# Gene Identification for Phototropin-dependent Photoprotection in Chlamydomonas Reinhardtii

Anthony Pang [*, #], Che Su [#], Yunqi Peng [#]

Basis International School of Guangzhou

* Corresponding Author Email: anthony.pang13417-bigz@basischina.com

[#]These authors contributed equally to this work.

**Abstract.** Photosynthetic organisms convert solar energy into chemical energy through photosynthesis, generating carbohydrates and lipids that are valuable for biofuels. Algae, renowned for their rapid growth and efficiency, have developed mechanisms to adapt to varying light conditions, ensuring their survival and prosperity. Among these mechanisms, photoprotection strategies such as Non-Photochemical Quenching (NPQ) enhance their tolerance to high light intensities. Excessive light intensity can cause detrimental overexcitation of the photosystems. In *Chlamydomonas,* one of the proteins, LHCSR3, provides a quick protective response known as energy-dependent quenching (qE), the fastest and most important component of NPQ. Deletion of LHCSR3 leads to cell death under high light conditions. Recent research has shown that blue light, perceived by phototropin (PHOT), mediates the photoprotection of the photosynthetic machinery under high light conditions in *Chlamydomonas reinhardtii*. Deletion of PHOT leads to compromised expression of LHCSR3 under high light conditions, therefore leading to cell death. However, the downstream signaling components of the PHOT-LHCSR3 pathway remain largely undiscovered. The objective of this project was to identify and characterize new actors involved in PHOT-dependent photoprotection in *Chlamydomonas*. Using forward genetics and omics analysis, we built a mutant library that could survive under high light intensity in a phot background. We also identified 8 putative PHOT-dependent photoprotection downstream signaling components. Overall, this project brings new insights into the acclimation of photosynthesis to high light. The mutant library could be further used for additional research. Furthermore, understanding photoprotection will not only help increase microalgae biofuel production productivity but could also provide new insights into the genetic engineering of crops for high light resistance.

**Keywords:** Biofuel, Stress response, UV mutagenesis, Green algae, Photoprotection.

## 1. Introduction

### 1.1. Chlamydomonas reinhardtii-Model Green microalgae

The model organism *Chlamydomonas reinhardtii* (**Figure 1**) used in this project is the most studied photosynthetic unicellular organism belonging to the green lineage. *Chlamydomonas reinhardtii* (hereafter *C. reinhardtii*) is a biflagellated microalgae which typically lives in freshwater environments, and is about 10 μm in size. It has a single large U-shaped chloroplast containing a pyrenoid for Carbon fixation and Carbon concentration. Additionally, it possesses an eyespot, that sense the light, which is essential for phototaxis[1]

As one of the most popular model organisms in scientific research, *C. reinhardtii* offers several advantages, such as a fast growth rate and a short life cycle. Additionally, its genome has been fully sequenced [2], and various genetic engineering [3, 4] tools are available, making it ideal for specific research purposes. Unlike higher plants like Arabidopsis, *C. reinhardtii* can use acetate as a carbon source, allowing it to survive even when key photosynthesis genes are knocked out, which is beneficial for photosynthesis research [4]. Thanks to these advantages, *C. reinhardtii* has been used to investigate metabolism[5], cell signaling pathways[6], and photosynthesis[7]. It can also provide biomass, with its lipid and starch content being useful for biofuel production, making it applicable in industrial settings[8]. Moreover, as part of the green lineage, research on *C. reinhardtii* provides transferable knowledge that can be applied to other green microalgae and higher plants[9].

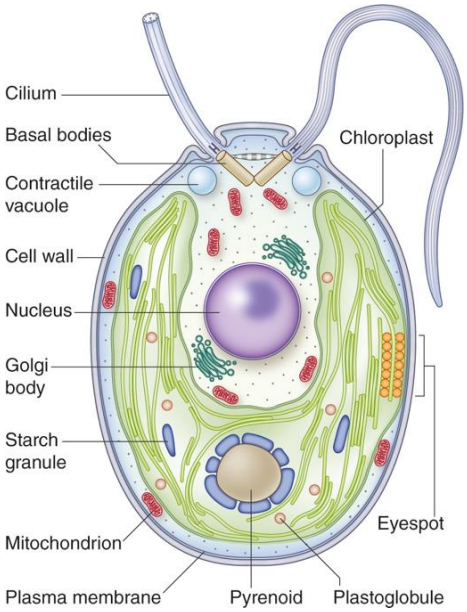



**Figure 1.** Structure of a vegetative Chlamydomonas reinhardtii cell [10].

### 1.2. The use of light in plants and algae

In photosynthetic eukaryotes, photosynthesis occurs by using sunlight as a source of energy to produce glucose. While low light limits growth by reducing photosynthetic productivity, excessive light can be toxic to cells and even lethal. To counter this, plants and algae have developed a mechanism called Non-Photochemical Quenching (NPQ) to protect themselves from high light damage[11]. When light is available, chlorophyll absorbs light energy and becomes excited. To return to a stable state, chlorophyll can dissipate the energy in three ways: a) driving photosynthesis (photochemistry), b) emitting it as fluorescence, or c) releasing it as heat, which forms the basis of NPQ[11].

NPQ is the major short-term response to excess light energy and includes components like qE (Energy-dependent NPQ), qT (State transition), qI (Photoinhibition), qZ (Zeaxanthin-dependent quenching) and others[12]. This project will focus on qE, the major component of NPQ, which dissipates excess light energy as heat. qE is triggered by lumen acidification, leading to the activation of specific qE effector proteins.

Effector proteins are found in all photoautotrophic eukaryotic organisms. Unlike *Arabidopsis*, where PSBS is the only qE effector protein [13], Chlamydomonas reinhardtii has three qE effector proteins: LHCSR1 [14, 15], LHCSR3 [16, 17], and PSBS [13]. Of these, LHCSR3 plays the primary role in qE responses [17]. The *npq4* mutant, which is defective in LHCSR3, shows a significantly reduced qE capacity compared to the wild type (WT), and cannot survive under high light conditions[16]. LHCSRs are encoded in the nucleus and then targeted to the chloroplast, where they are integrated into the thylakoid membrane. Previous research has shown that the induction of LHCSR3 requires active photosynthesis, as well as the $Ca^{2+}$-binding protein CAS (calcium sensor) and $Ca^{2+}$-induced signaling [18].

### 1.3. The use of light, not only light intensity, but also light quality

In addition to serving as an energy source for photosynthetic organisms, light also provides valuable environmental information. Beyond light intensity, light quality is crucial for both plants and algae. To perceive different light qualities, these organisms have developed various photoreceptors that sense a wide range of light wavelengths [19, 20]. By working together, these photoreceptors allow the organism to gather information from different parts of the light spectrum, enabling a comprehensive assessment of their surroundings and inducing corresponding physiological responses (**Figure 2**).

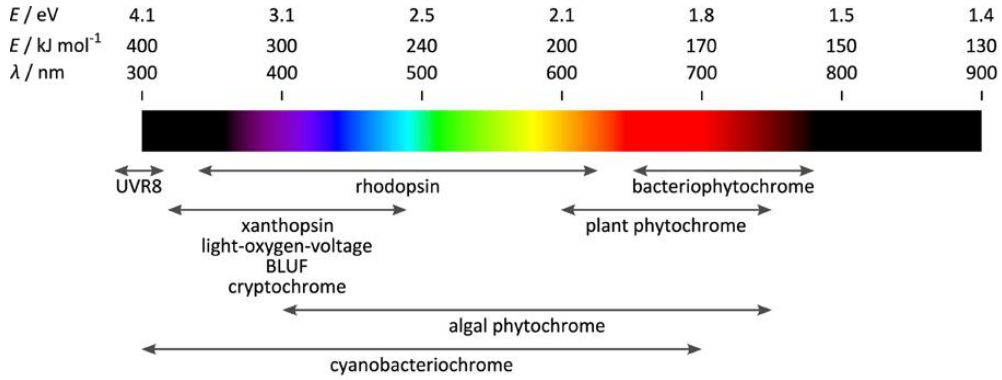



**Figure 2.** Spectral sensitivity of photoreceptors.

The spectral sensitivity of sensory photoreceptors ranges from the UV to the near-infrared region of the electromagnetic spectrum [21].

Photoreceptors detect light through different chromophores, converting light signals into biological signals that trigger downstream pathways, such as gene expression, phototropism [22], the cell cycle, and the circadian clock. In the green microalga *C. reinhardtii*, three photoreceptors have been identified: CRYs, PHOT, and UVR8[23].

UVR8 is a highly conserved photoreceptor that responds to UV-B light in both plants and algae[24]. Upon UV-B perception, the inactive UVR8 homodimer is disrupted, leading to UV-B-dependent reversible monomerization. The monomerized UVR8 then enters the nucleus to initiate UV-B molecular signaling pathways [24-26].

CRYs and PHOT are the two blue light photoreceptors found in *C. reinhardtii*, and our project focuses on PHOT mainly. PHOT are blue light photoreceptors found throughout the green lineage. The structure of PHOT consists of two main parts: the N-terminal half, which contains two flavin mononucleotide (FMN) chromophore-binding Photosensory Light–Oxygen–Voltage (LOV) domains and the C-terminal half, which includes a serine/threonine protein kinase domain responsible for signal output[27, 28]. At the molecular level, blue light induces the covalent binding of the FMN to the two LOV domains, leading to conformational changes that release the kinase domain, which had been inhibited by the photosensory domain. This release triggers the signal transduction pathway, ultimately regulating different responses[29].

### 1.4. Role of photoreceptors in photoprotection in Chlamydomonas reinhardtii

PHOT and UVR8 play crucial roles in photoprotection by controlling three qE effector proteins. In *C. reinhardtii*, PHOT is essential for regulating the expression of the LHCSR3 gene, which is critical in energy-dependent quenching (qE), a key photoprotective mechanism [30]. Under high light conditions, PHOT senses blue light through its LOV domains, which then activate its kinase domain. This activation triggers a signaling cascade that leads to the accumulation of LHCSR3, the main effector of qE. PHOT's kinase domain mediates signal transduction, likely involving cyclic nucleotides such as cAMP or cGMP [31]. This signal is integrated with chloroplast-derived signals reporting the amount of absorbed light not used for $CO_2$ fixation, ensuring that photoprotective responses are proportional to light intensity[32]. Additionally, downstream signaling components of PHOT, such as DET1 and DDB1, are involved in regulating photoprotection. These proteins form an E3 ubiquitin ligase complex (CUL4-DDB1-DET1)[33], which suppresses the expression of photoprotective genes in the dark. However, under high light conditions, PHOT signals inhibit this complex's activity, allowing for the activation of LHCSR3 and other photoprotective genes. This dual regulation enables precise control of photoprotection based on environmental light conditions.

UVR8 detects UV-B radiation and initiates a signaling pathway that induces photoprotective gene expression. In *C. reinhardtii*, UVR8 regulates the expression of genes such as PSBS and LHCSR1, both of which play important roles in photoprotection under UV-B and high-light conditions [26]. Upon sensing UV-B light, UVR8 forms a complex with COP1, which modulates the activity of the E3 ubiquitin ligase. This complex inhibits the degradation of key transcription factors that control

photoprotective genes, allowing PSBS and LHCSR1 to accumulate in response to UV-B exposure [34]. Unlike PHOT, UVR8 signaling is independent of photosynthetic electron transport, meaning it can activate photoprotection even when photosynthesis is limited.

While PHOT and UVR8 operate through distinct pathways, there is evidence of crosstalk between these photoreceptors in *Chlamydomonas* [35]. Under conditions where both high light and UV-B are present, the expression of photoprotective genes such as LHCSR1, LHCSR3, and PSBS is modulated by both pathways. This suggests that the UVR8 pathway may act as a complementary mechanism when the blue-light-induced photoprotection from PHOT is insufficient. The interaction between the PHOT and UVR8 pathways highlights the complexity of photoprotective mechanisms in photosynthetic organisms (**Figure 3**). The ability of these organisms to integrate signals from both blue light and UV-B ensures they can adapt to fluctuating light environments, optimizing their photoprotective responses to avoid photodamage and maintain photosynthetic efficiency.

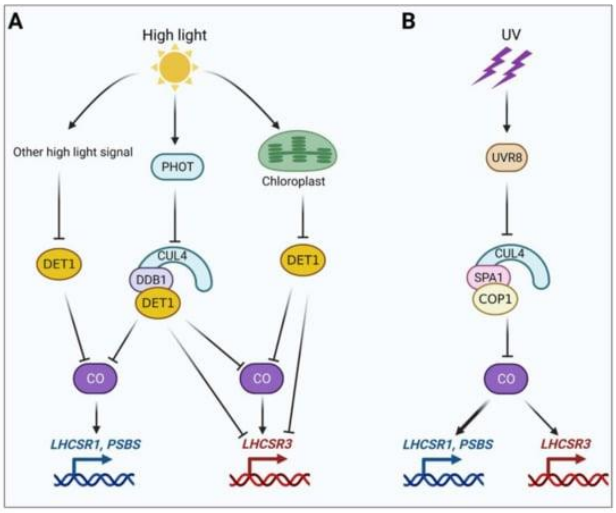



**Figure 3.** Models for the induction of LHCSR1, LHCSR3, and PSBS expression by high light and UV-B in *C. reinhardtii*[36].

### 1.5. Project presentation & Aims of the project

Photosynthetic organisms have developed the NPQ mechanism to protect themselves from excess light damage, allowing them to survive under various light conditions. Previous research has shown that NPQ is regulated not only by light intensity but also by light quality, which is sensed by different photoreceptors. In *C. reinhardtii*, PHOT and UVR8 are involved in NPQ regulation by controlling the expression of the three qE effector proteins: LHCSR3, LHCSR1, and PSBS. However, while photoprotection has been extensively studied from different perspectives, the link between photoreceptors and qE effector proteins remains unclear. For example, how does PHOT regulate LHCSR3? What are the upstream factors of CONSTANS (CO) in PHOT-dependent qE regulation [37].

In this project, our goal is to identify new factors involved in PHOT-dependent qE regulation in *C. reinhardtii*. By combining UV mutagenesis and multi-omics approaches, we aim to provide a list of candidates that may play a role in PHOT-dependent qE regulation in *C. reinhardtii*.

## 2. Materials and Methods

### 2.1. Algal Strains

The *Chlamydomonas reinhardtii* wild strains CC-125 and mutant strain *phot* (https://www.chlamycollection.org/product/cc-5391-%e2%88%86phot-c41-mt-ph13/) were obtained from the *Chlamydomonas Resource Center*. All the strains were maintained on solid TAP agar plates at 18℃ and 5 μE.

### 2.2. Growth Conditions and Medium

All the experiments in this project were performed using standard (Tris Acetate phosphate) TAP medium or Sueoka's high salt medium (HSM) medium. For High light treatment experiment, cells were initially grown in 25 or 50ml of liquid TAP medium in 150ml or 250ml flasks at 22°C with shaking at 120rpm under low light conditions (15 μE) until reaching sufficient cell density. Low light (LL) was defined as 15 μE, while high light (HL) was set at 1000 μE unless otherwise specified.

### 2.3. UV mutagenesis

UV mutagenesis of phot strain was performed on a Biometra Transilluminator (312 nm, ultraviolet light).

### 2.4. DNA extraction

For DNA extraction, we implement the method of CTAB to isolate DNA from the samples.

Note: The DNA absorption peak is at 260nm. The A260 value is used to determine DNA concentration, where A260=1 corresponds to approximately 50μg/ml of double-stranded DNA. The A260/A280 ratio is used to assess purity: a ratio of 1.8-2.0 indicates high purity. A lower ratio suggests protein contamination, while a higher ratio indicates the presence of RNA or fragmented DNA.

### 2.5. RNA extraction

RNA extraction was performed using the Spin Column Plant Total RNA Purification Kit provided in the experiment. The blots were developed with the ECL detection reagent, and images of the blots were obtained using a CCD imager (ChemiDoc MP System, Bio-Rad).

### 2.6. Omics analysis

Samples were collected and sent for omics analysis at Sangon Biotech Company.

## 3. Results

### 3.1. Chlamydomonas cultivation and phot mutant confirmation

We obtained both the wild type (WT) and *phot* mutant strains from the Chlamydomonas Resource Center in the US. No prior project or paper has specifically tested and confirmed the phenotype of this mutant. Therefore, we conducted extensive experiments to validate and study its phenotype in detail.

For cell replating, we prepared TAP plates containing 1.5% agar and HSM, and for further research, the samples were preserved in a solid culture medium.

After four days of cultivation, under the microscopy, both the WT and *phot* mutant, as reported in previous research[38], we can't observed any difference between WT and *phot* under light microscopy (**Figure 4**).

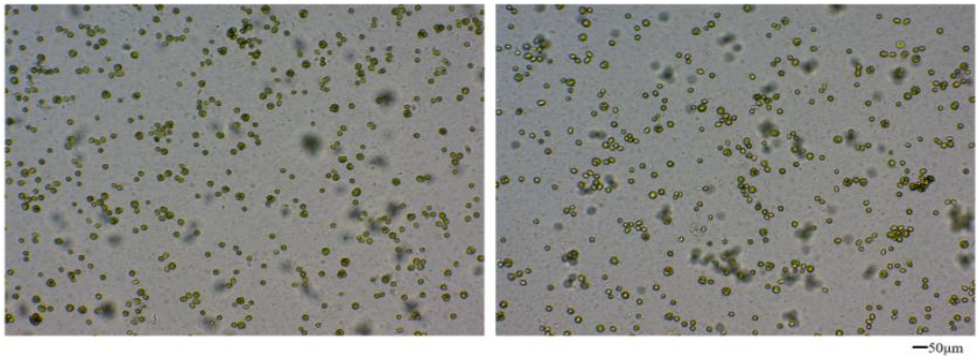



**Figure 4.** Representive microscopy picture of WT (Left) and *phot* (Right).

Following the cultivation of Chlamys, further testing was required to verify the *phot* mutant. Specifically, tests on their sensitivity to high light were conducted to validate the phenotype described in the literature. These experiments were crucial for confirming the *phot* mutant, as the sample was generated using the CRISPR-Cas9 method to knock out the *PHOT* gene, with the insertion of a paromomycin resistance gene, approximately 2.28 kb in length, into the 5th exon [4]. However, whether this gene was truly disrupted had not yet been tested. Additionally, the potential off-target effects of CRISPR-Cas9 could lead to disruptions in other genes, potentially affecting the mutant's phenotype.

We first tested whether the mutant would exhibit bleaching under high light intensity, as previously reported. Since photoprotection occurs only under photoautotrophic conditions, we grew both WT and *phot* mutant in TAP medium for several days to ensure sufficient cell growth for the high light test. Once an adequate number of cells were obtained, they were transferred from the TAP medium to HSM medium and placed under either low light or high light conditions for at least 24 hours to observe their responses.

As shown in **Figure 5**, both the WT and *phot* mutant grew normally and exhibited similar behavior under low light conditions. However, under high light conditions, while the WT continued to grow normally—though with a slightly more yellow appearance compared to low light conditions—the *phot* mutant could not survive [30].

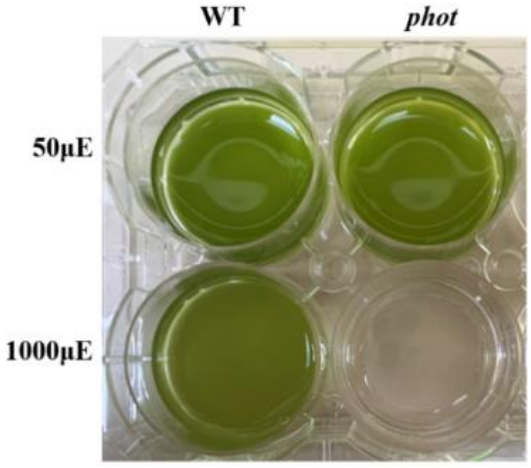



**Figure 5.** The bleaching phenotypes of WT and *phot* visualized in multi-well plate.

Representative cell cultures treated with low light (50μE; left wells) or high light (1000μE; right wells) after 24h.

We further tested the *phot* mutant under three different light intensities: 15, 300, and 1000μE, taking photographs at various time points during the light treatment. As shown in **Figure 6**, the *phot* mutant was able to survive at 15 and 300μE. However, under 1000μE conditions, the *phot* mutant began to turn yellow and pale, eventually bleaching completely and dying. Both experiments confirm the high light sensitivity and bleaching phenotype of the *phot* mutant used in this project.

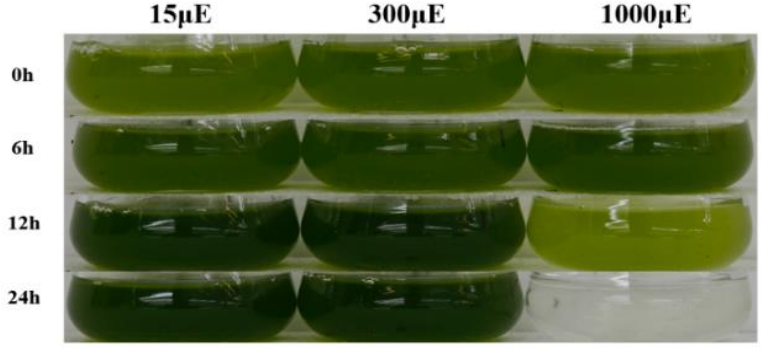



**Figure 6.** The bleaching phenotypes of *phot* visualized in flasks under different light intensity

To further confirm the *phot* mutant, we examined the protein level of PHOT by performing a western blot using the anti-LOV antibody [1], which targets the unique LOV domain of PHOT in *C. reinhardtii*. In the WT sample, a strong band corresponding to the correct size of approximately 100 kDa was observed (**Figure 7**). However, in the *phot* mutant, we detected a very weak band of a slightly smaller size (**Figure 7**). This indicates that PHOT protein expression is disrupted in the *phot* mutant. Although the band is smaller, the weak signal suggests either the presence of a very low abundance of PHOT protein in the *phot* mutant or the expression of a truncated version of the protein.

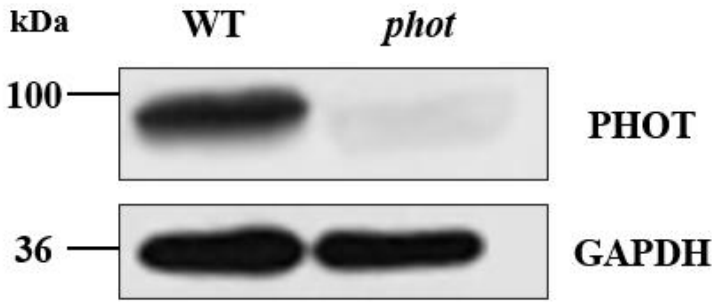



**Figure 7.** The protein level of PHOT in WT and phot by immunodetection. GAPDH is used as a loading control.

To confirm this will not affect the phenotype of *phot* (**Figure 7**), we also examined the expression levels of LHCSR3 in both WT and *phot* mutants under low and high light conditions. Previous research has reported that LHCSR3 expression is compromised in the *phot* mutant under high light conditions, which is the reason the *phot* mutant cannot survive under such conditions [30]. Since prior studies have shown that PHOT regulates LHCSR3 expression at the transcriptional level under high light conditions, we checked LHCSR3 transcription levels by performing RT-qPCR. We placed WT and *phot* cells under 300μE light conditions. In our previous tests, 300μE light intensity did not kill the *phot* mutant (**Figure 6**), allowing us to collect samples to check the transcription levels. Since transcription levels peak before protein levels, we collected samples at 0h and 1h after high light exposure [39].

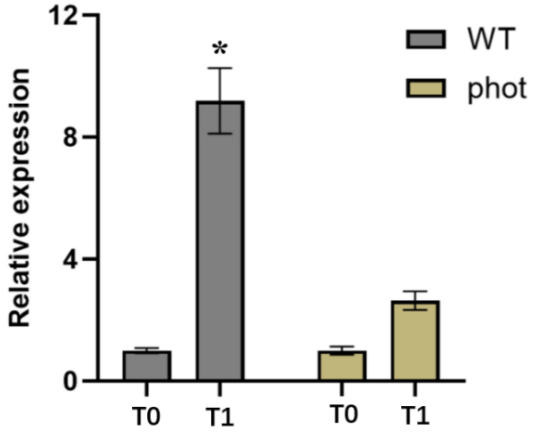



**Figure 8.** The LHCSR3 expression level in WT and *phot* under high light conditions. T0, 0h; T1, 1h high light treatment. * means P value<0.05.

As shown in **Figure 8**, LHCSR3 was upregulated in WT, consistent with previous reports, while LHCSR3 expression was significantly reduced in *phot*. These results confirm that LHCSR3 expression is compromised in our *phot* mutant under high light conditions.

After conducting a series of experiments, we confirmed the successful disruption of PHOT in the *phot* mutant, which exhibited high light sensitivity. This was further validated by western blot and RT-qPCR (**Figure 7 & 8**). Therefore, we can confidently proceed with using this mutant for the next step—UV mutagenesis.

### 3.2. UV mutagenesis

UV light induces DNA damage by causing chemical changes, particularly the formation of Pyrimidine dimers, such as thymine dimers, between adjacent bases. These lesions disrupt normal DNA replication and transcription, leading to mutations. When the cell attempts to repair this damage, errors can occur, resulting in permanent genetic mutations. The specific steps for UV mutagenesis are as follows:

1. The *phot* mutant is transferred into liquid TAP medium and cultured for 5 days. The cell concentration in the medium reaches 60 million cells/ml, with an OD680 value of 0.537.

2. 10 ml of each *phot* mutant culture is placed in separate triangular flasks. The flasks are positioned in the UV gel imaging system, using the ChampGe16000 machine from Beijing Sage Creation. The UV light source is located 30 cm directly above the flasks, emitting light at 3.0^18 W. Each flask is exposed to UV light for varying durations: 0, 3, 5, 10, and 15 minutes.

3. After UV mutagenesis, the cell pellets were collected by centrifugation at 2000 rpm for 5 minutes and then placed under low light conditions to grow overnight.

4. After overnight incubation, 100 µl of culture from each treatment was taken and spread onto HSM agar plates (**Figure 9**). The plates were then placed under high light conditions with an intensity of 1000 µE for one week of screening.

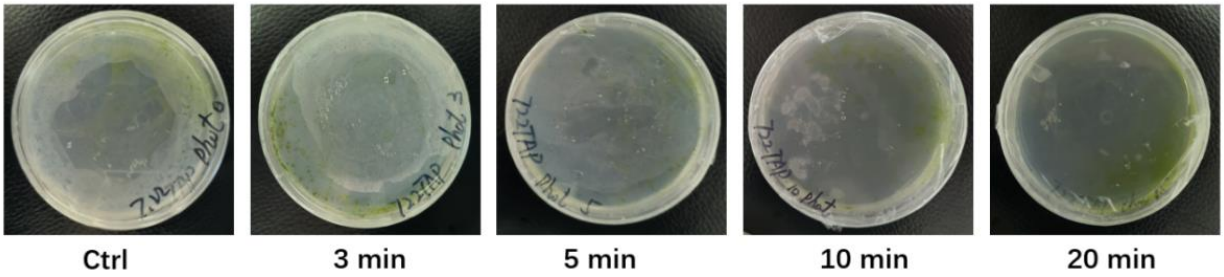



**Figure 9.** HSM agar plates after UV mutagenesis. UV light exposure times indicated for each plate.

5. After one week, each plate showed a certain number of transformants that survived the high light exposure. All plates had a similar number of surviving transformants, except for the *phot 15 min* plate, which exhibited a higher number of transformants. These mutants had undergone 15 minutes of UV irradiation

### 3.3. Colonies test

To confirm the success of UV mutagenesis, in the previous section, we transferred all the cells onto a new TAP agar plate and made two copies on HSM agar plates. These two HSM plates were then placed under 1000 µE light conditions.

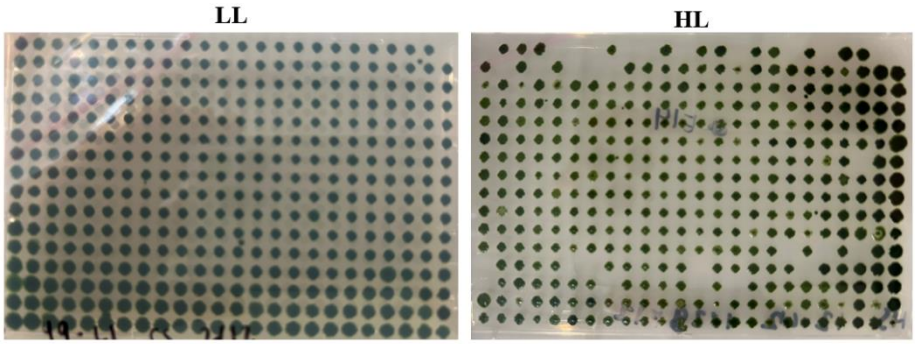



**Figure 10.** Transformant testing under low light (LL) and high light (HL) conditions on HSM agar plates.

After 48 hours of high light treatment, all colonies grew well under low light conditions. However, despite being selected from high light screening, some mutants were unable to grow under high light conditions (**Figure 10**). This could be due to uneven light distribution in the experimental

environment, where certain localized areas had lower light intensity. Nonetheless, more than 90% of the colonies survived under high light conditions, indicating that the mutant screening was successful.

Although all these colonies originated from the phot background and survived under high light conditions, this does not necessarily mean that the disrupted genes in each colony are all involved in the PHOT-dependent photoprotection pathway. It is possible that genes in other pathways might compensate for the reduced LHCSR3 expression in *phot* mutant, allowing them to survive under high light conditions. To prepare samples for omics analysis, a few colonies were picked, and their LHCSR3 expression was checked under the same conditions used in **Figure 8**.

From all the colonies, we selected three for further analysis, with one colony, named *phot15*, standing out.

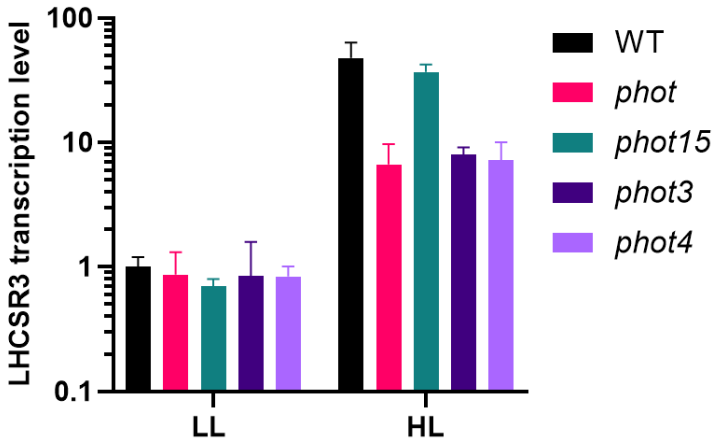



**Figure 11.** The LHCSR3 expression level in WT, *phot, phot3, phot4* and *phot15* under high light conditions. T0, 0h; T1, 1h high light treatment.

As shown in **Figure 11**, under low light conditions, all mutants displayed similarly low transcription levels of *LHCSR3*. Under high light conditions, *LHCSR3* transcription was significantly upregulated in the WT, while it remained low in the *phot* mutant. Among the selected colonies, *phot3* and *phot4* exhibited *LHCSR3* levels similar to *phot*, suggesting that in these two mutants, some non-PHOT-dependent photoprotection-related genes were disrupted, compensating for the low LHCSR3 levels in *phot*. In contrast, phot15 showed a much higher *LHCSR3* expression level, close to that of the WT. This suggests that the gene(s) mutated in *phot15* are involved in the PHOT-dependent photoprotection pathway. As a result, *phot15* was chosen for further omics analysis.

### 3.4. Omics analysis

For omics analysis, we collected sufficient *phot15* cells and extracted DNA, RNA, and protein, along with samples from WT and *phot*, under the same conditions. We first identified all the genes that were mutated exclusively in phot15, but not in WT or phot, resulting in a total of 1778 genes. After we excluded genes that were detected in either the transcriptome or proteome and genes that exhibited opposite expression trends in the transcriptome and proteome, the gene list was narrowed down to only 8 genes, as shown in **Table. 1**.

**Table 1.** Candidate gene list identified in this project.

| Gene ID | Description | mRNA level | Protein level |
|---|---|---|---|
| Cre07.g330900 | Endonuclease/exonuclease/phosphatase | UP | UP |
| Cre08.g364550 | ubiquitin-protein ligase E3 A | DOWN | DOWN |
| Cre09.g405100 | unknown | UP | UP |
| Cre10.g458350 | Scavenger receptor cysteine rich (SRCR) protein | DOWN | DOWN |
| Cre11.g483351 | Putative pherophorin-chlamydomonas homolog | DOWN | DOWN |
| Cre12.g505050 | unknown | DOWN | DOWN |
| Cre16.g693202 | Ferredoxin-sulfite reductase | DOWN | DOWN |
| Cre18.g750047 | Flagella Membrane Glycoprotein 1A | DOWN | DOWN |

These genes are promising candidates for further research into proteins involved in the PHOT-dependent photoprotection pathway. Individual genes can be isolated through cross-mating, or mutants of these genes can be ordered from the CLiP library [40] of for future studies. We also conducted a basic functional analysis of these genes, which will be discussed in the following discussion section.

## 4. Discussion

After further filtering out genes that exhibited incongruent expression trends between transcription and protein levels, we narrowed the list down to 8 candidate genes, all of which showed consistent regulation at both the RNA and protein levels.

The 8 candidate genes identified through omics analysis represent diverse functions, potentially linked to various cellular processes:

*1. Cre08.g364550* (Ubiquitin-protein ligase E3): This gene was significantly downregulated in the *phot15* mutant. Ubiquitin-protein ligases are crucial in protein degradation via the ubiquitin-proteasome system, regulating the stability of many cellular proteins. In *C. reinhardtii*, the E3 ubiquitin ligase complex CUL4-DDB1-DET1 is known to regulate photoprotection by suppressing photoprotective gene expression under low light conditions [33]. PHOT signaling inhibits this complex under high light, allowing proteins like LHCSR3 to accumulate for protection. The E3 complex SPA1-COP1-DDB1 is also involved in HL and UV induced photo protection [34]. There may be additional E3 ubiquitin ligases involved in these pathways. The downregulation of this gene in *phot15* suggests a possible role in light acclimation and stress response, making it a strong candidate for further investigation.

*2. Cre18.g750047* (Flagella membrane glycoprotein 1A): FMG1A is one of two paralogous genes in *C. reinhardtii* encoding glycoproteins associated with the flagellar membrane[41]. FMG1B is found predominantly in the ciliary membrane and is essential for force transduction and surface motility, acting as a key player in cilia's interaction with substrates and the surrounding environment. It also participates in the process of mating by helping with ciliary adhesion between gametes[41]. While FMG1B is well-characterized, little is known about FMG1A. FMG1A shares about 60% sequence identity with FMG1B. However, no direct evidence of FMG1A being transcribed or translated in *C. reinhardtii* has been found. Interestingly, in our study, FMG1A was highly upregulated in *phot* compared to WT at both the transcriptional and protein levels, but this upregulation was reversed in the *phot15* mutant. This suggests that FMG1A may play an important inhibitory role in high light protection, making it a promising target for future research.

The identification of these candidate genes opens new possibilities for understanding the regulation of photoprotection in Chlamydomonas and other photosynthetic organisms. The PHOT-LHCSR3 pathway is a well-established component of the qE response, but our findings suggest that alternative pathways may also compensate for the loss of PHOT under certain conditions. The upregulation of LHCSR3 in the phot15 mutant, despite the absence of functional PHOT, indicates that these alternative pathways can effectively mediate photoprotection.

## References

[1] M. Schmidt et al., "Proteomic analysis of the eyespot of Chlamydomonas reinhardtii provides novel insights into its components and tactic movements," (in eng), Plant Cell, vol. 18, no. 8, pp. 1908-30, Aug 2006, doi: 10.1105/tpc.106.041749.

[2] S. S. Merchant et al., "The Chlamydomonas genome reveals the evolution of key animal and plant functions," (in eng), Science, vol. 318, no. 5848, pp. 245-50, Oct 12 2007, doi: 10.1126/science.1143609.

[3] K. Baek et al., "DNA-free two-gene knockout in Chlamydomonas reinhardtii via CRISPR-Cas9 ribonucleoproteins," Sci Rep, vol. 6, p. 30620, Jul 28 2016, Art no. 30620, doi: 10.1038/srep30620.

[4] A. Greiner, S. Kelterborn, H. Evers, G. Kreimer, I. Sizova, and P. Hegemann, "Targeting of Photoreceptor Genes in Chlamydomonas reinhardtii via Zinc-Finger Nucleases and CRISPR/Cas9," Plant Cell, vol. 29, no. 10, pp. 2498-2518, Oct 2017, doi: 10.1105/tpc.17.00659.

[5] X. Johnson and J. Alric, "Central carbon metabolism and electron transport in Chlamydomonas reinhardtii: metabolic constraints for carbon partitioning between oil and starch," Eukaryot Cell, vol. 12, no. 6, pp. 776-93, Jun 2013, doi: 10.1128/EC.00318-12.

[6] S. Wakao and K. K. Niyogi, "Chlamydomonas as a model for reactive oxygen species signaling and thiol redox regulation in the green lineage," Plant Physiol, vol. 187, no. 2, pp. 687-698, Oct 5 2021, doi: 10.1093/plphys/kiab355.

[7] S. Wakao et al., "Discovery of photosynthesis genes through whole-genome sequencing of acetate-requiring mutants of Chlamydomonas reinhardtii," PLoS Genet, vol. 17, no. 9, p. e1009725, Sep 2021, doi: 10.1371/journal.pgen.1009725.

[8] M. I. Khan, J. H. Shin, and J. D. Kim, "The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products," Microbial Cell Factories, vol. 17, no. 1, p. 36, 2018/03/05 2018, doi: 10.1186/s12934-018-0879-x.

[9] A. Onodera et al., "Phototropin from Chlamydomonas reinhardtii is functional in Arabidopsis thaliana," Plant Cell Physiol, vol. 46, no. 2, pp. 367-74, Feb 2005, doi: 10.1093/pcp/pci037.

[10] S. Sasso, H. Stibor, M. Mittag, and A. R. Grossman, "From molecular manipulation of domesticated Chlamydomonas reinhardtii to survival in nature," eLife, vol. 7, p. e39233, 2018/11/01 2018, doi: 10.7554/eLife.39233.

[11] P. Muller, X. P. Li, and K. K. Niyogi, "Non-photochemical quenching. A response to excess light energy," Plant Physiol, vol. 125, no. 4, pp. 1558-66, Apr 2001, doi: 10.1104/pp.125.4.1558.

[12] E. H. Murchie and K. K. Niyogi, "Manipulation of photoprotection to improve plant photosynthesis," Plant Physiol, vol. 155, no. 1, pp. 86-92, Jan 2011, doi: 10.1104/pp.110.168831.

[13] P. Redekop et al., "PsbS contributes to photoprotection in Chlamydomonas reinhardtii independently of energy dissipation," Biochim Biophys Acta Bioenerg, vol. 1861, no. 5-6, p. 148183, Jun 1 2020, doi: 10.1016/j.bbabio.2020.148183.

[14] E. Dinc, L. Tian, L. M. Roy, R. Roth, U. Goodenough, and R. Croce, "LHCSR1 induces a fast and reversible pH-dependent fluorescence quenching in LHCII in Chlamydomonas reinhardtii cells," Proc Natl Acad Sci U S A, vol. 113, no. 27, pp. 7673-8, Jul 5 2016, doi: 10.1073/pnas.1605380113.

[15] K. Kosuge et al., "LHCSR1-dependent fluorescence quenching is mediated by excitation energy transfer from LHCII to photosystem I in Chlamydomonas reinhardtii," Proc Natl Acad Sci U S A, vol. 115, no. 14, pp. 3722-3727, Apr 3 2018, doi: 10.1073/pnas.1720574115.

[16] G. Peers et al., "An ancient light-harvesting protein is critical for the regulation of algal photosynthesis," Nature, vol. 462, no. 7272, pp. 518-21, Nov 26 2009, doi: 10.1038/nature08587.

[17] L. Girolomoni, S. Cazzaniga, A. Pinnola, F. Perozeni, M. Ballottari, and R. Bassi, "LHCSR3 is a nonphotochemical quencher of both photosystems in Chlamydomonas reinhardtii," Proc Natl Acad Sci U S A, vol. 116, no. 10, pp. 4212-4217, Mar 5 2019, doi: 10.1073/pnas.1809812116.

[18] D. Petroutsos et al., "The chloroplast calcium sensor CAS is required for photoacclimation in Chlamydomonas reinhardtii," Plant Cell, vol. 23, no. 8, pp. 2950-63, Aug 2011, doi: 10.1105/tpc.111.087973.

[19] A. Kianianmomeni and A. Hallmann, "Algal photoreceptors: in vivo functions and potential applications," Planta, vol. 239, no. 1, pp. 1-26, Jan 2014, doi: 10.1007/s00425-013-1962-5.

[20] [20] F. W. Li and S. Mathews, "Evolutionary aspects of plant photoreceptors," J Plant Res, vol. 129, no. 2, pp. 115-22, Mar 2016, doi: 10.1007/s10265-016-0785-4.

[21] T. Ziegler and A. Möglich, "Photoreceptor engineering," (in English), Frontiers in Molecular Biosciences, Review vol. 2, 2015-June-17 2015, doi: 10.3389/fmolb.2015.00030.

[22] J. M. Christie et al., "Arabidopsis NPH1: a flavoprotein with the properties of a photoreceptor for phototropism," (in eng), Science, vol. 282, no. 5394, pp. 1698-701, Nov 27 1998, doi: 10.1126/science.282.5394.1698.

[23] D. Petroutsos, "Chlamydomonas Photoreceptors: Cellular Functions and Impact on Physiology," in Chlamydomonas: Biotechnology and Biomedicine, M. Hippler Ed., (Microbiology Monographs. Cham: Springer International Publishing, 2017, ch. Chapter 1, pp. 1-19.

[24] M. Heijde and R. Ulm, "UV-B photoreceptor-mediated signalling in plants," Trends Plant Sci, vol. 17, no. 4, pp. 230-7, Apr 2012, doi: 10.1016/j.tplants.2012.01.007.

[25] K. Tilbrook et al., "UV-B Perception and Acclimation in Chlamydomonas reinhardtii," Plant Cell, vol. 28, no. 4, pp. 966-83, Apr 2016, doi: 10.1105/tpc.15.00287.

[26] G. Allorent et al., "UV-B photoreceptor-mediated protection of the photosynthetic machinery in Chlamydomonas reinhardtii," Proc Natl Acad Sci U S A, vol. 113, no. 51, pp. 14864-14869, Dec 20 2016, doi: 10.1073/pnas.1607695114.

[27] E. Liscum, S. K. Askinosie, D. L. Leuchtman, J. Morrow, K. T. Willenburg, and D. R. Coats, "Phototropism: Growing towards an Understanding of Plant Movement," The Plant Cell, vol. 26, no. 1, pp. 38-55, 2014, doi: 10.1105/tpc.113.119727.

[28] J. M. Christie, M. Salomon, K. Nozue, M. Wada, and W. R. Briggs, "LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): binding sites for the chromophore flavin mononucleotide," Proc Natl Acad Sci U S A, vol. 96, no. 15, pp. 8779-83, Jul 20 1999, doi: 10.1073/pnas.96.15.8779.

[29] J. M. Christie, "Phototropin blue-light receptors," Annu Rev Plant Biol, vol. 58, no. 1, pp. 21-45, 2007/06/01 2007, doi: 10.1146/annurev.arplant.58.032806.103951.

[30] D. Petroutsos et al., "A blue-light photoreceptor mediates the feedback regulation of photosynthesis," Nature, vol. 537, no. 7621, pp. 563-566, Sep 22 2016, doi: 10.1038/nature19358.

[31] Y. T. Jiang et al., "Vacuolar H(+)-ATPase and BZR1 form a feedback loop to regulate the homeostasis of BR signaling in Arabidopsis," Mol Plant, Oct 12 2023, doi: 10.1016/j.molp.2023.10.007.

[32] M. Águila Ruiz-Sola et al., "Light-independent regulation of algal photoprotection by CO2 availability," Nature Communications, vol. 14, no. 1, 2023, doi: 10.1038/s41467-023-37800-6.

[33] Y. Aihara, K. Fujimura-Kamada, T. Yamasaki, and J. Minagawa, "Algal photoprotection is regulated by the E3 ligase CUL4-DDB1(DET1)," Nat Plants, vol. 5, no. 1, pp. 34-40, Jan 2019, doi: 10.1038/s41477-018-0332-5.

[34] S. T. Gabilly et al., "Regulation of photoprotection gene expression in Chlamydomonas by a putative E3 ubiquitin ligase complex and a homolog of CONSTANS," Proc Natl Acad Sci U S A, vol. 116, no. 35, pp. 17556-17562, Aug 27 2019, doi: 10.1073/pnas.1821689116.

[35] P. Redekop, E. Sanz-Luque, Y. Yuan, G. Villain, D. Petroutsos, and A. R. Grossman, "Transcriptional regulation of photoprotection in dark-to-light transition-More than just a matter of excess light energy," Sci Adv, vol. 8, no. 22, p. eabn1832, Jun 3 2022, doi: 10.1126/sciadv.abn1832.

[36] D. Lu, Y. Zhang, A. Zhang, and C. Lu, "Non-Photochemical Quenching: From Light Perception to Photoprotective Gene Expression," Int J Mol Sci, vol. 23, no. 2, Jan 8 2022, doi: 10.3390/ijms23020687.

[37] R. Tokutsu, K. Fujimura-Kamada, T. Matsuo, T. Yamasaki, and J. Minagawa, "The CONSTANS flowering complex controls the protective response of photosynthesis in the green alga Chlamydomonas," Nat Commun, vol. 10, no. 1, p. 4099, Sep 10 2019, doi: 10.1038/s41467-019-11989-x.

[38] J. Trippens et al., "Phototropin influence on eyespot development and regulation of phototactic behavior in Chlamydomonas reinhardtii," Plant Cell, vol. 24, no. 11, pp. 4687-702, Nov 2012, doi: 10.1105/tpc.112.103523.

[39] D. Strenkert et al., "Multiomics resolution of molecular events during a day in the life of Chlamydomonas," Proc Natl Acad Sci U S A, vol. 116, no. 6, pp. 2374-2383, Feb 5 2019, doi: 10.1073/pnas.1815238116.

[40] X. Li et al., "A genome-wide algal mutant library and functional screen identifies genes required for eukaryotic photosynthesis," (in English), Nat Genet, vol. 51, no. 4, pp. 627-635, Apr 2019, doi: 10.1038/s41588-019-0370-6.

[41] R. A. Bloodgood, "Chapter 10 - The Chlamydomonas ciliary membrane and its dynamic properties," in The Chlamydomonas Sourcebook (Third Edition), S. K. Dutcher Ed.: Academic Press, 2023, pp. 247-271.