ARTICLE

Supporting information for: The cyanobacterial phytochrome 2 regulates the expression of motility-related genes through the second messenger cyclic di-GMP

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Thomas Wallner,[a] Laura Pedroza,[a,b] Karsten Voigt,[a] Volkhard Kaever[c] and Annegret Wilde*[a]

Supplemental methods and material

Physiology

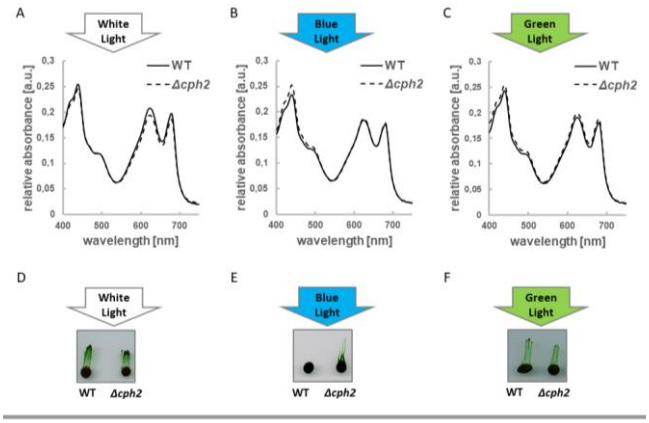

Figure S1 **Whole cell absorption spectra and phototactic behaviour of *Synechocystis* 6803 WT and *Δcph2* cells in response to different light qualities.** A-C Cells were grown mixotrophically with 11 mM glucose under continuous illumination of either 10 µmol photons m^{-2} s^{-1} of white light (A), 5 µmol photons m^{-2} s^{-1} of blue light (Lee filters #119) (B) or 5 µmol photons m^{-2} s^{-1} of green light (Lee filters #089) (C), respectively. Spectra were recorded with cells adjusted to the same $OD_{750\,nm}$ in a spectrophotometer equipped with an integrating sphere (Shimadzu UV2450 PC). D-E Cells of *Synechocystis* 6803 WT and *Δcph2* were spotted onto BG11 agar plates (0.5% (w/v)) supplemented with 11 mM glucose and 10 mM TES buffer (pH 8.0) and placed under diffuse white light (50 µmol photons m^{-2} s^{-1}) for 24 h. Afterwards the plates were illuminated unidirectional with 5 µmol photons m^{-2} s^{-1} of white (D), blue (E) or green light (F), respectively.

Supporting data sets

Supporting data set 1 (pdf-file). Genome-wide visualization of the blue light microarray results. The microarray compares the transcriptomes of *Synechocystis* 6803 WT and *Δcph2* cells grown mixotrophically in the presence of 11 mM glucose under 5 µmols of photons m^{-2} s^{-1} of blue light (Lee filter #119, dark blue). Both strands are shown with the location of annotated genes (blue), 5´-UTRs and 3´-UTRs (white), internal sense RNA transcripts (light blue), antisense RNAs (red) and potential non-coding RNAs (yellow). The read numbers from an RNAseq experiment [1] derived from cells grown in exponential (dark grey) or stationary phases (light grey) are given in a log_2 scale. The microarray expression values for WT (WT, light blue) and *Δcph2* cells (delta_cph2, red) are also given in a log_2 scale.

Supporting data Table 1 (excel-spreadsheet). Microarray dataset for *Synechocystis* 6803 WT and the *Δcph2* mutant grown at 5 µmols of photons m^{-2} s^{-1} of blue light supplemented with 11 mM glucose. The table reports log_2 fold changes (FC) of transcripts in the *Δcph2* mutant relative to the WT (stated in the column header). Features are separated into mRNAs, antisense RNAs (asRNAs), potentially trans-encoded non-coding RNAs (ncRNAs), 5'-UTRs and transcripts derived from internal (within CDS) transcriptional start sites (int). Statistical significance of a FC is assigned in an additional column (1/-1 = significant, 0 = not significant). Fold changes were regarded as significant if the log_2 value was ≤ -0.8 or ≥ 0.8 and the corresponding adjusted p-value ≤ 0.05. Shades of blue and green colour indicate the most differentially expressed transcript that met our significance criteria.

Supporting data set 2 (pdf-file). Genome-wide visualization of the green light microarray results. The microarray compares the transcriptomes of *Synechocystis* 6803 WT and *Δcph2* cells grown mixotrophically in the presence of 11 mM glucose under 5 µmols of photons m^{-2} s^{-1} of green light (Lee filter #089, moss green). The visualization of genomic features is the same as in supplemental data set 1. The microarray expression values for WT (WT, light blue) and *Δcph2* cells (cph2, red) are also given in a log_2 scale.

Supporting Data Table 2 (excel spreadsheet). Microarray dataset for *Synechocystis* 6803 WT and the *Δcph2* mutant

[a] *Molecular Genetics of Prokaryotes, Institute of Biology III, University of Freiburg, Freiburg, Germany*
[b] *current address: Microbiology II, Center of Medical Biotechnology, Faculty of Biology, University Duisburg-Essen, Essen, Germany*
[c] *Research Core Unit Metabolomics, Hannover Medical School, Hannover, Germany*
*To whom correspondence should be addressed

grown at 5 µmols of photons m^{-2} s^{-1} of green light supplemented with 11 mM glucose. The table reports log_2 FC of transcripts in the *Δcph2* mutant relative to the WT (stated in the column header). Features are separated into mRNAs, asRNAs, potentially trans-encoded ncRNAs, 5'-UTRs and transcripts derived from internal (within CDS) transcriptional start sites (int). Statistical significance of a FC is assigned in an additional column (1/-1 = significant, 0 = not significant). Foldchanges were regarded as significant if the log2 value was ≤ -0.8 or ≥ 0.8 and the corresponding adjusted p-value ≤ 0.05. Shades of blue and green colour indicate the most differentially expressed transcripts that met our significance criteria.

Supporting data set 3 (pdf-file). Genome-wide visualization of the cellular response of WT and *Δcph2* cells to blue or green light. The microarray compares the transcriptomes of *Synechocystis* 6803 WT cells or *Δcph2* cells grown mixotrophically in the presence of 11 mM glucose under 5 µmols of photons m^{-2} s^{-1} of either blue or green light (Lee filter #119 or #089; dark blue or moss green). The visualization of genomic features is the same as in supplemental data set 1. The microarray expression values for WT cells (WT_b, dark blue) and *Δcph2* cells (d_cph2_b, violet) grown under blue light or green light (WT_g, dark green and d_cph2_g, light green) are given in a log_2 scale.

Supporting Data Table 3 - Microarray dataset for *Synechocystis* 6803 WT or the Δ*cph2* mutant grown at 5 µmols of 5 µmols of photons m^{-2} s^{-1} of either blue or green light, supplemented with 11 mM glucose. The table reports log_2 FC of transcripts in the WT or *Δcph2* mutant after blue light treatment relative to the green light treatment (stated in the column header). Features are separated into mRNAs, potential asRNAs, potentially trans-encoded ncRNAs, 5'-UTRs and transcripts derived from internal (within CDS) transcriptional start sites (int). Statistical significance of the respective FCs is assigned in additional columns (1/-1 = significant, 0 = not significant). Fold changes were regarded as significant if the log_2 value was ≤ -0.8 or ≥ 0.8 and the corresponding adjusted p-value ≤ 0.05. Shades of blue and green color indicate the most differentially expressed features.

Mutagenesis

The discistronic operon *pilA5-pilA6* (gene locus *slr1924-slr1924* [2]; transcriptional unit (TU) TU2300 according to Kopf *et al*. (2014)[1]) was inactivated by replacing the two coding sequences of *pilA5* and *pilA6* with a chloramphenicol resistance cassette via homologous recombination (**supporting Figure S2A**). Therefore, an upstream DNA fragment (600 bp) and a downstream DNA fragment (583 bp) were amplified from genomic DNA of *Synechocystis* 6803 WT by polymerase chain reaction (PCR) using the primer pairs P1 and P2 or P3 and P4 (sequences are listed in the **supporting information table S1**), respectively. Primer P2 added NsiI and SalI recognition sequences to the 3´-end of the upstream element replacing the original start codon of the *pilA5* gene. Primer P3 added NsiI and a SalI recognition sequences to the 5´-end of the downstream element substituting the original stop codon of the *pilA6* gene. Both fragments were fused together by PCR with primer pair P1 and P4 and subsequently cloned into the pJET1.2 vector (Thermo Scientific, Germany), yielding pJET-US-DS-TU2300.

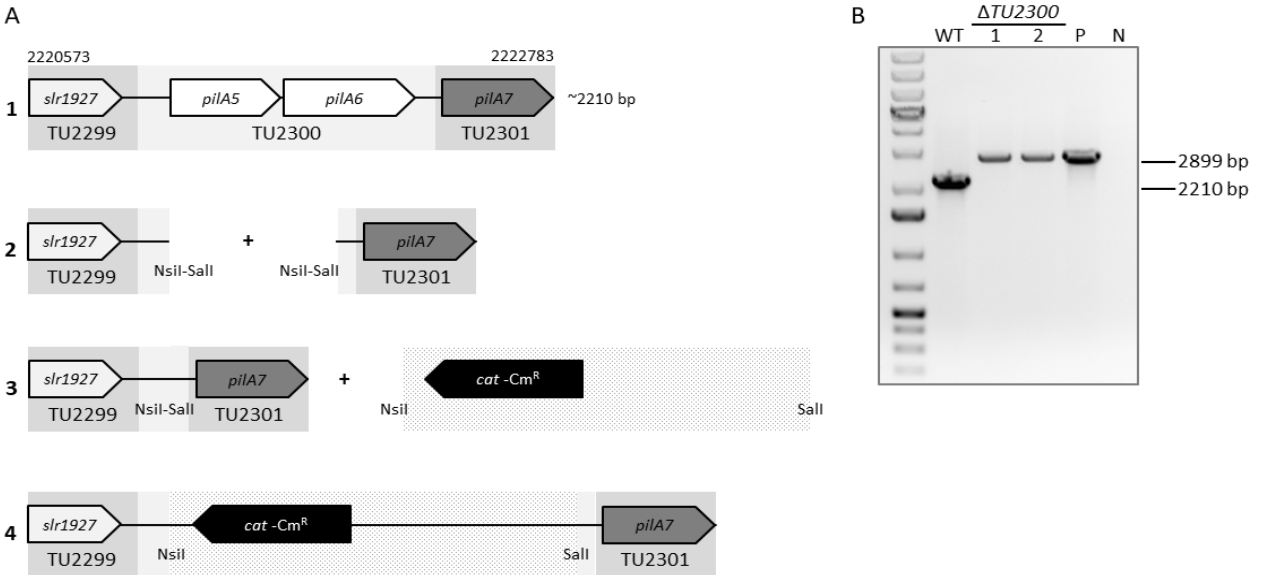

Figure S2. Schematic representation and verification of the inactivation of the *pilA5-pilA6* operon, (Δ*pilA5-pilA6*) A1 Gene loci of the *pilA5-pilA6* operon and the adjacent genes *slr1927 and pilA7*. Arrows indicate the length and orientation of the genes. The dark and light grey boxes represent the transcriptional units according to Kopf *et al*. (2014)[2]. **A2** An upstream fragment, containing the neighboring *slr1927* gene and a downstream fragment including *pilA7* were generated by PCR from genomic DNA using primer pairs P1 and P2 or P3 and P4, respectively (**supporting information table S1**). **A3** The fusion of both fragments was achieved by overlapping PCR using primers P1 and P4. The cassette (dotted box) containing the chloramphenicol resistance gene was amplified by PCR using primers P5 and P6. **A4** Final fragment used for the inactivation with the chloramphenicol resistance cassette inserted into the NsiI and SalI cleavage sites. **B** Verification of the complete segregation of the Δ*pilA5-pilA6* knockout via PCR using primer pair P1 and P4 and chromosomal DNA of the WT, of two individual transformants of Δ*pilA5-pilA6* and the plasmid DNA which was used for the transformation (P). Water serves as non-template control (N); the sizes of the fragments are indicated.

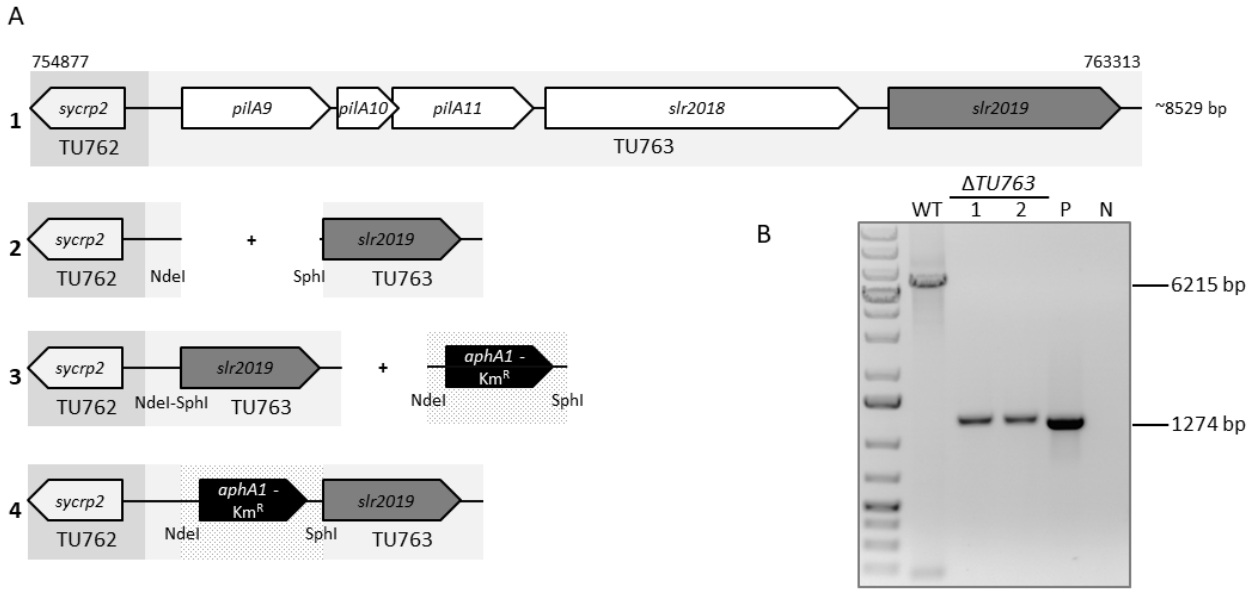

Figure S3. Schematic representation and verification of the inactivation of the *pilA9-slr2019* operon, (Δ*pilA9-slr2019*) A1 Gene arrangement of the *pilA9-slr2019* operon and the adjacent *sycrp2* gene. Arrows indicate the length and orientation of the genes. The dark and light grey boxes represent the transcriptional units according to Kopf *et al.* (2014)[2]. **A2** The upstream element contains the entire *sycrp2* gene and the intergenic region upstream of the *pilA9* start codon and was amplified by PCR from genomic DNA using primer pair P7 and P8 (**supporting information table S1**). The downstream element harboring the second half of *slr2019* was generated by PCR using primer pair P9 and P10. **A3** Both DNA fragments were fused by overlapping PCR using primer pair P7 and P10. The NdeI and SphI restriction sites were added to the cassette conferring resistance to kanamycin using primer pair P11 and P12. **A4** The kanamycin resistance cassette was inserted into the NdeI and SphI cleavage sites replacing TU763. **B** Verification of the complete segregation of the *pilA9-slr2019* operon knockout via PCR using primer pair P13 and P14 and chromosomal DNA of the wild type (WT), of two individual transformants of Δ*pilA9-slr2019* and the plasmid DNA which was used for the transformation (P). Water serves as non-template control (N); the sizes of the fragments are indicated.

The correctness of the DNA sequence was verified by sequencing. A cassette containing a chloramphenicol resistance gene was amplified by PCR from the pVZ321 vector [3] using primer pair P5 and P6, thereby adding a SalI recognition sequence to the 5´-end and a NsiI recognition sequence to the 3´-end of the cassette, respectively. The cassette was then ligated into the pJET-US-DS-TU2300 vector cut with NsiI and SalI. The final vector pJET-ΔTU2300-CmR-rev was used for transformation of the *Synechocystis* 6803 WT as described by Ermakova-Gerdes and W. Vermaas (1999) [4]. Transformants were re-streaked on BG11 agar plates with increasing concentrations of chloramphenicol starting from 1.4 µg ml^{-1} to final 7 µg ml^{-1}. Full segregation of the mutated genomic DNA fragment was validated by PCR using primer pair P1 and P4 (**supporting Figure S2B**).

The inactivation of the polycistronic operon *pilA9-pilA11-slr2019* (gene locus *slr2015-slr2019* [2]*;* TU763 according to Kopf *et al*. (2014)[1]) was carried out as described in Conradi *et al*. (2019)[5] using primers P7-P12 (**supporting Figure S3A**). The final vector pJET-ΔTU763-KmR-fw was used for transformation of the *Synechocystis* 6803 WT and transformants were re-streaked on BG11 agar plates with increasing concentrations of kanamycin starting from 1 µg ml^{-1} to final 40 µg ml^{-1}. The full segregation of the mutated DNA fragment was monitored and verified by PCR using primer pair P13 and P14 (**supporting Figure S3B**).

The inactivation of the gene *sycrp2* (locus *sll1924* [2]*;* TU762 according to [1]) was achieved by replacing almost the entire coding sequence of *sycrp2* with a chloramphenicol resistance cassette (**supporting Figure S4A**). The two DNA fragments were amplified using the primer pairs P15 and P16 or P17 and P18, respectively. The upstream fragment (1557 bp) covers the entire adjacent *slr2013* gene, the intergenic spacer between *slr2013* and *sll1924* as well as the last 91 bp of the 3´-end of *sycrp2*. Primer P15 added a SalI recognition sequences to the 3´-end just downstream of an intrinsic StuI recognition sequence. The downstream fragment (1647 bp) comprises the entire *slr2015* with the 450 bp long intergenic spacer between the *sll1924* and *slr2105* genes. Primer pair P17 and P18 was used for amplification, thereby adding the recognition sequences of StuI and SalI to the 5´-end of the downstream DNA fragment. Both fragments were fused together using the overlap during PCR with primer pair P15 and P18. The resulting US-DS-*sycrp2* DNA fragment (3128 bp) was cloned into the pJET1.2 vector yielding pJET-US-DS-*sycrp2* and the correctness of the DNA sequence verified. A cassette containing a chloramphenicol resistance gene was amplified by PCR from the pACYC184 vector [6] using primer pair P19 and P20, thereby adding a StuI and a SalI recognition sequence to the 5´-end and to the 3´-end of the cassette, respectively. The resulting DNA fragment was cleaved using StuI and SalI restriction enzymes and subsequently ligated into pJET-US-DS-*sycrp2* that was cut with the same enzymes. The final vector pJET-*Δsycrp2*-CmR-rev was used for transformation of the *Synechocystis* 6803 WT as described in penultimate section. The full segregation of the mutated DNA fragment was verified by PCR using primer pair P21 and P22 (**supporting Figure S4B**).

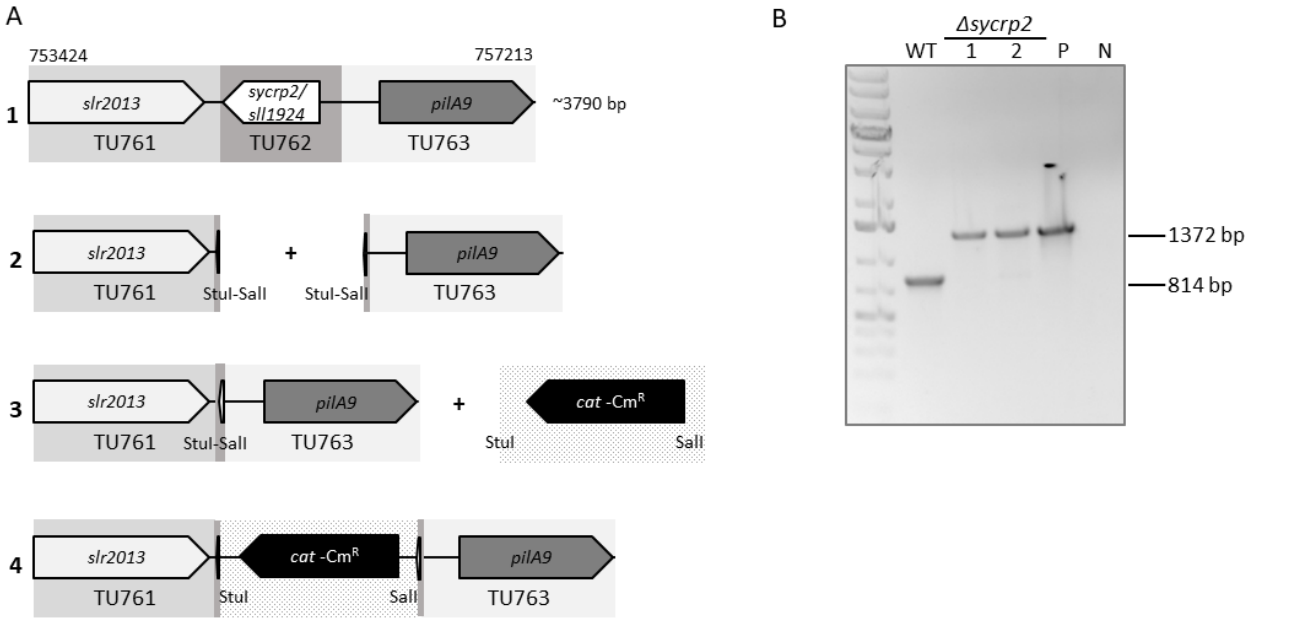

Figure S4. Schematic representation and verification of the inactivation of the *sycrp2 gene*, (*Δsycrp2*) A1 Genomic arrangement of the *sycrp2* gene and its adjacent genes *slr2013* and *pilA9*. Arrows indicate the length and orientation of the genes. Light, middle and dark grey boxes represent the transcriptional units according to Kopf *et al*. (2014)[2]. **A2** The upstream fragment containing the entire *slr2013* gene and the last 91 bp of the *sycrp2* open reading frame including an intrinsic StuI recognition sequence was amplified from genomic DNA using primer pairs P15 and P16 (**supporting information table S1**). Primer pair P17 and P18 was used to create a downstream element, that compromises *pilA9* and the intergenic spacer, and to add the StuI and SalI recognition sites. **A3** PCR-mediated fusion of the two fragments and chloramphenicol resistance cassette (dotted box) amplified by PCR using primer pair P19 and P20. **A4** Final construct used for inactivation including the chloramphenicol resistance cassette that was ligated into the StuI and SalI cleavage sites. **B** Verification of the complete segregation of the *sycrp2* knockout via PCR using primer pair P21 and P22 and chromosomal DNA of the wild type (WT), of two individual transformants of *Δsycrp2* and the plasmid DNA which was used for the transformation (P). Water serves as non-template control (N); the sizes of the fragments are indicated.

Complementation of *Δsycrp2* was achieved by expression of an N-terminal FLAG-tagged SyCRP2 from a modified pVZ321 conjugative plasmid [7]. The coding sequence of SyCRP2 was amplified from genomic DNA using primer pair P28 and P29 thereby replacing the original ATG start codon with an EcoRI recognition site and adding a BamHI recognition site directly after the stop codon (**supporting figure S5**). The PCR product was ligated into the pJET1.2 vector, excised using EcoRI and BamHI and subsequently ligated into a modified conjugative pVZ321 vector. The resulting pUR-N_FLAG-*sycrp2* plasmid was conjugated into *Δsycrp2* cells via tri-parental mating ensuing *Synechocystis* 6803 strain *Δsycrp2/sycrp2*$^{+}$ *that* allows expression of N-terminal FLAG-tagged SyCRP2 in a copper-dependent manner driven from the promoter of *Synechocystis* 6803 *petJ* gene[8].

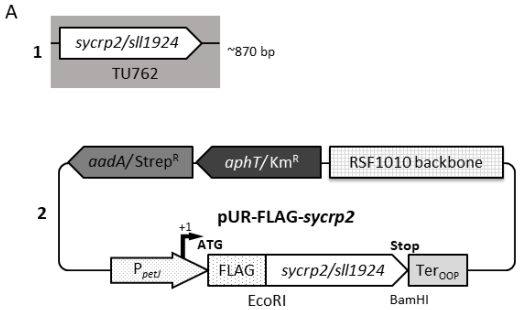

Figure S5. Schematic representation of the complementation of the *Δsycrp2 mutant*. A1 The *sycrp2* open reading frame was amplified from genomic DNA using primer pairs P28 and P29 (supplementary table S1) replacing the original start codon with an EcoRI restriction site sequence and adding a BamHI restriction site sequence downstream of the stop codon. The resulting PCR product was ligated into the pJET1.2 vector, excised using EcoRI and BamHI and subsequently ligated into a modified conjugative pVZ321 vector.[3,7]. **A2** The final construct pUR-FLAG-SyCPR2 was conjugated into *Δsycrp2* cells using tri-parental mating and allows expression of N-terminal FLAG-tagged SyCRP2 in a copper-dependent manner driven from the promoter of *Synechocystis* 6803 *petJ*[8].

Filter sets used in this study

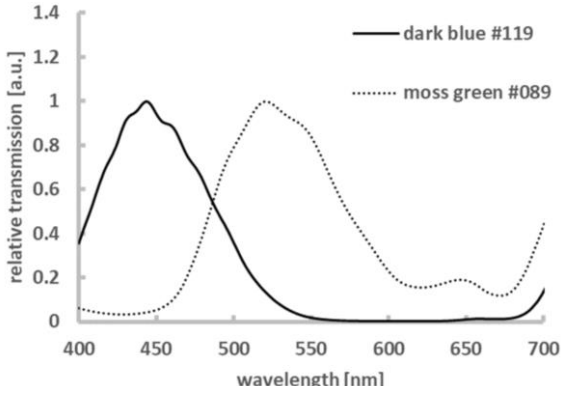

Figure S6. Transmission spectra of the filters used for the microarray experiment. Light transmission through the dark blue (solid line, Lee Filters #119) and moss green filter (dashed line, Lee filters #089) used for the microarray experiment. Transmission spectra were recorded in a spectrophotometer (Shimadzu UV2450 PC) and normalised to their respective transmission maximum.

Supporting information Table S1: Oligonucleotides used in this study

	Name	Sequence (5'-3')†	Purpose‡
P1	US-TU2300-fw	GGTCAGCAGAAATTTTTGAAG	MU, CP
P2	US-TU2300-NsiI-SalI	AA*GTCGAC*TT*ATGCAT*GGTGAAACCCTGCTGAG	MU
P3	DS-TU2300-NsiI-SalI-fw	CC*ATGCAT*AA*GTCGAC*TTTGTTAAGTAAAATTAAGTTTTGTGAATA	MU
P4	DS-TU2300-rev	GCCAAACAATAACTTACTGTGG	MU, CP
P5	SalI-cat-fw	TA*GTCGAC*GGCATCGTGGTGTCAC	MU
P6	NsiI-cat-rev	TA*ATGCAT*GAATTTCTGCCATTCATCC	MU
P7	US763-fw	TCAGATCGAAGTGGGATTGC	MU
P8	NdeI-US763-rev	*GCATGC*TA*CATATG*GGTCGATTGGGGTTTTTG	MU
P9	SphI-DS763-fw	*CATATG*TA*GCATGC*CTATCGGGACAGTAATC	MU
P10	DS763-rev	CATTGGGTAAGGGTAAGGATTTC	MU
P11	NdeI-KmR-fw	GC*CATATG*TTGTGTCTCAAAATCTCTGATG	MU
P12	KmR-fw-SphI	TA*GCATGC*TGAGGTCTGCCTCGTG	MU
P13	TU-763-Col-fw	CACGGTCTTTGCTGACTTC	CP
P14	TU-763-Col-rev	CCCGTTGATTACTGTCCC	CP
P15	US-slr2013-fw	GCCATGGTTCCCACTCTCC	MU
P16	SalI-StuI-rev	GCC*GTCGAC*AAAA*AGGCCT*GATTTTGC	MU
P17	StuI-SalI-fw	ATC*AGGCCT*TTTT*GTCGAC*GGCTTTTGTG	MU
P18	DW-slr2015-rev	GGTTGAGAATGTAGGAAACTATTTTTTACCC	MU
P19	StuI-CmR	C*AGGCCT*CAGGCATTTGAGAAG	MU
P20	SalI-CmR	GC*GTCGAC*AGCTGATAGAAACAGAAG	MU
P21	*sycrp2*-col-fw	CCAGCGGACTTTTCGACC	CP
P22	*sycrp2*-col-rev	GGGTTTGAGAGGGTCTAGGG	CP
P23	*slr1928-pilA5*-fw	CCCCAGGCTTTTATCTCTCG	PR
P24	T7-*slr1928-pilA5*-rev	**TAATACGACTCACTATAGGG**GGCGTCAGCACTATTGTTTG	PR
P25	*slr2015-pilA9*-fw	CTAGCGACCGATACCACCAT	PR
P26	T7-*slr2015/pilA9*-rev	**TAATACGACTCACTATAGGG**ACGCCGTTATTGCATTTTTC	PR
P27	5S-rRNA-oligo	GCATCGGACTATTGTGCCGTG	LC
P28	EcoRI-*sycrp2*-fw	AA*GAATTC*GCACCACAAAAGCCG	Co
P29	BamHI-*sycrp2*-rev	AA*GGATCC*TCAGATCGAAGTGGGATTG	Co

† Sequences of restriction endonucleases are italic and underlined; sequence of the T7 promoter is bold and underlined

‡ MU = mutagenesis; CP = control PCR to verify the full segregation of the mutated DNA fragment; PR = generation of template DNA for RNA probes including the T7 promoter; LC = control hybridisation to assure equal loading; Co = complementation

References

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