SUPPLEMENTARY MATERIALS

Eukaryotic-like Ser/Thr protein kinases SpkC/F/K are involved in phosphorylation of GroES in the cyanobacterium *Synechocystis*

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Mutation of *spk* genes

***Synechocystis* strains used for mutation of *spk* genes.** A *Synechocystis* sp. PCC 6803 GS-strain[30] was used for mutation of all the *spk* genes, except the *spkA*. In this strain the *spkA* gene is spontaneously mutated by a frame shift.[25]

The *spkB*::Km^r mutant. A DNA fragment of 893 bp that contained part of the *spkB* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pGEM®-T Easy vector (Promega). The resultant plasmid was digested with the restriction enzyme *Stu* I and the cleaved fragment was ligated with a Km^r cassette that included the *aphA* gene from plasmid pUC4K.[34] This gene encodes aminoglycoside 3'-phosphotransferase, which confers resistance to kanamycin.

The *spkC*::Cm^r mutant. A DNA fragment of 1,143 bp that contained part of the *spkC* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pGEM®-T vector. The resultant plasmid was digested with the restriction enzyme *Kpn* I and the cleaved fragment was ligated

with the Cm^r cassette that included the *cat* gene from plasmid pACYC184.[35] This gene encodes chloramphenicol acetyltransferase and confers resistance to chloramphenicol.

The *spkD*::Km^r mutant. A DNA fragment of 1,256 bp that contained part of the *spkD* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pT7Blue-T vector (Novagen). The resultant plasmid was used for insertion, with an EZ::TN™ <KAN-2> Tnp Transposome™ Kit (Epicentre), of a Km^r transposon that contained the same Km^r cassette as we used for mutation of the *spkB* gene. The location and orientation of insertion of the cassette were determined by sequencing DNA in the region that flanked the cassette.

The *spkE*::Gm^r mutant. A DNA fragment of 1,070 bp that contained part of the *spkE* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pGEM®-T. The resultant plasmid was digested with the restriction enzyme *Hin*d III and the cleaved fragment was ligated with a Gm^r cassette that included the *aacC1* gene from plasmid pUCGm.[37] This gene encodes gentamycin acetyltransferase and confers resistance to gentamycin.

The *spkF*::Sp^r mutant. A DNA fragment of 1,677 bp that contained part of the *spkF* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pGEM®-T Easy vector. The resultant plasmid was digested with the restriction enzymes *Eco*N I and *Cla* I and the cleaved fragment was ligated with a Sp^r cassette that included the *aadA1* gene. This gene encodes aminoglycoside adenylyltransferase and confers resistance to streptomycin and spectinomycin.[36]

The *spkG*::Km^r mutant. A DNA fragment of 885 bp that contained part of the *spkG* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pUC18 vector.[33] The resultant

plasmid was digested with the restriction enzyme *Bam*H I and the cleaved fragment was ligated with the same Km^r cassette as we used for mutation of the *spkB* gene.

The *spkH*::Sp^r mutant. A DNA fragment of 2,022 bp that contained part of the *spkH* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pT7Blue-T vector. The resultant plasmid was digested with the restriction enzyme *Nco* I and the cleaved fragment was ligated with the same Sp^r cassette as we used for mutation of the *spkF* gene.

The *spkI*::Cm^r mutant. A cosmid clone cs0838, which contained the *spkI* gene,[17] was amplified in *E. coli*. A transposon that included a Cm^r cassette was randomly inserted into the cs0838 cosmid using the Genomic Priming System (pGPS2.1; New England BioLabs). A *spkI*::Cm^r mutant clone was selected from the resultant mutants by sequencing DNA in the region that flanked the cassette.

The *spkJ*::Cm^r mutant. A cosmid clone cs0282, which contained the *spkJ* gene,[17] was amplified in *E. coli*. A transposon that included a Cm^r cassette was randomly inserted into the cs0282 cosmid using the Genomic Priming System. A *spkJ*::Cm^r mutant clone was selected from the resultant mutants by sequencing DNA in the region that flanked the cassette.

The *spkK*::Sp^r mutant. A DNA fragment of 1,681 bp that contained part of the *spkK* gene was amplified by PCR with the primers indicated in Table S1 and DNA from *Synechocystis* as template. The fragment was cloned into the pT7Blue-T vector. The resultant plasmid was digested with the restriction enzyme *Nco* I and the cleaved fragment was ligated with the same Sp^r cassette as we used for mutation of the *spkF* gene.

The *spkL*::Cm^r mutant. A cosmid clone cs0352, which contained the *spkL* gene,[17] was amplified in *E. coli*. A transposon that included a Cm^r cassette was randomly inserted into the cs0352 cosmid using the Genomic Priming System. A *spkL*::Cm^r mutant clone was selected from the resultant mutants by sequencing DNA in the region that flanked the cassette.

Table S1

Forward and reverse primers used for amplification by PCR of partial sequences of *spk* genes and the sites of insertion and antibiotic-resistance gene cassettes used for mutation of *spk* genes of *Synechocystis*

Gene	ORF	**Forward and reverse primers (5'-3') for amplification by PCR**	**Insertional mutation**		
			Site[1]	Restriction enzyme	Antibiotic-resistance cassette
spkB	*slr1697*	TTTATCGGCATTGGGGTGTTTTTC ATTGCTTGACACTCCCCTCGGTAA	65	*Stu*I	Km^r
spkC	*slr0599*	ATCTAGAGGCGATCGCCCAG GGGTGTCGTTGGCGTTGTAG	556	*Kpn*I	Cm^r
spkD	*sll0776*	TAATAAAAGAGTCCTGACGGAATGA GATTTTAGATACATGACTTTGCCAG	439	-	$Tn(Km^r)$[2]
spkE	*slr1443*	CTTTCGCCCCTGACCGTATT CGCCATCAAGCCCAGTAAGA	332	*Hin*dIII	Gm^r
spkF	*slr1225*	CTAAGGGCAGAAACCCGCAGTC CCTGGCGGAGCTTTACCCTGAT	157-195	*Eco*NI and *Cla*I 38 bp deleted	Sp^r
spkG	*slr0152*	TTATTGATGGGGATGGAG CAGGGAAAAGGCTCAATG	867	*Bam*HI	Km^r
spkH	*sll0005*	TTACCACCGCCGAACAAGAT AGGCTGCATTGCGGATTTTG	866-1352	*Nco*I 486 bp deleted	Sp^r
spkI	*sll1770*	CCGGGATAACCAGGGTTAACCG TGGAACAAGTATGCTCGGGCCC	248	-	$Tn(Cm^r)$[2]
spkJ	*slr0889*	GCCATGTGGCTCACTGGTAGCT GGGTGGGGATCCGCATGGAAAA	138	-	$Tn(Cm^r)$
spkK	*slr1919*	CCTAACCCCAAACCCCTGC TGGTGCTCACGCCGGCC	702	*Nco*I	Sp^r
spkL	*sll0095*	CCACCATCCGCATGGTAGTTGC TGCCAGCGGTTTCCAAGGCTTG	183	-	$Tn(Cm^r)$

[1] Positions counted from the initiation codon.

[2] $Tn(Km^r)$ and $Tn(Cm^r)$ indicate transposons that included a Km^r and a Cm^r cassette, respectively.

Table S2

Forward and reverse primers used for amplification by PCR of mutated *spk* genes

Gene	ORF	**Forward and reverse primers (5'-3')**
spkB	*slr1697*	AATTTATCGGCATTGGGGTGTTTTT TTTGATTTTTCACTGCGCCAAAATC
spkC	*slr0599*	AAACTACTCAACAATCGCTACAGAA TAAACTCTTGAGTGTAAACCTTGGT
spkD	*sll0776*	The same as in Table S1
spkE	*slr1443*	TTTTGCAATATCACTTCTACTTGCC GGACCATATTTTTTACTTTTGCGGA
spkF	*slr1225*	The same as in Table S1
spkG	*slr0152*	GAGATTTATGTTTGGCGGGAAAAAT AAACCTTCATAGTCCAACAAAGCTA
spkH	*sll0005*	The same as in Table S1
spkI	*sll1770*	The same as in Table S1
spkJ	*slr0889*	The same as in Table S1
spkK	*slr1919*	The same as in Table S1
spkL	*sll0095*	The same as in Table S1

Table S3

Forward and reverse primers used for amplification by PCR of *spk* genes for cloning in pVZ321 and complementation of mutations in *spkC*, *spkF*, and *spkK*.

Gene	ORF	**Forward and reverse primers (5'-3')**
spkC	*slr0599*	CCTAGAACAGGCTTCCTTTCC
		CCTCTCCTCGGCTTAATATTG
spkF	*slr1225*	CTAGTACCGAAGGGTCCAAC
		CCAAGCGGTGACCGATAG
spkK	*slr1919*	CCATAGACCAAATCCCTG
		GACGTTTTGTAACATCAAG

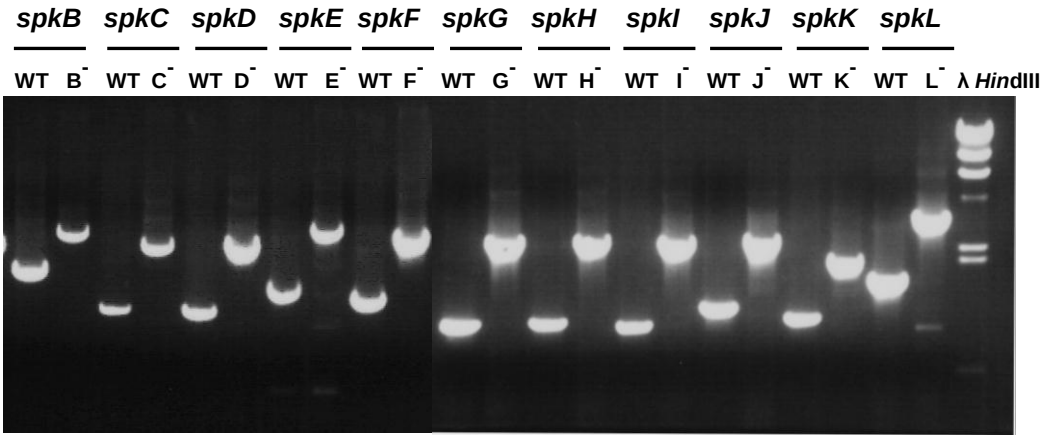

Fig. S1. PCR analysis of the replacement of each wild-type gene by a mutated gene in individual *spk* mutants. Chromosomal DNA was isolated from wild-type (WT) and mutant (from B^- to L^-) cells and used as template for PCR amplification with pairs of primers as indicated in Fig. 1 and Supplementary Table 2. A *Hin*d III digest of DNA from bacteriophage λ is included as a size markers.

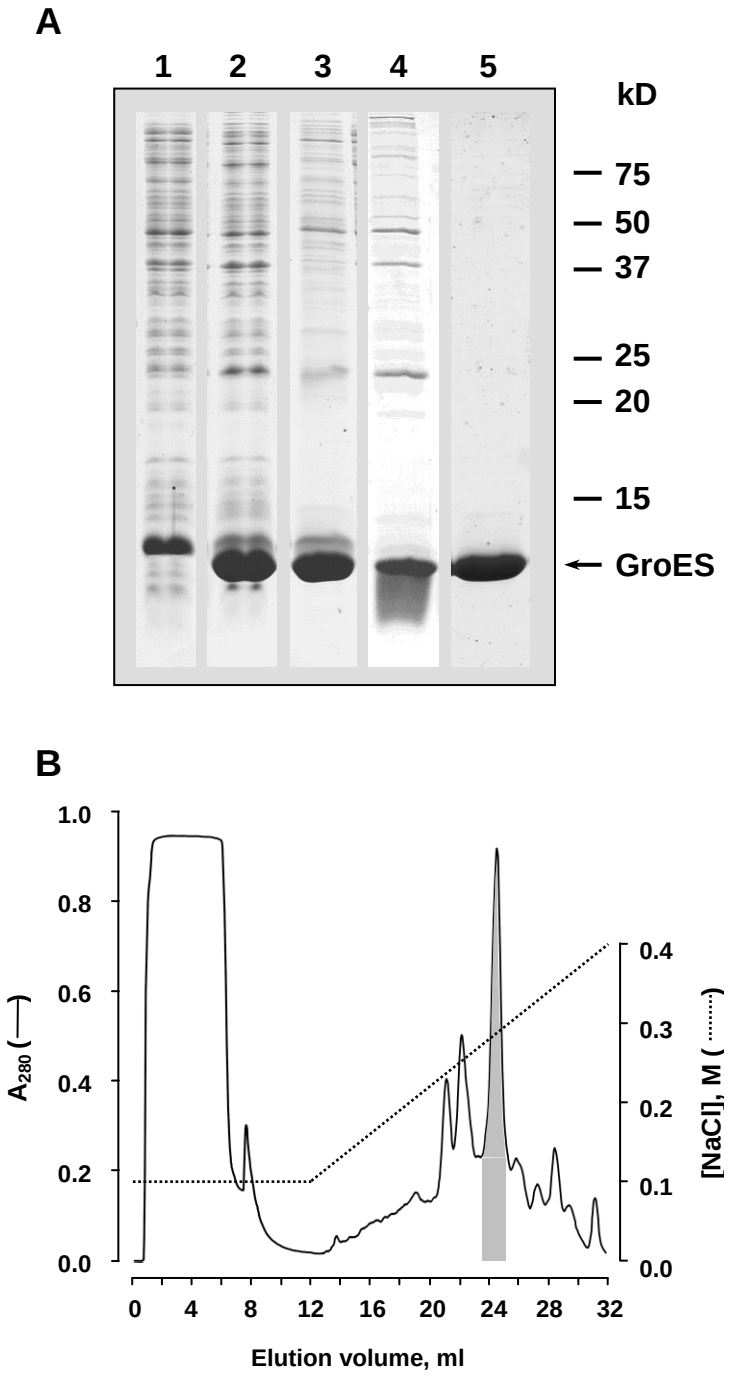

Fig. S2. Purification of recombinant GroES. (A) SDS-PAGE (18%) stained with Coomassie R-250 of crude lysate of non-induced *E. coli* cells carrying pET-22b with *groES* gene of *Synechocystis* (lane 1); crude lysate of induced *E. coli* cells carrying pET-22b with *groES* (lane 2); soluble protein fraction from induced *E. coli* cells (lane 3), GroES-containing protein fraction purified on Protino Ni-IDA 2000 column (lane 4), GroES purified on Mono Q column (lane 5). (B) Purification of recombinant GroES on Mono Q column. Peak of GroES is gray-shaded.

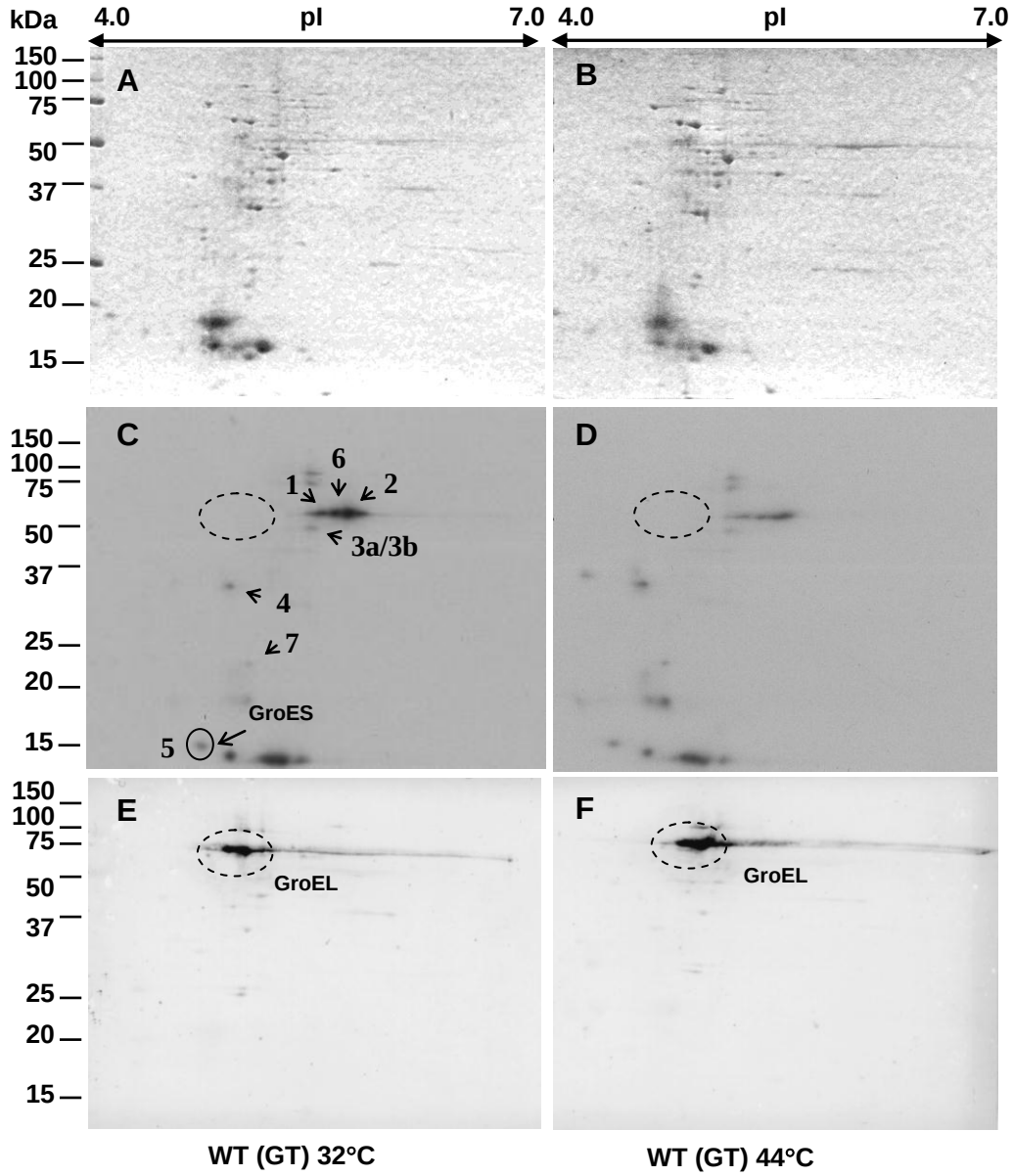

Fig. S3. Phosphorylation *in vitro* of a soluble protein fraction isolated from *Synechocystis* glucose tolerant (GT) strain. CBB-stained proteins from cells that had been grown at 32°C (A) and treated at 44°C for 30 min (B). Autoradiographs of phosphoprotein patterns at 32°C (C) and 44°C (D) and the corresponding western blots (E and F) developed with anti-Hsp60 (GroEL) antibodies are presented. Dashed circles designate the positions of GroEL.