we also analyzed whether MpCRY could affect ABA-responsive gene expression. Four ABA-responsive genes, namely Mp*LEA1/2/3* and Mp*DHN1*, have been presented in a previous report (Ghosh *et al.*, 2016; Eklund *et al.*, 2018). RT–qPCR analysis showed that the mRNA expression levels of all four genes were increased in both the Mp*cry*^{ge} gemmae compared with the WT and the ABA-treated WT gemmae compared with the mock-treated gemmae (Fig. 7h,i). At the same time, the mRNA expression levels of all four genes were decreased in the $_{pro}$*EF*:Mp*CRY* gemmae compared with the WT gemmae (Fig. 7h). There was no expression change in Mp*LEA1/2/3* and Mp*DHN1* between Mp*cry*^{ge} and WT gemmae cultured under red light conditions (Fig. S11b). Moreover, the mRNA expression levels of all eight ABA signaling-related genes in the gemmae inside the gemma cup were not significantly different between the Mp*cry*^{ge} and WT (Fig. S12a,b), although some gene mRNA expression levels were reduced in the $_{pro}$*EF*:Mp*CRY* gemmae compared with the WT gemmae (Fig. S12a,b). Taken together, these results revealed that MpCRY was involved in the ABA signaling pathway and transcriptionally repressed ABA signaling-related genes under blue light to suppress gemma dormancy and promote dormancy release after gemma dispersal from the gemma cup in *M. polymorpha*.

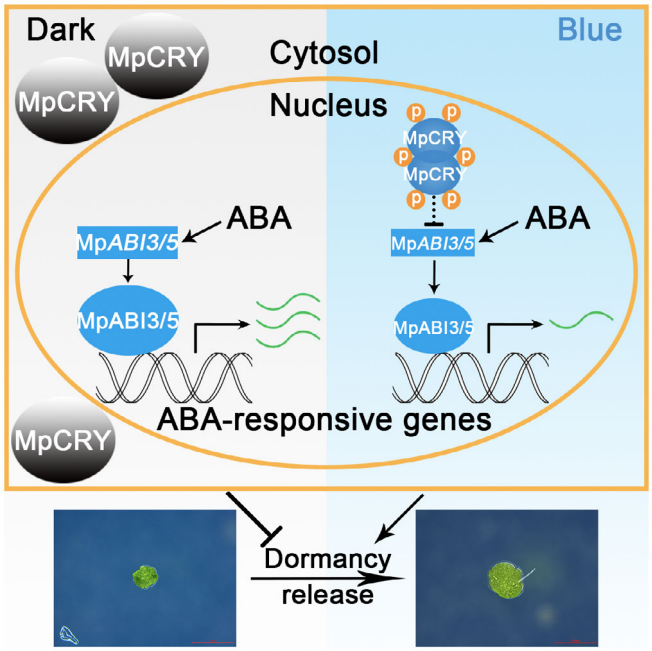


Fig. 8 Proposed model of cryptochrome-modulated abscisic acid (ABA) signaling in blue light in the liverwort *Marchantia polymorpha*. The schematic illustration shows that MpCRY autonomously promotes gemma dormancy release after gemmae dispersal from the gemma cup by transcriptionally repressing ABA signaling-regulated genes in blue light in *M. polymorpha*. The blunt ended dashed line means indirectly repressed, the blunt ended solid line means directly repressed, and the arrowheaded solid lines mean directly promoted.

Discussion

The findings in this study elucidated elements of a proposed MpCRY-mediated signaling pathway during gemma dormancy and prompted us to propose the following working model: when dormant gemmae disperse in darkness, MpCRY exists as an inactive monomer and/or oligomeric protein in the cytosol. After blue light illumination, nucleus-localized MpCRY self-interaction is enhanced and phosphorylation occurs to activate the protein, which transcriptionally represses the expression of both ABA signaling-regulated and ABA-responsive genes, further weakening the ABA inhibitory effect on gemma dormancy release (Fig. 8). Thus, in the liverwort *M. polymorpha*, MpCRY-mediated blue light signaling modulates ABA signaling to regulate gemma dormancy release, which provides insights into the distinct blue light environmental adaptation mechanism of plants in various surroundings.

MpCRY has distinct photooligomerization and protein degradation characteristics in *M. polymorpha*

Bryophytes and vascular plants diverged from their common ancestor, which was a *bona fide* early land plant (McDaniel,

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2021). *Marchantia polymorpha*, which is a member of the Bryophyta, is a shade-loving plant (Mache & Loiseaux, 1973) and may have acquired many derived traits to adapt to such environments over the past million years. MpCRY was first identified as a blue light receptor in the *M. polymorpha* genome (Bowman *et al.*, 2017), and MpCRY was involved in asymmetrical thallus growth, sucrose metabolism, and carbon fixation in *M. polymorpha* under blue light conditions, but it is unclear how it transduces blue light signaling (Li *et al.*, 2021; Zhang *et al.*, 2021). In this study, we first found that MpCRY self-interaction occurs under dark conditions and that blue light promotes this process (Figs 2, S2), which is different from blue light-dependent oligomerization in vascular plants (Liu *et al.*, 2020). In vascular plants, CRY photooligomerization is necessary for blue light signal transduction, phosphorylation, light-dependent proteolysis, and interaction with proteins (Liu *et al.*, 2020). It has also been reported that MpCRY interacts with MpSPA in the nucleus under both dark and blue light conditions (Zhang *et al.*, 2021). These results suggest that MpCRY undergoes oligomerization and interaction with cryptochrome-interacting proteins in darkness, which might be in preparation for subsequent signal transduction after blue light irradiation. In addition, we found that compared with the degradation of both CRY1 and CRY2 in *Arabidopsis*, it took much longer for blue light-induced degradation of MpCRY to occur in *M. polymorpha* (Fig. 3; Liu *et al.*, 2016). In *Arabidopsis*, blue light inhibitors of cryptochromes can inactive cryptochrome photooligomerization and subsequently inhibit cryptochrome photoactivation to repress the known biochemical and physiological activities in blue light (Wang *et al.*, 2016; Wang & Lin, 2020). Interestingly, the *M. polymorpha* genome does not encode the homologs of blue light inhibitors of cryptochromes (Wang *et al.*, 2017). Thus, although MpCRY have conserved physiology function in *Arabidopsis* (Fig. S1d,e), a distant regulatory mechanism may exist for cryptochrome photoactivation and inactivation in *M. polymorpha* – in contrast to what occurs in *Arabidopsis* – that could be tested in the future.

MpCRY has a specific nuclear localization mechanism

Photoactivated CRYs transduce blue light signals to downstream targets, primarily in the nucleus, via transcriptional regulation. For example, CRY2 localizes in the nucleus all the time, and nuclear-targeted CRY1 regulates most CRY1-mediated responses in *Arabidopsis*, although CRY1 localizes equally in the cytoplasm and nucleus (Cashmore *et al.*, 1999; Wu & Spalding, 2007; Yu *et al.*, 2007a). We found that MpCRY exhibited cytoplasmic and plasma membrane localization under dark, red, or far-red light conditions (Fig. S5) but showed nuclear localization under blue light illumination (Figs 4a, S5). These results indicate that MpCRY is a distinct class of cryptochromes and localizes differently from the known CRY localization in land plants. However, MpCRY has a nuclear localization exclusive to *N. benthamiana* leaf epidermal cells (Fig. 2d), indicating that there is a specific nuclear localization mechanism in *M. polymorpha*. In addition, there is plasma membrane localization signaling of MpCRY under nonblue light conditions (Figs 4a, S5). *Arabidopsis* CRY1 interacts with the G-protein β subunit AGB1, which is usually localized in the plasma membrane in all eukaryotes (Gilman, 1987; Lian *et al.*, 2018), suggesting that MpCRY probably has other important functions that need to be further studied. MpCRY and its distant relative from vascular plants which shared with conserved physiology functions evolved from one ancient ancestor (Figs 1, S1d,e) but sense blue light in different light environments. Therefore, blue light-specific MpCRY nuclear localization together with oligomerization in darkness, longer degradation, and conserved phosphorylation constitute a new blue light signal transduction pathway in *M. polymorpha*, which may help it adapt to its own ecological environment that consists of shaded areas (Mache & Loiseaux, 1973; Fraser *et al.*, 2016).

MpCRY plays a key role in gemma dormancy release after its dispersion

Light is one of the most important environmental cues regulating dormancy; for example, phytochrome-mediated red/far-red light signaling plays various roles in seed dormancy induction, maintenance, release, and germination in *Arabidopsis* (Jiang *et al.*, 2016; Yang *et al.*, 2019, 2020; Liao *et al.*, 2022). CRY-mediated blue light signaling also has roles in seed dormancy and germination regulation in barley and *Arabidopsis* (Barrero *et al.*, 2014; Stawska & Oracz, 2019), indicating that both blue and red light participate in seed dormancy and germination regulation in vascular plants. It has been reported that MpPHY is involved in gemma germination in a conserved photoreversible way (Inoue *et al.*, 2016). In our study, we did not find different dormancy levels between WT and Mp*cry-33*^{ge} gemmae in gemma cups under blue light (Fig. 5). We speculate that many complicated factors, such as hormones, signals produced by the apex of the paternal plant, and other unknown signals from the base of the gemma cup, may inhibit the dormancy regulation function of MpCRY in gemma cups (Eklund *et al.*, 2015, 2018), although overexpressed MpCRY can enable the high dormancy status to be overcome (Figs 5, S6e). In addition, MpCRY protein content is negatively regulated by blue light (Figs 3, 4a, S4), which causes the gemmae to maintain high dormancy in the gemma cup even when the light intensity reaches 200 μmol m^{-2} s^{-1} (Fig. S12c,d). Once the gemmae are dispersed from the gemma cup, the functional inhibition of MpCRY is removed, which mediates blue signaling and plays key roles in dispersed gemma dormancy release (Figs 6, S6f, S8). We found that the rhizoid emergence rate of dispersed gemmae increased with increasing blue light intensity, and this rate in Mp*cry*^{ge} was significantly lower than that in the WT under blue light conditions (Fig. 6e). Coincidentally, MpCRY phosphorylation and protein degradation in dispersed gemmae are also correlated with blue light intensity and exposure time (Fig. 3). More importantly, MpCRY nuclear localization, photobody formation, and gene expression regulation are also dependent on blue light irradiation in dispersed gemmae (Figs 4, S5). These results suggest that photoexcited MpCRY-mediated blue light signaling regulates gemma dormancy release, indicating that both MpCRY and MpPHY modulate gemma dormancy/germination in *M. polymorpha*.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

MpCRY-mediated blue light interacts with ABA signaling during dormancy release

Due to the lack of an ABA-binding PYL homolog, ABA-dependent responses cannot be analyzed in algae, although ABA synthesis among algal species is widespread (Sun *et al.*, 2019). Abscisic acid signaling is specific to land plants and is closely related to drought stress and dormancy regulation (Hauser *et al.*, 2011). The interaction between ABA synthesis/signaling transduction and environmental cues modifies the adaptability of plants to the external environment. In barley, the ABA synthesis-related gene *HvNCED1* and ABA metabolism-related gene *HvABA8′OH-1*, which are regulated by HvCRY1, mediate blue light signaling to promote dormancy and inhibit seed germination (Barrero *et al.*, 2014). The ABA signaling pathway, which is conserved in *M. polymorpha*, regulates dormancy induction and/or maintenance and is specific to gemmae rather than gemma cups (Eklund *et al.*, 2018). Our data further showed that the gemma as an asexual reproductive organ can independently respond to MpCRY-mediated blue light cues to repress the key ABA signaling genes and promote dormancy release in *M. polymorpha* (Figs 6–8). These results indicate that CRY-modulated ABA biosynthesis or signal transduction is a conserved environmental adaptation mechanism, although it is still unknown how CRY regulates ABA biosynthesis/signaling gene expression.

We also observed that red light may also function in gemma dormancy release (Fig. S8e), but the shaded areas where *M. polymorpha* survived have a high far-red ratio (Mache & Loiseaux, 1973; Casal, 2013), which reverses red light signal transduction (Inoue *et al.*, 2016). MpPIF has also been identified in *M. polymorpha*, it will be degraded via interacting with the active form of MpPHY in red light condition, although it is stable in dark and far-red condition (Inoue *et al.*, 2016). It has been reported that CRYs interacts with PIF4/5 in limiting blue light condition to promote hypocotyl elongation (Pedmale *et al.*, 2016). Given that only one MpPIF protein is encoded by the *M. polymorpha* genome and the conserved function of MpCRY on hypocotyl and floral origination in *Arabidopsis* (Fig. S1d,e), it is likely that MpCRY may interact with MpPIF to regulate some biological events of gemma in blue light condition. Hence, both MpCRY and MpPHY may interact MpPIF to regulate gemma dormancy release and germination in different light conditions, which maintaining *M. polymorpha* propagation and expansion.

Acknowledgements

We very much appreciate the preliminary guidance and constructive suggestions on the data analysis made by Prof. Yoshito Oka (Kyoto University). We thank Prof. Takayuki Kohchi (Kyoto University) for providing *M. polymorpha* subsp. *ruderalis* Tak-1. We also thank Prof. Huanquan Zheng (Department of Biology, McGill University) for his expert assistance in protein localization analysis. This study was supported by the National Natural Science Foundation of China (31770325 and 31870661) and the Natural Science Foundation of Jiangsu Province (BK20221408), Start-Up Funds for Scientific Research of Yancheng Teachers University (204670015), the Opening Foundation of Jiangsu Key Laboratory for Bioresources of Saline Soils (206670144) and the Forestry Peak Discipline Construction Project of Fujian Agriculture and Forestry University (72202200205).

Competing interests

None declared.

Author contributions

BL and JL contributed to the conceptualization. JL, BD, BL, QY, YL, YZ, JC, XW and MVK contributed to the investigation. JL and BL writing – original draft. BL, JL, BD, DL, Z-JL, M-ZL and LG contributed to the writing – review and editing. BL contributed to the funding acquisition. DL, LG and BL contributed to the project administration. JL and BD contributed equally to this work.

ORCID

Ban Deng https://orcid.org/0000-0002-8216-9330
Lianfeng Gu https://orcid.org/0000-0002-3810-2411
Markus V. Kohnen https://orcid.org/0000-0001-5075-575X
Jiakai Liao https://orcid.org/0000-0002-9595-6181
Deshu Lin https://orcid.org/0000-0003-4591-583X
Bobin Liu https://orcid.org/0000-0001-7133-6888
Zhong-Jian Liu https://orcid.org/0000-0003-4390-3878
Meng-Zhu Lu https://orcid.org/0000-0001-9245-1842
Xiaqin Wang https://orcid.org/0000-0002-4866-7483
Qixin Yang https://orcid.org/0000-0001-5104-6453

Data availability

Sequence data of this article can be found in the *M. polymorpha* genome database (http://marchantia.info) with the following accession nos.: Mp*CRY* (Mp2g17590); Mp*ABI3A* (Mp5g08310); Mp*ABI5B* (Mp2g22820); Mp*MFT* (Mp2g12650); Mp*LEA1* (Mp5g23710); Mp*LEA2* (Mp6g13390); Mp*LEA3* (Mp6g03080); Mp*DHN1* (Mp6g07540); and Mp*APT* (Mp3g25140). The gene and transgene nomenclature guidelines for *M. polymorpha* were reported previously (Bowman *et al.*, 2016). RNA-seq data are available in the SRA database under accession no. PRJNA682681.

References

Agrawal SC. 2009. Factors affecting spore germination in algae – review. *Folia Microbiologia* **54**: 273–302.

Barrero JM, Downie AB, Xu Q, Gubler F. 2014. A role for barley CRYPTOCHROME1 in light regulation of grain dormancy and germination. *Plant Cell* **26**: 1094–1104.

Bowman JL, Araki T, Arteaga-Vazquez MA, Berger F, Dolan L, Haseloff J, Ishizaki K, Kyozuka J, Lin SS, Nagasaki H *et al.* 2016. The naming of names: guidelines for gene nomenclature in *Marchantia*. *Plant & Cell Physiology* **57**: 257–261.

Bowman JL, Kohchi T, Yamato KT, Jenkins J, Shu S, Ishizaki K, Yamaoka S, Nishihama R, Nakamura Y, Berger F *et al.* 2017. Insights into land plant evolution garnered from the *Marchantia polymorpha* genome. *Cell* **171**: 287–304.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Carol CB, Jerry MB. 2014. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA, USA: Academic Press.

Casal JJ. 2013. Photoreceptor signaling networks in plant responses to shade. *Annual Review of Plant Biology* **64**: 403–427.

Cashmore AR, Jarillo JA, Wu YJ, Liu D. 1999. Cryptochromes: blue light receptors for plants and animals. *Science* **284**: 760–765.

Chang C, Bowman JL, Meyerowitz EM. 2016. Field guide to plant model systems. *Cell* **167**: 325–339.

Clark JW, Donoghue PCJ. 2018. Whole-genome duplication and plant macroevolution. *Trends in Plant Science* **23**: 933–945.

Dring MJ. 1988. Photocontrol of development in algae. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**: 157–174.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Eklund DM, Ishizaki K, Flores-Sandoval E, Kikuchi S, Takebayashi Y, Tsukamoto S, Hirakawa Y, Nonomura M, Kato H, Kouno M *et al.* 2015. Auxin produced by the indole-3-pyruvic acid pathway regulates development and gemmae dormancy in the liverwort *Marchantia polymorpha*. *Plant Cell* **27**: 1650–1669.

Eklund DM, Kanei M, Flores-Sandoval E, Ishizaki K, Nishihama R, Kohchi T, Lagercrantz U, Bhalerao RP, Sakata Y, Bowman JL. 2018. An evolutionarily conserved abscisic acid signaling pathway regulates dormancy in the liverwort *Marchantia polymorpha*. *Current Biology* **28**: 3691–3699.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171**: 501–523.

Finkelstein R. 2013. Abscisic acid synthesis and response. *Arabidopsis Book* **11**: e0166.

Finkelstein R, Reeves W, Ariizumi T, Steber C. 2008. Molecular aspects of seed dormancy. *Annual Review of Plant Biology* **59**: 387–415.

Forbes-Stovall J, Howton J, Young M, Davis G, Chandler T, Kessler B, Rinehart CA, Jacobshagen S. 2014. *Chlamydomonas reinhardtii* strain CC-124 is highly sensitive to blue light in addition to green and red light in resetting its circadian clock, with the blue-light photoreceptor plant cryptochrome likely acting as negative modulator. *Plant Physiology and Biochemistry* **75**: 14–23.

Fraser DP, Hayes S, Franklin KA. 2016. Photoreceptor crosstalk in shade avoidance. *Current Opinion in Plant Biology* **33**: 1–7.

Gamborg OL, Miller RA, Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**: 151–158.

Ghosh TK, Kaneko M, Akter K, Murai S, Komatsu K, Ishizaki K, Yamato KT, Kohchi T, Takezawa D. 2016. Abscisic acid-induced gene expression in the liverwort *Marchantia polymorphais* mediated by evolutionarily conserved promoter elements. *Physiologia Plantarum* **156**: 407–420.

Gilman AG. 1987. G proteins: transducers of receptor-generated signals. *Annual Review of Biochemistry* **56**: 615–649.

Gouy M, Guindon S, Gascuel O. 2010. SEAVIEW v.4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**: 221–224.

Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ. 2012. Molecular mechanisms of seed dormancy. *Plant, Cell & Environment* **35**: 1769–1786.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PHYML 3.0. *Systematic Biology* **59**: 307–321.

Han X, Chang X, Zhang Z, Chen H, He H, Zhong B, Deng XW. 2019. Origin and evolution of core components responsible for monitoring light environment changes during plant terrestrialization. *Molecular Plant* **12**: 847–862.

Hauser F, Waadt R, Schroeder JI. 2011. Evolution of abscisic acid synthesis and signaling mechanisms. *Current Biology* **21**: R346–R355.

Imaizumi T, Kadota A, Hasebe M, Wada M. 2002. Cryptochrome light signals control development to suppress auxin sensitivity in the moss *Physcomitrella patens*. *Plant Cell* **14**: 373–386.

Imaizumi T, Kanegae T, Wada M. 2000. Cryptochrome nucleocytoplasmic distribution and gene expression are regulated by light quality in the fern *Adiantum capillus-veneris*. *Plant Cell* **12**: 81–96.

Inoue K, Nishihama R, Kataoka H, Hosaka M, Manabe R, Nomoto M, Tada Y, Ishizaki K, Kohchi T. 2016. Phytochrome signaling is mediated by PHYTOCHROME INTERACTING FACTOR in the liverwort *Marchantia polymorpha*. *Plant Cell* **28**: 1406–1421.

Ishizaki K, Chiyoda S, Yamato KT, Kohchi T. 2008. *Agrobacterium*-mediated transformation of the haploid liverwort *Marchantia polymorpha* L., an emerging model for plant biology. *Plant & Cell Physiology* **49**: 1084–1091.

Jiang Z, Xu G, Jing Y, Tang W, Lin R. 2016. Phytochrome B and REVEILLE1/2-mediated signalling controls seed dormancy and germination in Arabidopsis. *Nature Communications* **7**: 12377.

Kato H, Kouno M, Takeda M, Suzuki H, Ishizaki K, Nishihama R, Kohchi T. 2017. The roles of the sole activator-type auxin response factor in pattern formation of *Marchantia polymorpha*. *Plant & Cell Physiology* **58**: 1642–1651.

Kato H, Yasui Y, Ishizaki K. 2020. Gemma cup and gemma development in *Marchantia polymorpha*. *New Phytologist* **228**: 459–465.

Kenrick P, Crane PR. 1997. The origin and early evolution of plants on land. *Nature* **389**: 33–39.

Kianianmomeni A, Hallmann A. 2014. Algal photoreceptors: *in vivo* functions and potential applications. *Planta* **239**: 1–26.

Kubota A, Ishizaki K, Hosaka M, Kohchi T. 2013. Efficient *Agrobacterium*-mediated transformation of the liverwort *Marchantia polymorpha* using regenerating thalli. *Bioscience, Biotechnology, and Biochemistry* **77**: 167–172.

Kucera B, Cohn MA, Leubner-Metzger G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Science Research* **15**: 281–307.

Li T, Zhang L, Su S, Li S, Zhang J, Yang Z, Zuo Z. 2021. Roles of a cryptochrome in carbon fixation and sucrose metabolism in the liverwort *Marchantia polymorpha*. *Cells* **10**: 3387.

Lian H, Xu P, He S, Wu J, Pan J, Wang W, Xu F, Wang S, Pan J, Huang J *et al.* 2018. Photoexcited CRYPTOCHROME 1 interacts directly with G-protein β subunit AGB1 to regulate the DNA-binding activity of HY5 and photomorphogenesis in *Arabidopsis*. *Molecular Plant* **11**: 1248–1263.

Liao J, Deng B, Cai X, Yang Q, Hu B, Cong J, Zhang Y, Wang G, Xin G, Li Y *et al.* 2022. Time-course transcriptome analysis reveals regulation of Arabidopsis seed dormancy by the transcription factors WOX11/12. *Journal of Experimental Botany* **74**: 1090–1106.

Lin C, Shalitin D. 2003. Cryptochrome structure and signal transduction. *Annual Review of Plant Biology* **54**: 469–496.

Lin C, Todo T. 2005. The cryptochromes. *Genome Biology* **6**: 220.

Linkies A, Graeber K, Knight C, Leubner-Metzger G. 2010. The evolution of seeds. *New Phytologist* **186**: 817–831.

Liu Q, Su T, He W, Ren H, Liu S, Chen Y, Gao L, Hu X, Lu H, Cao S *et al.* 2020. Photooligomerization determines photosensitivity and photoreactivity of plant cryptochromes. *Molecular Plant* **13**: 398–413.

Liu Q, Wang Q, Deng W, Wang X, Piao M, Cai D, Li Y, Barshop WD, Yu X, Zhou T *et al.* 2017. Molecular basis for blue light-dependent phosphorylation of Arabidopsis cryptochrome 2. *Nature Communications* **8**: 15234.

Liu Q, Wang Q, Liu B, Wang W, Wang X, Park J, Yang Z, Du X, Bian M, Lin C. 2016. The blue light-dependent polyubiquitination and degradation of Arabidopsis cryptochrome2 requires multiple E3 ubiquitin ligases. *Plant & Cell Physiology* **57**: 2175–2186.

Liu S, Zhang L, Gao L, Chen Z, Bie Y, Zhao Q, Zhang S, Hu X, Liu Q, Wang X *et al.* 2022. Differential photoregulation of the nuclear and cytoplasmic CRY1 in Arabidopsis. *New Phytologist* **234**: 1332–1346.

Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li J, He ZH. 2013. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **110**: 15485–15490.

Mache R, Loiseaux S. 1973. Light saturation of growth and photosynthesis of the shade plant *Marchantia polymorpha*. *Journal of Cell Science* **12**: 391–401.

Mao Z, He S, Xu F, Wei X, Jiang L, Liu Y, Wang W, Li T, Xu P, Du S *et al.* 2020. Photoexcited CRY1 and phyB interact directly with ARF6 and ARF8 to regulate their DNA-binding activity and auxin-induced hypocotyl elongation in Arabidopsis. *New Phytologist* **225**: 848–865.

Martin-Fontecha ES, Tarancon C, Cubas P. 2018. To grow or not to grow, a power-saving program induced in dormant buds. *Current Opinion in Plant Biology* **41**: 102–109.

McDaniel SF. 2021. Bryophytes are not early diverging land plants. *New Phytologist* **230**: 1300–1304.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

McGrath CL, Gout JF, Johri P, Doak TG, Lynch M. 2014. Differential retention and divergent resolution of duplicate genes following whole-genome duplication. *Genome Research* **24**: 1665–1675.

Mishler BD, Churchill SP. 1984. A cladistic approach to the phylogeny of the "Bryophytes". *Brittonia* **36**: 406–424.

Molisch H. 1922. *Pflanzenphysiologie als theorie der gärtnerei*. Jena, Germany: Gustav Fisher.

Muller N, Wenzel S, Zou Y, Kunzel S, Sasso S, Weiss D, Prager K, Grossman A, Kottke T, Mittag M. 2017. A plant cryptochrome controls key features of the *Chlamydomonas* circadian clock and its life cycle. *Plant Physiology* **174**: 185–201.

Ozkan-Dagliyan I, Chiou YY, Ye R, Hassan BH, Ozturk N, Sancar A. 2013. Formation of Arabidopsis Cryptochrome 2 photobodies in mammalian nuclei: application as an optogenetic DNA damage checkpoint switch. *Journal of Biological Chemistry* **288**: 23244–23251.

Pedmale UV, Huang SS, Zander M, Cole BJ, Hetzel J, Ljung K, Reis PA, Sridevi P, Nito K, Nery JR *et al.* 2016. Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* **164**: 233–245.

Penfield S. 2017. Seed dormancy and germination. *Current Biology* **27**: R874–R878.

Qiu YL, Li L, Wang B, Chen Z, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J *et al.* 2006. The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences, USA* **103**: 15511–15516.

Saint-Marcoux D, Proust H, Dolan L, Langdale JA. 2015. Identification of reference genes for real-time quantitative PCR experiments in the liverwort *Marchantia polymorpha*. *PLoS ONE* **10**: e0118678.

Sang Y, Li QH, Rubio V, Zhang YC, Mao J, Deng XW, Yang HQ. 2005. N-terminal domain-mediated homodimerization is required for photoreceptor activity of Arabidopsis CRYPTOCHROME 1. *Plant Cell* **17**: 1569–1584.

Sawers RG. 2016. Dormancy: illuminating how a microbial sleeping beauty awakens. *Current Biology* **26**: R1139–R1141.

Serrano-Bueno G, Romero-Campero FJ, Lucas-Reina E, Romero JM, Valverde F. 2017. Evolution of photoperiod sensing in plants and algae. *Current Opinion in Plant Biology* **37**: 10–17.

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.

Shalitin D, Yu X, Maymon M, Mockler T, Lin C. 2003. Blue light-dependent *in vivo* and *in vitro* phosphorylation of Arabidopsis cryptochrome 1. *Plant Cell* **15**: 2421–2429.

Shimamura M. 2016. *Marchantia polymorpha*: taxonomy, phylogeny and morphology of a model system. *Plant & Cell Physiology* **57**: 230–256.

Shu K, Liu XD, Xie Q, He ZH. 2016. Two faces of one seed: hormonal regulation of dormancy and germination. *Molecular Plant* **9**: 34–45.

Singh NK, Sonani RR, Rastogi RP, Madamwar D. 2015. The phycobilisomes: an early requisite for efficient photosynthesis in cyanobacteria. *EXCLI Journal* **14**: 268–289.

Stawska M, Oracz K. 2019. phyB and HY5 are involved in the blue light-mediated alleviation of dormancy of Arabidopsis seeds possibly via the modulation of expression of genes related to light, GA, and ABA. *International Journal of Molecular Sciences* **20**: 5882.

Sugano SS, Nishihama R. 2018. CRISPR/Cas9-based genome editing of transcription factor genes in *Marchantia polymorpha*. *Methods in Molecular Biology* **1830**: 109–126.

Sugano SS, Nishihama R, Shirakawa M, Takagi J, Matsuda Y, Ishida S, Shimada T, Hara-Nishimura I, Osakabe K, Kohchi T. 2018. Efficient CRISPR/Cas9-based genome editing and its application to conditional genetic analysis in *Marchantia polymorpha*. *PLoS ONE* **13**: e0205117.

Sugano SS, Shirakawa M, Takagi J, Matsuda Y, Shimada T, Hara-Nishimura I, Kohchi T. 2014. CRISPR/Cas9-mediated targeted mutagenesis in the liverwort *Marchantia polymorpha* L. *Plant & Cell Physiology* **55**: 475–481.

Sun Y, Harpazi B, Wijerathna-Yapa A, Merilo E, de Vries J, Michaeli D, Gal M, Cuming AC, Kollist H, Mosquna A. 2019. A ligand-independent origin of abscisic acid perception. *Proceedings of the National Academy of Sciences, USA* **116**: 24892–24899.

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. 2016. W-IQ-Tree: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* **44**: W232–W235.

Vesty EF, Saidi Y, Moody LA, Holloway D, Whitbread A, Needs S, Choudhary A, Burns B, McLeod D, Bradshaw SJ *et al.* 2016. The decision to germinate is regulated by divergent molecular networks in spores and seeds. *New Phytologist* **211**: 952–966.

de Vries J, Archibald JM. 2018. Plant evolution: landmarks on the path to terrestrial life. *New Phytologist* **217**: 1428–1434.

Wang F, Gao Y, Liu Y, Zhang X, Gu X, Ma D, Zhao Z, Yuan Z, Xue H, Liu H. 2019. BES1-regulated BEE1 controls photoperiodic flowering downstream of blue light signaling pathway in Arabidopsis. *New Phytologist* **223**: 1407–1419.

Wang Q, Barshop WD, Bian M, Vashisht AA, He R, Yu X, Liu B, Nguyen P, Liu X, Zhao X *et al.* 2015. The blue light-dependent phosphorylation of the CCE domain determines the photosensitivity of Arabidopsis CRY2. *Molecular Plant* **8**: 631–643.

Wang Q, Lin C. 2020. Mechanisms of cryptochrome-mediated photoresponses in plants. *Annual Review of Plant Biology* **71**: 103–129.

Wang Q, Zuo Z, Wang X, Gu L, Yoshizumi T, Yang Z, Yang L, Liu Q, Liu W, Han YJ *et al.* 2016. Photoactivation and inactivation of Arabidopsis cryptochrome 2. *Science* **354**: 343–347.

Wang X, Wang Q, Han Y-J, Liu Q, Gu L, Yang Z, Su J, Liu B, Zuo Z, He W *et al.* 2017. A CRY-BIC negative-feedback circuitry regulating blue light sensitivity of Arabidopsis. *The Plant Journal* **92**: 426–436.

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R, Group NEGW. 2014. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist* **203**: 300–309.

Wu G, Spalding EP. 2007. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. *Proceedings of the National Academy of Sciences, USA* **104**: 18813–18818.

Xie P. 2013. *Scaling ecology to understand natural design of life systems and their operations and evolutions – integration of ecology, genetics and evolution through reproduction.* Beijing, China: Science Press.

Xu F, He S, Zhang J, Mao Z, Wang W, Li T, Hua J, Du S, Xu P, Li L *et al.* 2018. Photoactivated CRY1 and phyB interact directly with AUX/IAA proteins to inhibit auxin signaling in Arabidopsis. *Molecular Plant* **11**: 523–541.

Yan A, Chen Z. 2017. The pivotal role of abscisic acid signaling during transition from seed maturation to germination. *Plant Cell Reports* **36**: 689–703.

Yang L, Jiang Z, Jing Y, Lin R. 2020. PIF1 and RVE1 form a transcriptional feedback loop to control light-mediated seed germination in Arabidopsis. *Journal of Integrative Plant Biology* **62**: 1372–1384.

Yang L, Jiang Z, Liu S, Lin R. 2019. Interplay between REVEILLE1 and RGA-LIKE2 regulates seed dormancy and germination in *Arabidopsis*. *New Phytologist* **225**: 1593–1605.

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

Yu X, Liu H, Klejnot J, Lin C. 2010. The cryptochrome blue light receptors. *Arabidopsis Book* **8**: e0135.

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.

Yu X, Shalitin D, Liu X, Maymon M, Klejnot J, Yang H, Lopez J, Zhao X, Bendehakkalu KT, Lin C. 2007b. Derepression of the NC80 motif is critical for the photoactivation of *Arabidopsis* CRY2. *Proceedings of the National Academy of Sciences, USA* **104**: 7289–7294.

Zhang L, Li T, Su S, Peng H, Li S, Li K, Ji L, Xing Y, Zhang J, Du X *et al.* 2021. Functions of COP1/SPA E3 ubiquitin ligase mediated by MpCRY in the liverwort *Marchantia polymorpha* under blue light. *International Journal of Molecular Sciences* **23**: 158.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 PHR domain of cryptochromes is conserved, and the important residues of the CCE domain are conserved in *Marchantia polymorpha*.

Fig. S2 PHR domain-mediated self-interaction of MpCRY is promoted under blue light in yeast cells, related to the content of Fig. 2.

Fig. S3 Negative control experiment for the BiFC assay for MpCRY self-interaction in *Nicotiana benthamiana* cells, related to the content of Fig. 2.

Fig. S4 Phosphorylation of the MpCRY protein in a blue light-dependent manner in *Marchantia polymorpha*, related to the content of Fig. 3.

Fig. S5 Blue light induces the translocation of the MpCRY protein into the nucleus in *Marchantia polymorpha*, related to the content of Fig. 4.

Fig. S6 Dormancy phenotype of Mp*cry*^{ge} and $_{pro}$*EF*:Mp*CRY* plants of *Marchantia polymorpha*, related to the content of Figs 5 and 6.

Fig. S7 MpCRY does not affect gemma dormancy in the gemma cup under red light in *Marchantia polymorpha*, related to the content of Fig. 5.

Fig. S8 MpCRY represses dormancy and promotes dormancy release of gemmae specifically under blue light in *Marchantia polymorpha*, related to the content of Fig. 6.

Fig. S9 Abscisic acid treatment dosage effect on delaying gemma rhizoid formation under blue light in *Marchantia polymorpha*, related to the content of Fig. 7.

Fig. S10 MpCRY acts antagonistically with abscisic acid to suppress gemma dormancy under blue light in *Marchantia polymorpha*, related to the content of Fig. 7.

Fig. S11 Abscisic acid-related gene mRNA levels of gemmae grown under red light in *Marchantia polymorpha*, related to the content of Fig. 7.

Fig. S12 Abscisic acid-related gene mRNA levels of gemmae inside the gemma cup grown under blue light in *Marchantia polymorpha*, related to the content of Figs 5 and 7.

Methods S1 Supplementary details about the materials and methods.

Table S1 Plants used for construction of the phylogenetic tree in this study of *Marchantia polymorpha*, related to the content of Fig. 1.

Table S2 PCR primers used in this study of *Marchantia polymorpha*.

Table S3 Gene Ontology results related to the content of Fig. 4.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

© 2023 The Authors
New Phytologist © 2023 New Phytologist Foundation

14698137, 0, Downloaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18815 by Lancaster University The Library, Wiley Online Library on [09/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License