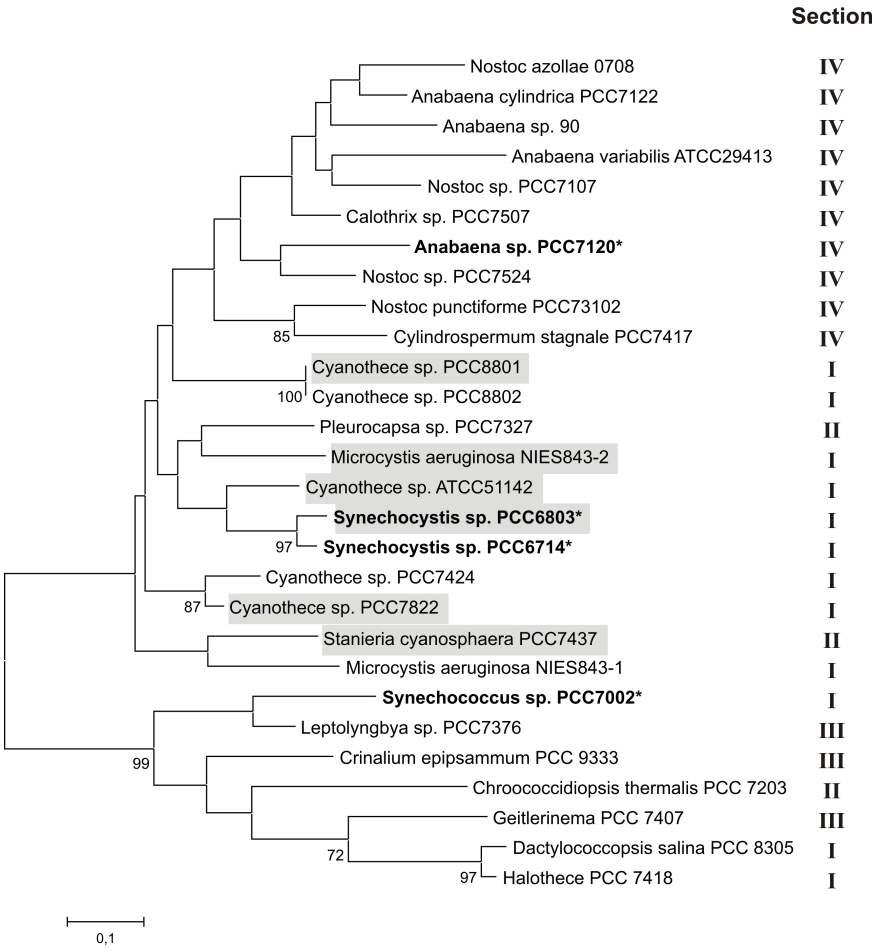
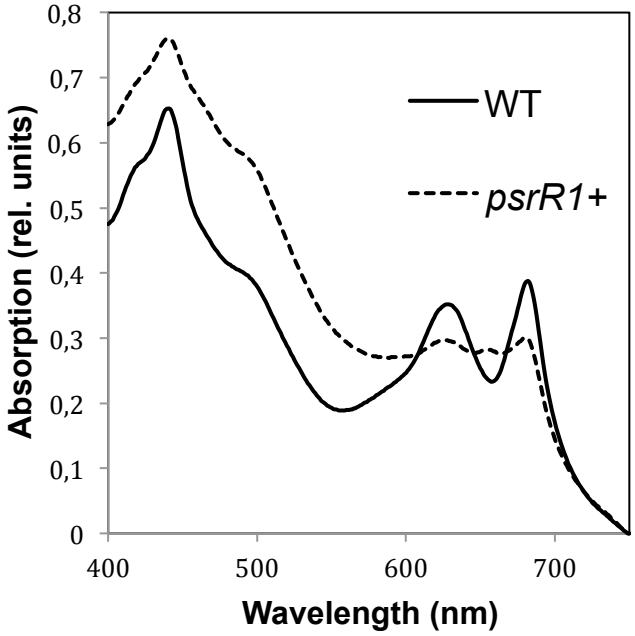


Supplemental Figure 1.

Visualization of the sequence relatedness among 28 PsrR1 homologs in an un-rooted neighbor-joining phylogenetic tree. Roman numerals indicate the cyanobacterial sections [3] to which the respective strain belongs to. The organisms chosen for the CopraRNA prediction are highlighted by a grey box, those for which transcriptome data have confirmed the presence of PsrR1, are in boldface letters and labelled by an asterisk. The optimal tree with the sum of branch length = 4.9822 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches when ≥70%. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. There were a total of 121 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [4].



Supplemental Figure 2.

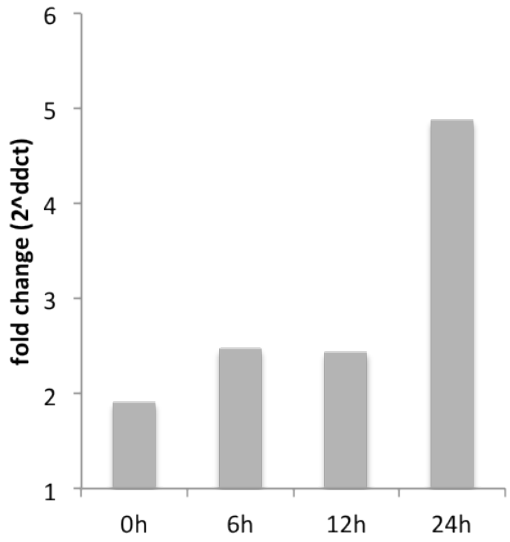
Absorption spectrum of the *psrR1*$^{+}$ over-expressor strain. Whole cell absorption spectra of wild-type and *psrR1*$^{+}$ mutant strains after five days of copper depletion. Under these conditions the PsrR1 sRNA is highly induced due to overexpression using the *petJ* promoter, whereas the wild type does not show bleaching.

Supplemental Figure 3.

Figure legend see next page.

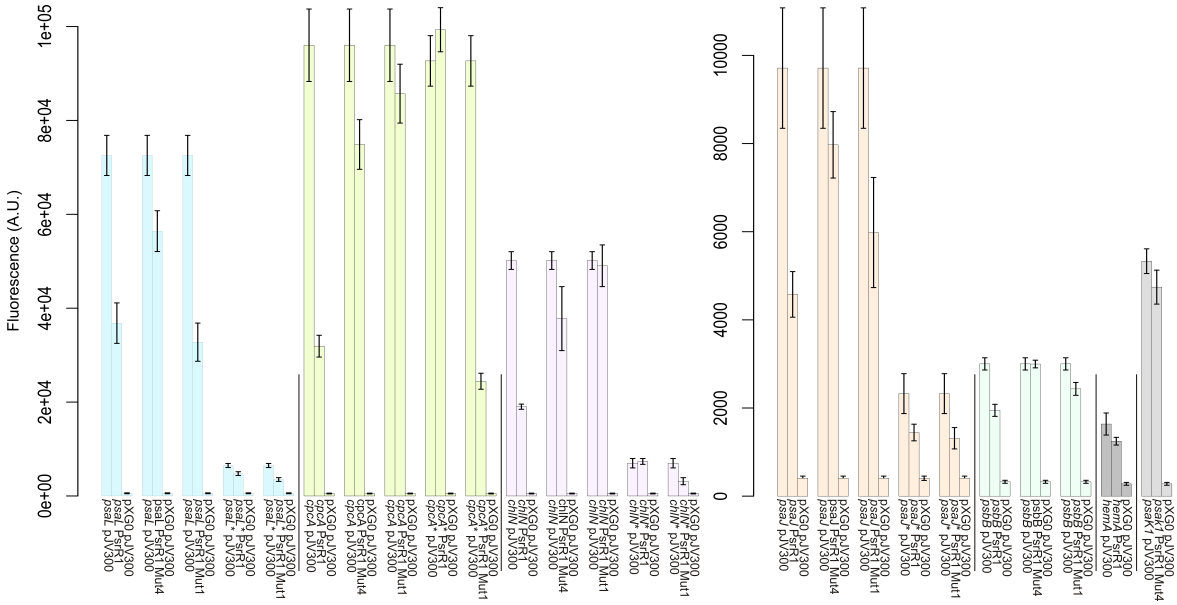
Supplemental Figure 3.

Visualization of functional enrichment of the PsrR1 target prediction by the DAVID webserver [5] as provided by CopraRNA [6]. The 60 targets of the top 85 list of the comparative CopraRNA prediction which have homologs in *Synechocystis* sp. PCC 6803 have been subjected to functional enrichment. The figure shows all members of the 11 clusters with a DAVID enrichment score ≥ 1 in specific color. Each row represents a gene and each column a specific functional term. If the gene can be assigned to a term, the corresponding square is filled/colored. Closely related terms are assigned to a cluster and have the same color. The opacity of the color depends on the p-value of the CopraRNA prediction. A more intense color represents a more significant p-value. The “Fold enrichment” gives the enrichment of a term in the prediction group in relation to the whole genome background (e.g. the PsrR1 prediction contains 6.51 times more genes belonging to the term “GO:0015979~photosynthesis” than the background). The enrichment scores give a measure of the biological significance of the cluster. The DAVID enrichment score for a cluster is the log transformed geometric mean of all enrichment p-values from the terms belonging to the respective cluster. A higher score represents a more statistically significant enrichment. The individual p-values for the terms are calculated by a modified Fisher's exact test. The length of the bars next to the groups of enriched genes corresponds to the size of the enrichment score. The publication on the DAVID webserver suggests to investigate clusters with an enrichment score of ≥ 1.3 [5].



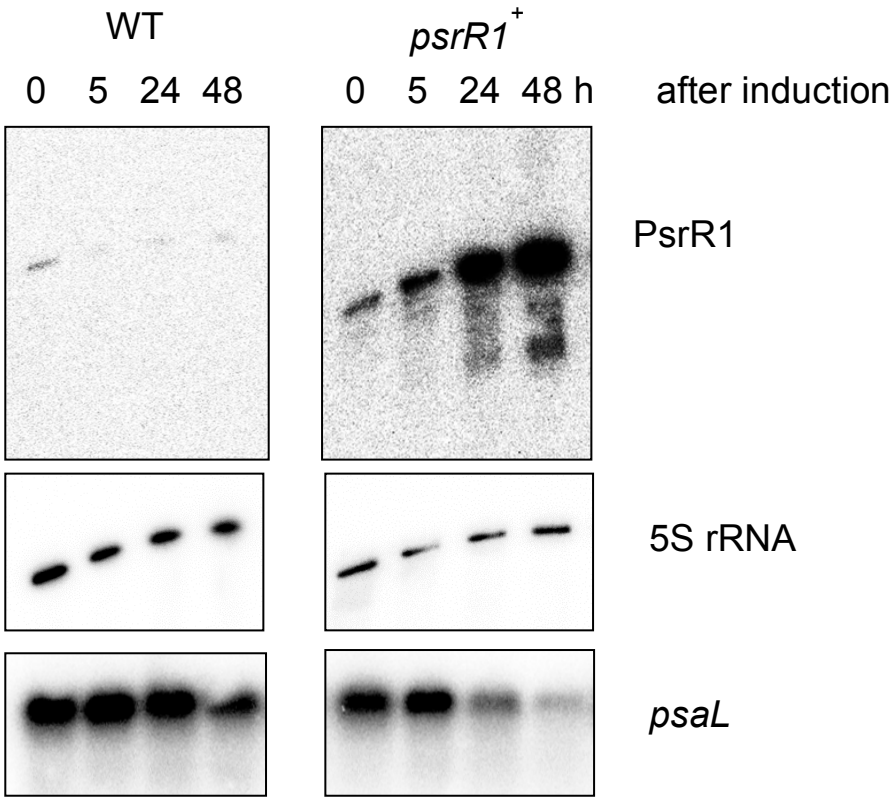
Supplemental Figure 4.

Induction pattern of PsrR1 under control of the *petJ* promoter in the *psrR1*$^{+}$ strain upon copper depletion. PsrR1 abundance was quantified in *psrR1*$^{+}$ and wild-type cell by qRT-PCR analysis using *rnpB* as an internal control. The amount of PsrR1 is expressed as a fold change relative to its level in the wild type under the same growth conditions.



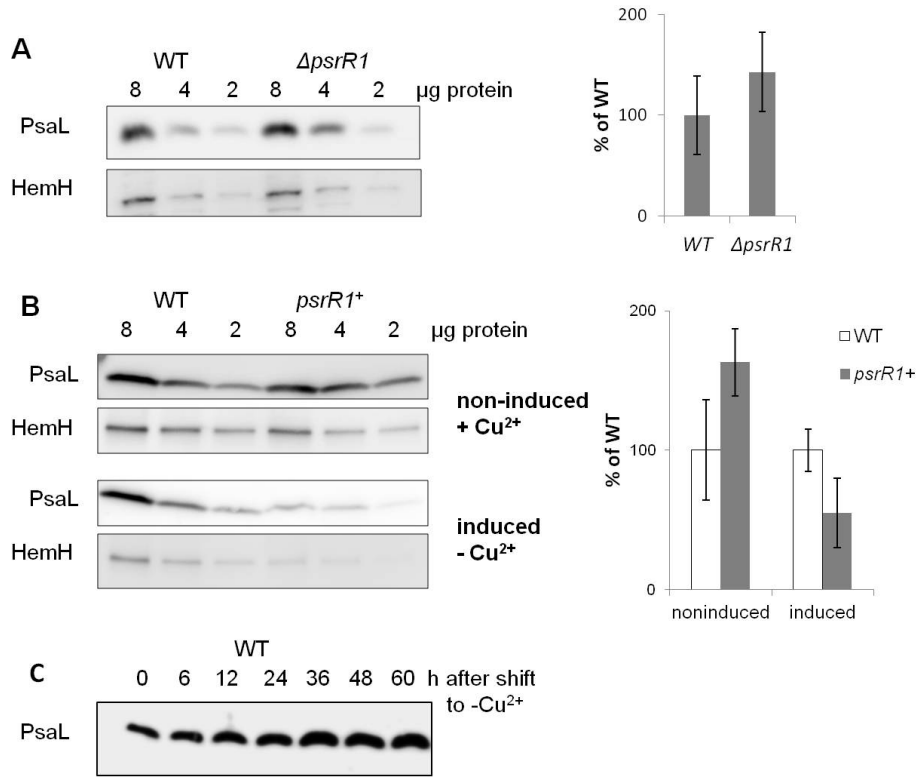
Supplemental Figure 5.

Reporter gene assay. Mean GFP fluorescence for the wild type or mutated 5′-UTR–s*gfp* fusions in the absence (pJV300) and presence of PsrR1 or the mutated versions of PsrR1 as indicated. The background fluorescence is shown with the negative control (pXG0 pJV300).



Supplemental Figure 6.

Verification of the PsrR1 impact on *psaL* expression in cyanobacteria. The RNA gel blot hybridization shows the level of *psaL* mRNA after over-expression of PsrR1 in comparison to the wild-type control. *Synechocystis* sp. PCC 6803 cells were subjected to copper step down during exponential growth phase. Immediately before (0 h), as well as after 5, 24 and hours of copper depletion, cultures were sampled for analysis of PsrR1 and *psaL* mRNA accumulation. 5S rRNA served as loading control.

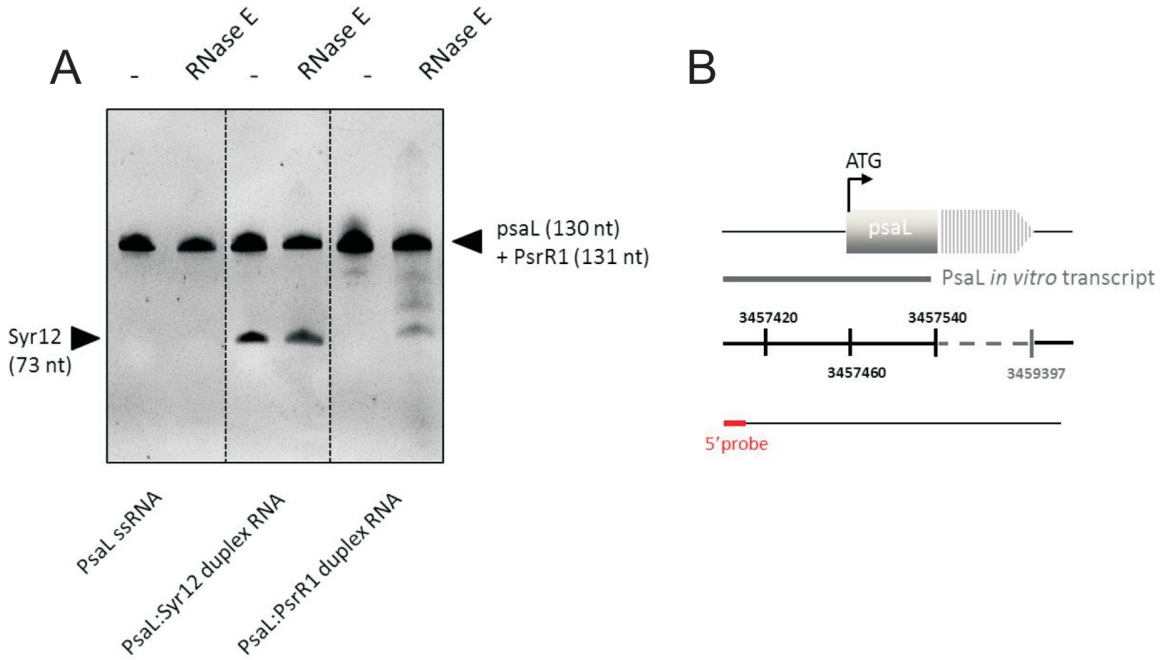


Supplemental Figure 7.

Western Blot analysis of wild-type (WT), *ΔpsrR1* and *psrR1*$^{+}$ cell extracts using PsaL and HemH antisera. **(A)** WT and *ΔpsrR1* mutant strains were grown in standard BG-11 medium under moderate light conditions. Different amounts of protein extracts were separated by SDS-PAGE and the level of PsaL protein was quantified based on the gel blot image by Fusion-SL4 and the Fusion-Capt Advance software (Peqlab) and normalized with the HemH immunosignal. Samples corresponding to 100, 50 and 25 % of the protein loading were analyzed.

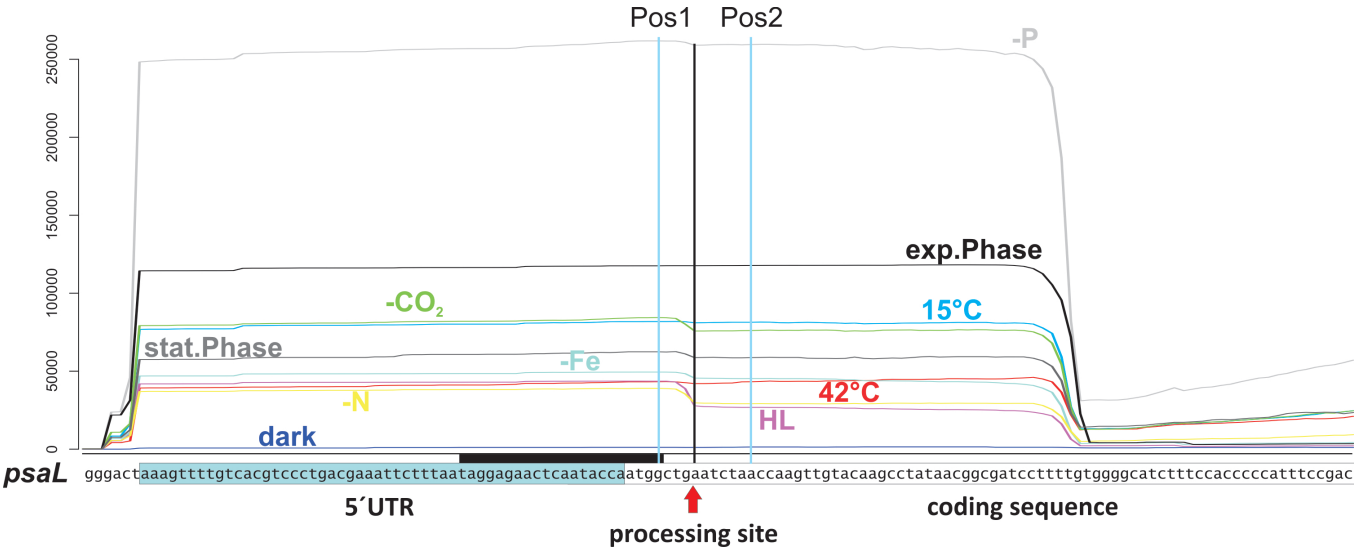
(B) WT and *psrR1*$^{+}$ mutant strains were grown in medium containing 5 µM $CuSO_4$ (non-induced), washed and incubated for 48 h in copper-free medium for induction of *psrR1*$^{+}$ (induced). Different amounts of protein extracts were separated in a pre-casted gradient SDS-PAGE gel (Biorad) and the level of PsaL protein was quantified based on the gel blot image by Fusion-SL4 and the Fusion-Capt Advance software (Peqlab) and normalized with the HemH immunosignal. Samples corresponding to 100, 50 and 25 % of the protein loading were analyzed.

(C) WT cells were grown in BG-11 medium containing 5 µM $CuSO_4$ (time point 0), washed and further grown in BG-11 medium lacking $CuSO_4$ for up to 60 h. 5 µg of protein were loaded per lane.



Supplemental Figure 8.

PsrR1 dependent processing of *psaL* by RNase E. (A) Ethidium bromide stained 8M urea 10% PAA gel of *in vitro* transcribed single-stranded *psaL* RNA (left panel), duplex *psaL* RNA:SyR12 (middle panel) and duplex *psaL* RNA:PsrR1 (right panel), incubated without (-) and with (+) recombinant PCC 6803 RNase E, respectively. PsrR1 and *psaL* have similar sizes and run in one band. (B) Scheme of the *psaL in vitro* transcript and the location of the oligonucleotide probe.



Supplemental Figure 9.

Normalized Solexa transcriptome sequencing reads for the *psaL* 5' region for all 10 conditions investigated in [7]. Conditions as described in [7]: Phosphate limitation (-P, grey, cells washed with phosphate free BG11, 12h cultivation), exponential phase (exp. Phase, black, OD_{750} = ~0.6-0.8, 50 - 80 µmol photons $m^{-2}s^{-1}$), CO_2 depletion (-CO_2, green, cells washed with CO_2 free medium), cold stress (15°C, blue, 30 min 15°C), stationary phase (stat. Phase, grey, OD_{750} = 4.7), iron stress (-Fe, cyan, iron was chelated with DFB), heat stress (42°C, red, cells were incubated for 30 min at 42°C), nitrogen depletion (-N, yellow, cells were washed in nitrogen free BG11, 12h cultivation), high light stress (HL, purple, cells were exposed 470 µmol photons $m^{-2}s^{-1}$ for 30 min), dark induction (dark, dark blue, no light for 12h). The 5'UTR of *psaL* and the interaction site with PsrR1 is indicated. Sequence coverage in the e.g. HL or -N data show a sharp drop, which indicates the processing site, and is marked by a red arrow and a black vertical line. As a measure for the amount of processed 5' fragment we compare the read coverage from a nucleotide position 5' of the processing site with the read coverage from a nucleotide position 3' of the processing site (Pos1 and Pos2).

Supplemental Table 1. Combined List of mRNAs and 5'UTRs Significantly Affected by PsrR1 Overexpression.

			MA[b]	CopraRNA[b]		
Locus tag	**Gene**	**Description**	**PsrR1-WT**	**R**	**p-Value**	**E**
sll1578	*cpcA*	phycocyanin alpha subunit	0.11	1	1.5E-05	-14.4
slr1459	*apcF*	phycobilisome core component	0.02	2	2.0E-05	-10.8
slr1655	*psaL*	PSI subunit XI (*psaL-psaI* operon)	**-0.82**	3	3.8E-05	-24.3
sll1091	*chlP*	geranylgeranyl reductase	0.24	4	1.3E-04	-9.5
sll1995		family 2 glycosyl transferase	-0.14	5	2.8E-04	-13.2
ssr0390	*psaK1*	PSI subunit X1	**-1.37**	6	3.4E-04	-15.5
slr0610	*evrC*	ethyl viologen export system permease	-0.12	7	3.7E-04	-10.6
slr1808	*hemA*	glutamyl-tRNA reductase	0.14	8	4.6E-04	-11.9
sll1138		haloacid dehalogenase superfamily protein	-0.11	9	7.1E-04	-11.4
sll0374	*urtE*	urea transport system ATP-binding protein	-0.16	10	9.8E-04	-11.8
ssr3451	*psbE*	PSII cytochrome b559 subunit alpha	-0.28	12	1.1E-03	-16.5
sll0634	*btpA*	PSI biogenesis protein	-0.11	13	1.2E-03	-17.4
sll1577	*cpcB*	phycocyanin beta subunit	0.44	14	1.6E-03	-14.0
sll1796	*petJ*	cytochrome c-553	**-1.17**	15	1.7E-03	-12.0
sll0109	*aroH*	chorismate mutase	0.04	17	2.2E-03	-15.5
sll1580	*cpcC1*	phycobilisome rod linker polypeptide Lr	0.07	18	2.4E-03	-9.3
slr0749	*chlL*	protochlorophyllide reductase	-0.15	24	3.7E-03	-22.4
slr0750	*chlN*	protochlorophyllide reductase	-0.25	34	5.1E-03	-11.8
sll1194	*psbU*	PSII 12 kD extrinsic protein	0.37	37	5.8E-03	-7.9
slr0906	*psbB*	PSII CP47 protein	0.25	38	5.9E-03	-9.6
sml0008	*psaJ*	PSI reaction center subunit IX	-0.56	41	6.0E-03	-12.7
sll0258	*psbV*	PSII cytochrome c550	-0.20	50	7.6E-03	-9.3
ssl3044		hydrogenase subunit	-0.53	63	1.2E-02	-8.5
ssl2009		thylakoid membrane protein	0.10	67	1.3E-02	-10.5
slr0335	*apcE*	phycobilisome LCM core-membrane linker	0.64	72	1.4E-02	-11.7
slr2094	*glpX*	fructose-1,6-/sedoheptulose-1,7-bisphosphatase	-0.13	80	1.7E-02	-16.5
sll1873		unknown protein	**-0.96**	286	6.2E-02	-13.6
ssr2194[a]		hypothetical protein	**-1.11**	476	1.1E-01	-9.9
slr1704[a]		hypothetical protein	**-1.13**	518	1.2E-01	-10.0
sll0849	*psbD1*	PSII D2 protein	**0.83**	1022	2.5E-01	-8.2
sll0982		uncharacterized thylakoid-associated protein	**1.30**	1120	2.7E-01	-14.5
smr0004	*psaI*	PSI subunit VIII (*psaL-psaI*	**-1.01**	1533	3.7E-01	-8.1

		operon)				
slr0342	*petB*	Cytochrome b6	**0.80**	1759	4.3E-01	-6.9
sll0629	*psaK2*	PSI subunit X2	**-1.16**	2036	5.1E-01	-7.5
sll0851	*psbC*	PSII 44kDa reaction center protein	**0.88**	2331	5.9E-01	-13.6
ssl2615	*atpH*	ATP synthase subunit C	**0.88**	3082	8.0E-01	-6.0
sll0849-5'UTR	*psbD1*	PSII D2 protein	**0.89**	-	-	-
ssr2595-5'UTR[a]	*hliB*	High light inducible protein	**-1.39**	-	-	-
ssr0390-5'UTR	*psaK1*	PSI subunit X1	**-1.24**	-	-	-
sll1652-5'UTR[a]		hypothetical protein	**-1.05**	-	-	-
ssr2153-5'UTR		unknown protein	**-0.97**	-	-	-
sll1873-5'UTR		unknown protein	**-0.84**	-	-	-
slr1655-5'UTR	*psaL*	PSI subunit XI (*psaL-psaI* operon)	**0.85**	-	-	-
[c]slr1897-5'UTR	*srrA*	part of the introduced *petJ* promoter	**2.74**	-	-	-
NC-0690	*psrR1*	regulatory sRNA	**3.76**	-	-	-
sll0630		*psaK2-sll0630* operon	**-1.29**	NA	NA	NA
ssr2595[a]	*hliB*	High light inducible protein	**-1.26**	NA	NA	NA
sll1651[a]		hypothetical protein	**-0.99**	NA	NA	NA
slr1544	*lilA*	*lilA-hliB* operon	**-0.93**	NA	NA	NA

[a]Features that showed differential expression prior to PsrR1 induction by copper depletion.

[b]The results of microarray (MA) experiments at t=24h are shown, along with a list of the targets predicted by CopraRNA. The respective genes and 5'UTRs are indicated by their locus tags and gene names, if available. The $\log_2$ fold changes are given in the column PsrR1-WT (significant changes are in bold), followed by the respective rank (R) and p-value from the comparative target prediction and the specific interaction energies in kcal mol^{-1} for *Synechocystis* 6803 (Wright et al., 2013). "NA" indicate genes for which a prediction was not possible and dashes indicate 5'UTRs detected by microarray analysis for which a prediction was not meaningful.

[c]The strong signal for the *srrA*-5'UTR in *psrR1*$^+$ was an experimental artifact, since the corresponding sequence was part of the inserted fragment harboring the P*petJ* promoter.

Supplemental Table 2. Oligonucleotides used in this study

Sequence (5´-3´)	Name	Purpose
AGGCTATGGAAACCCGACAG	qRT-syr1-fw	qPCR
CACCGAGGGCATATCTAGGA	qRT-syr1-rev	qPCR
AGGGAATCTGAGGAAAGTCCG	qRT-rnpB-fw	qPCR
GGCGGTATTTTTCTGTGGCA	qRT-rnpB-rev	qPCR
TAATACGACTCACTATAGGGATAAGGCTATGGAAACCC GACAG	T7-PsrR1-fw-GS	EMSA
ATACCCCGGCATCACCGAGGG	PsrR1-rev-GS1	EMSA
TACCCCGGCATCACCGAGGGCATATCCCCCAACTCAAT	GS-PsrR1-Mu-rev	EMSA
TAATACGACTCACTATAGGGAAAGTTTTGTCACGTCCCT G	GS-T7-UTR-psaL-fw	EMSA
TGGTATTGAGTTCTCCTATT	GS-UTR-psaL-rev	EMSA
TTCAGCCATTGGTATTGAGTTC	GS-UTR+9-psaL-rev	EMSA
GGCTTGTACAACTTGGTTAG	GS-UTR+30-psaL-rev	EMSA
TAATACGACTCACTATAGGGAATCCTGACAATATTATTTT	GS-psaC-5-UTR-fw	EMSA
ACCAATACAGGTATCGTAAA	GS-psaC-+39	EMSA
TAATACGACTCACTATAGGGaatcctgacaatattatttt	GS-psaC-5-UTR-fw	EMSA
ACCAATACAGGTATCGTAAA	GS-psaC-+39	EMSA
TGTCACGTCCCTGACGAA	psaL-fw	RNA gel blot
TAATACGACTCACTATAGGGCCCCTTGGAAGGTAACCA GT	T7-psaL-rev	RNA gel blot
GGCTATGGAAACCCGACAGAATTC	PsrR1-fw	RNA gel blot
TAATACGACTCACTATAGGGCCGAGGGCATATCTAGGA GAAC	T7-PsrR1-rev	RNA gel blot
TAATACGACTCACTATAGGACCCGACAAGGAATTTCGCT ACC	23SrRNA_for	RNA gel blot
AGACGCAGTATGGGGGCTGAC	23SrRNA_rev	RNA gel blot
CCACAGTGCCCAGCAAGCCG	fabF-fw	mutation
CATAGATCTCTGTCGGGTTTCCATAGCC	BglII-PsrR1-rev	mutation
CAGAGATCTATGCCCTCGGTGATGCCG	BglII-PsrR1-fw	mutation
GCTGGAGGTGTGGCCACGTCC	hoxH-rev	mutation
TTAATGCATAAAGTTTTGTCACGTCCCTGACG	psaL_5_NsiI	pXG10_psaL
TTAGCTAGCATGCCCCACAAAAGGATCGCC	psaL_3_NheI	pXG10_psaL
ATAAGGCTATGGAAACCCGACAGA	PsrR1_5_phos	pPsrR1
GTTTTTTCTAGAGCGGGATTAAACAAAAAACATTCAGAC	PsrR1_3_xbaI	pPsrR1
CggggAGATATGCCCTCGGTGATGCC	PsrR1_mut1_for	pPsrR1mut1
TCTccccGAACTCAATGTCAATGGAAGGTACC	PsrR1_mut1_rev	pPsrR1mut1
GTTCTCgTAGATATGCCCTCGGTGATG	PsrR1_mut2_for	pPsrR1mut2
TCTAcGAGAACTCAATGTCAATGGAAGGTAC	PsrR1_mut2_rev	pPsrR1mut2
AATAcGAGAACTCAATACCAATGGCTGAATCTAAC	psaL_mut_for	pXG10_psaL*

TTCTCGTATTAAAGAATTTCGTCAGGGACGTGA	psaL_mut_rev	pXG10_psaL*
TTAATGCATGCCCTGGACATCGTTAACGATC	cpcA_5_NsiI	pXG30_cpcA
TTAGCTAGCAGCTTGACGTAGACGACCGAAAG	cpcA_3_NheI	pXG30_cpcA
ATCTAcGAAAATTTTCACATTCTAACGGGAGATACCA	cpcA_mut_for	pXG30_cpcA*
TTTTCgTAGATATGTCAGCTTTAAGCTGGATTTTCTC	cpcA_mut_rev	pXG30_cpcA*
TTAATGCATTTACGATTTACCAACGATCAAGTTATTG	chlN_5_NsiI	pXG30_chlN
TTAGCTAGCTTGATAAAGCCAAGATACGCAACTAATG	chlN_3_NheI	pXG30_chlN
CCTAcGAGAACCTAAACTATGACTGTTGC	chlN_mut_for	pXG30_chlN*
TTCTCgTAGGTATAGTTTTTATTTTGCCTTCCTAACC	chlN_mut_rev	pXG30_chlN*
TTAATGCATATGTTGGGTGGTTTCCTTTGGC	psaJ_5_NsiI	pXG30_psaJ
TTAGCTAGCAGTCAACAAAGCCATGATCATGACC	psaJ_3_NheI	pXG30_psaJ
ATTTTcGAGACTCAATTTATGGACGGTTTGAAATCC	psaJ_mut_for	pXG30_psaJ*
GTCTCgAAAATTGACAAAGGTAAACAAAAATAATGTCGAAATTG	psaJ_mut_rev	pXG30_psaJ*
TTAATGCATAAATATTCTCACTTTGTAAGGGATAATGGATAA	psbB_5_NsiI	pXG10_psbB
TTAGCTAGCCACTAGGGCAGTGTGCATTAAATG	psbB_3_NheI	pXG10_psbB
TTAATGCATATTAGAGAAACTTGTTTAACAAAAAACGTCG	hemA_5_NsiI	pXG10_hemA
TTAGCTAGCCCGCAGATGGGTTAGCGCTTC	hemA_3_NheI	pXG10_hemA
TTAATGCATGATTTTTAAAGACCCCCATTAGCGTG	psaK1_5_NsiI	pXG10_psaK1
TTAGCTAGCAACTTTAGGGCTCCAGGACAGG	psaK1_3_NheI	pXG10_psaK1
TAATACGACTCACTATAGGGAAAGTTTTGTCACGTCCCTGAC	psaL fw	In vitro transcription/ RNase E assay
GTCGGAAATGGGGGTGGAAA	psaL rev	In vitro transcription/ RNase E assay
TAATACGACTCACTATAGGGATAAGGCTATGGAAACCCGACAG	PsrR1 fw	In vitro transcription/ RNase E assay
AAAAAAATACCCCGGCATCACCG	PsrR1 rev	In vitro transcription/ RNase E assay
TAATACGACTCACTATAGGGAAGACATAAAGTCAATATCACCC	SyR12 fw	In vitro transcription/ RNase E assay
AAAAAGAAAGCCGCCACTG	SyR12 rev	In vitro transcription/ RNase E assay
CAGGGACGTGACAAAACTTT	5'probe psaL	RNA gel blot oligo probe RNase E assay

Supplemental Table 3. Plasmids used in this study

Plasmid name	Comment	Origin, marker	Reference
control	Control plasmid, expresses a ~50 nt nonsense transcript.	ColE1, Amp^R	[5]
pXG10_psaL	sfGFP reporter plasmid. Carries the *psaL* 5'UTR and the first 57nt of the coding sequence	pSC101*, Cm^R	This study
pPsrR1	PsrR1 expression plasmid	ColE1, Amp^R	This study
pPsrR1mut1	Derivate of pPsrR1	pSC101*, Cm^R	This study
pPsrR1mut2	Derivate of pPsrR1	pSC101*, Cm^R	This study
pXG10_psaL*	Derivate of pXG10_psaL	pSC101*, Cm^R	This study
pXG30_cpcA	sfGFP reporter plasmid. Carries the last 108nt of *cpcB* the *cpcB-cpcA* intergenic region and the first 102nt of the *cpcA* coding sequence	pSC101*, Cm^R	This study
pXG30_cpcA*	Derivate of pXG30_cpcA	pSC101*, Cm^R	This study
pXG30_chlN	sfGFP reporter plasmid. Carries the last 102 nt of *ssr1251* the *ssr1251-chlN* intergenic region and the first 102 nt of the *chlN* coding sequence	pSC101*, Cm^R	This study
pXG30_chlN*	Derivate of pXG30_chlN	pSC101*, Cm^R	This study
pXG30_psaJ	sfGFP reporter plasmid. Carries the last 99 nt of *psaF* the *psaF-psaJ* intergenic region and the first 60 nt of the *psaJ* coding sequence	pSC101*, Cm^R	This study
pXG30_psaJ*	Derivate of pXG30_psaJ	pSC101*, Cm^R	This study
pXG10_psbB	sfGFP reporter plasmid. Carries the *psbB* 5'UTR and the first 90nt of the coding sequence	pSC101*, Cm^R	This study
pXG10_hemA	sfGFP reporter plasmid. Carries the *hemA* 5'UTR and the first 108 nt of the coding sequence	pSC101*, Cm^R	This study
pXG10_psaK1	sfGFP reporter plasmid. Carries the *psaK1* 5'UTR and the first 60 nt of the coding sequence	pSC101*, Cm^R	This study

Supplemental Methods

Reverse transcription and quantitative real-time PCR experiments. RNA extracted from *psrR1*$^{+}$ the isogenic wild-type control strains before and after removal of copper was reverse-transcribed by using the Superscript III kit (Fermentas). Reverse transcription was performed according to the manufacturer's protocol. PsrR1 was reverse transcribed using the specific primer qRT-syr1-rev (Table S1). In parallel non-template controls were run for each RNA sample. Quantitative PCR was carried out in duplicates with a QuantiFast SYBR Green PCR Kit (Qiagen) according to the two-step cycling protocol in the manufacturer's instructions using a 7500 Fast System (Applied Biosystems, Foster City, CA, USA). In each reaction volume of 10µl 1µM of primer (Table S1) and 1ng of the synthesized cDNA was mixed. Data were analyzed with the 7500 Fast System SDS software (Applied Biosystems), threshold and baseline values were set automatically by the software. Relative transcript quantities were calculated by the ddCT method [2]. Normalized expression values were calculated relative to the endogenic control gene *rnpB*.

Supplemental References

[1] Mitschke J*, et al.* (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC6803. *Proc Natl Acad Sci U S A* **108**:2124-2129.

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