Supporting Information for

[2Fe-2S] proteins in chlorosomes: CsmI and CsmJ participate in light-dependent control of energy transfer in chlorosomes of *Chlorobaculum tepidum*

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Materials and Methods Addenda:

Cell viability testing. Cells in late exponential phase ($OD_{600\ nm}$ ~1.5) were diluted 100-fold in anoxic or oxic K-phosphate buffer, pH 7.0. Tubes containing anoxic cell suspensions were wrapped in foil (to maintain dark conditions) and were incubated in an anoxic chamber. Oxic cell suspensions were placed in darkness or under ambient (room) light (~50 μmol photons m^{-2} s^{-1}) with their caps attached loosely. After 3 or 6 days, the cell suspensions were diluted 1×10^4-fold, and an aliquot (30 μL) of cells was plated on CL plates solidified with 1.5% (w/v) phytagel. The plates were incubated in the light at ~40°C for 6 days in an anoxic chamber, and the resulting colonies were counted.

Pigment and quinone analyses. BChls, carotenoids, and quinones were extracted with acetone-methanol (150 μL, 7:2, v/v) from an aliquot of chlorosomes (1 μL). After mixing and filtration, ammonium acetate (0.1 volume of 1.0 M) was added prior to loading the extracts onto a 4.5 mm × 25 cm Discovery C_{18} reversed-phase, high-performance liquid chromatography (HPLC) column (Supelco, Bellefonte, PA). The Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA) was controlled with Agilent ChemStation software. HPLC analyses were performed as described (*1, 2*). Pigment concentrations were determined by absorption spectroscopy in methanol using the following absorption coefficients: BChl *c*, 20 L· g^{-1} cm^{-1} at 635 nm; BChl *a*, 60 L · g^{-1} cm^{-1} at 770 nm; carotenoids, 265 L · g^{-1} cm^{-1} at 491 nm; chlorobiumquinones (1′-oxomenaquinone-7 and 1′-hydroxymenaquinone-7), 17 L · g^{-1} cm^{-1} at 270 nm; menaquinone-7, 26 L· g^{-1} cm^{-1} at 270 nm.

References for Supplement:

1. Frigaard, N.-U., Takaichi, S.,Hirota, M., Shimada, K., Matsuura, K. 1997. Quinones in chlorosomes of green sulfur bacteria and their role in the redox-dependent fluorescence studied in chlorosome-like bacteriochlorophyll *c* aggregates. *Arch. Microbiol. 167*, 343-349.

2. Frigaard, N.-U., Li, H., Milks, K. J., Bryant, D. A. 2004. Nine mutants of *Chlorobium tepidum* each unable to synthesize a different chlorosome protein still assemble functional chlorosomes. *J. Bacteriol. 186*, 646-53.

3. Li, H., Jubilerer, S., Garcia Costas, A. M., Frigaard, N.-U., and Bryant D. A. (2009) Multiple antioxidant proteins protect *Chlorobaculum tepidum* against oxygen and reactive oxygen species. *Arch. Microbiol. 191*, 853-867.

Table S1. Plasmids used for mutant construction in this study.

Name	Size (kbp)	Description	Antibiotic Resistance[a]
pHP45Ω	4.4	Contains a 2.1-kb *aadA* streptomycin and spectinomycin cassette	Ap^R Sm^R Sp^R
pMS255	3.7	Contains a 1.1-kb *aacC1* gentamicin cassette	Ap^R Gm^R
pRL409	5.4	[b]Contains a 2.8-kb *cat-ermC* cassette	Ap^R Cm^R Em^R
pET3d*::csmI::aadA*	7.3	Constructed by insertion of *aadA* cassette from pHP45Ω into the *Xcm*I site of *csmI* in pET3d*::csmI*	Ap^R Sm^R Sp^R
pET32a*::csmX::aadA*	8.6	Constructed by insertion of *aadA* cassette from pHP45Ω into the *Mfe*I site of *csmX* in pET32a*::csmX*	Ap^R Sm^R Sp^R
pET3d*::csmI::cat-ermC*	8.1	Constructed by deletion of *aadA* marker from pET3d::*csmI*::*aadA*, and then inserting *cat-ermC* marker at the same XcmI site	Ap^R Cm^R Em^R
[c]pET3d*::csmJ::aacC1*	6.4	Constructed by insertion of *aacC1* from pMS255 into the ApoI site of *csmJ* in pET3d*::csmJ*	Ap^R Gm^R

[a]Ap^R, ampicillin resistance; Em^R, erythromycin resistance; Gm^R, gentamicin resistance; Sm^R, streptomycin resistance; Sp^R, spectinomycin resistance.

[b]The Cm^R marker is functional in *E. coli,* and the Em^R marker is functional in *Cba. tepidum*.

[c]The EcoRI site within pET3d*::csmJ* was previously deleted.

Table S2. Pigment and quinone contents of chlorosomes from WT and mutant strains[a]

	BChl *a*	Carotenoids	Chlorobium-quinones	Mena-quinone-7	Total Quinones
wild type	11 ± 1	67 ± 7	54 ± 4	10 ± 2	64 ± 6
csmI	12 ± 1	70 ± 3	46 ± 7	17 ± 5	63 ± 12
csmJ	15 ± 4	64 ± 2	62 ± 9	14 ± 2	76 ± 11
csmX	12 ± 3	67 ± 2	51 ± 11	12 ± 1	63 ± 12
csmI csmX	11 ± 1	56 ± 6	60 ± 11	13 ± 5	73 ± 16
csmJ csmX	13 ± 1	57 ± 2	66 ± 10	14 ± 4	80 ± 14
csmI csmJ	14 ± 2	52 ± 5	36 ± 8	25 ± 5	61 ± 13
csmI csmJ csmX	14 ± 1	53 ± 3	38 ± 8	28 ± 7	66 ± 15

[a] Values are reported as mg per g of BChl *c*. Contents were determined by HPLC analysis as previously described (*2*). All values are the averages and standard deviations of at least two measurements for two separate chlorosome preparations from different cell cultures.

Table S3. Growth rates of WT and mutant strains at three light intensities. [a]

	low irradiance 8 μmol photons m^{-2} s^{-1}	moderate irradiance 30 μmol photons m^{-2} s^{-1}	high irradiance 150 μmol photons m^{-2} s^{-1}
WT	0.047 ± 0.004	0.203± 0.007	0.277± 0.008
csmI	0.039 ± 0.005	0.203± 0.005	0.263± 0.010
csmJ	0.039± 0.002	0.170± 0.005	0.236± 0.005
csmX	0.044± 0.004	0.193± 0.006	0.275± 0.004
csmI csmX	0.041± 0.003	0.174± 0.012	0.248± 0.007
csmJ csmX	0.043± 0.003	0.168± 0.007	0.229± 0.006
csmI csmJ [b]	0.040± 0.004	0.168 ± 0.005	0.163 ± 0.007
csmI csmJ csmX	0.042± 0.005	0.174± 0.004	0.254± 0.009

[a] Specific growth rates (μ, h^{-1}) are reported for 47°C. The values are averages and standard errors for at least two independent cultures.

[b] Three independent *csmI csmJ* mutants were isolated and characterized. One *csmI csmJ* mutant failed to grow at high irradiance and grew much more slowly at moderate irradiance. This strain was also shown to be temperature-sensitive. Two other strains of the *csmI csmJ* mutant grew a little more slowly than wild type at moderate irradiance but grew more slowly (~60%) than other mutants and wild type at high irradiance. The values reported here are the average values for these other two strains.

Table S4. Cell viability after 3 and 6 days under anoxic and oxic conditions (± room light).

Strain/conditions[a]	72 h/ 3 d		144 h/ 6 d	
	Colonies[b]	Viability (%)[c]	Colonies[b]	Viability (%)[c]
WT (dark, anoxic)	86 ± 0	100	85 ± 5	99
WT (dark, oxic)	50 ± 4	58	24 ± 2	28
WT (light, oxic)	27 ± 3	31	0	≤1
csmI csmJ csmX (dark, anoxic)	90 ± 3	100	62 ± 3	69
csmI csmJ csmX (dark, oxic)	44 ± 8	49	12 ± 1	13
csmI csmJ csmX (light, oxic)	14 ± 4	16	0	≤1

[a] Strains and conditions: Wild type (WT) and *csmI csmJ csmX* mutant cells were incubated in the dark or under ambient room light (~50 µmol photons m^{-2} s^{-1}) for 3 d or 6 d (72 and 144 h, respectively) under oxic or anoxic conditions as specified (see supplemental Materials and Methods for additional details).

[b] Each number is the average of the colonies that appeared on two plates after an identical dilution was performed (see supplemental Materials and Methods for additional details).

[c] Viability was calculated as the percentage of colony-forming units relative to the control culture maintained under dark anoxic conditions for 72 h. Preliminary studies showed that no loss of viability occurred during this time (also see Li et al. (*3*)).

Figure S1

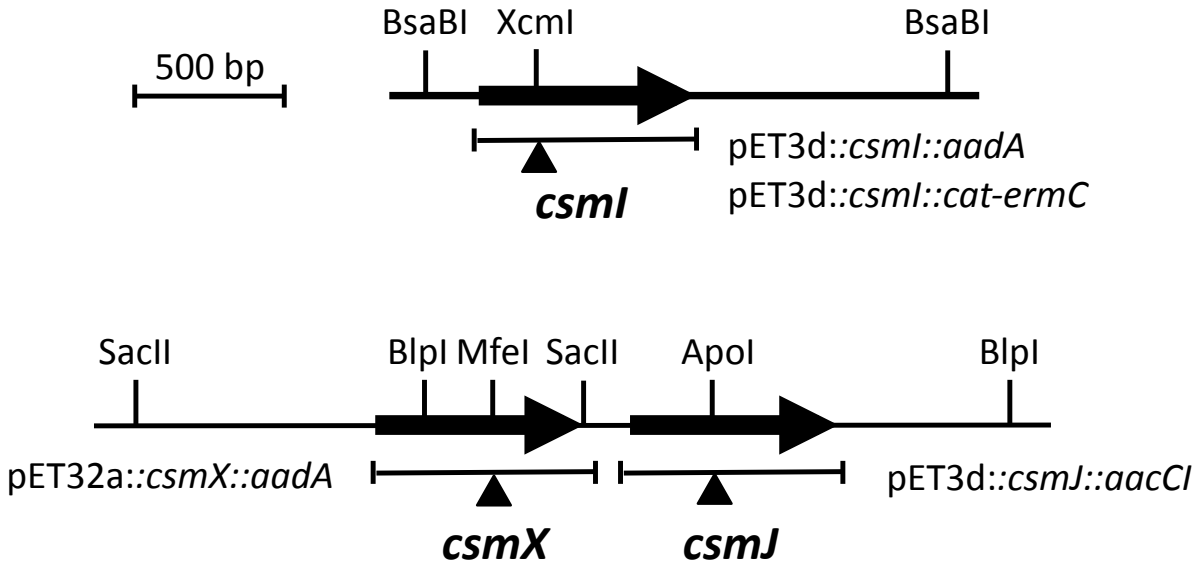

Figure S1. Restriction maps showing the physical maps of constructions used for inactivation of the *csmI*, *csmJ*, and *csmX* genes (see Table S1 and (*2*)).