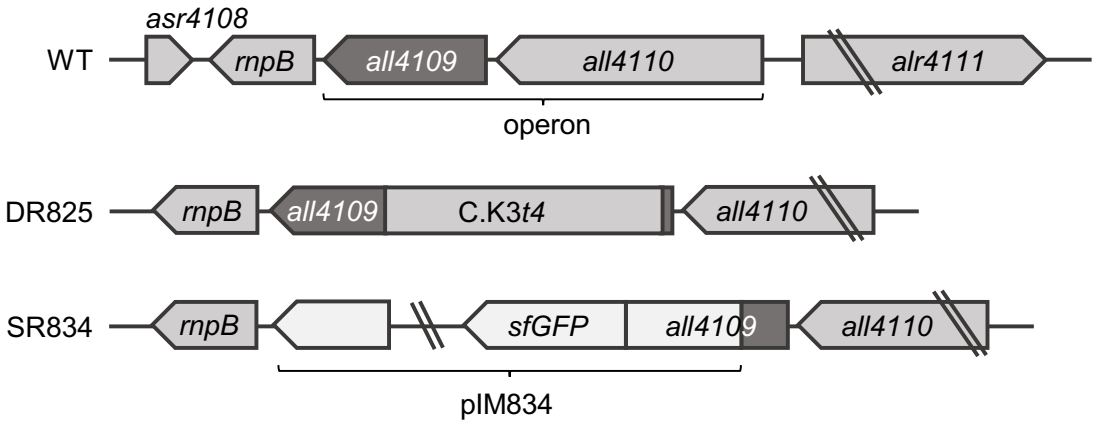
SepN is essential for assembly and gating of septal junctions in *Nostoc* sp. PCC 7120

Ann-Katrin Kieninger, Piotr Tokarz, Gregor L. Weiss, Martin Pilhofer and Iris Maldener

Supplemental data:



Supplement Figure 1: Schematic representation of the *all4109* genomic region.

For inactivation of *all4109* the neomycin resistance cassette C.K3*t*4 was inserted into the ORF of *all4109* via double homologous recombination creating strain DR825. Genomic *all4109* was exchanged for an *all4109-sfgfp* translation fusion via single homologous recombination with plasmid pIM834 yielding strain SR834.

Supplement Table 1: Strains and plasmids used in this work.

***Nostoc* sp. strains**	Relevant characteristics	Reference
PCC 7120	wild type	Rippka *et al.,* 1979
CSVT2	Δ*fraD* (Δ*alr2393*)	Merino-Puerto *et al.,* 2010
7120.800	P_{fraCDE}-*gfpmut2*, Sm^r, Sp^r	This study
DR825	*all4109*::C.K3*t4* cassette, Nm^r	This study
SR834	*all4109*-5xGS-*sfgfp*, Sm^r, Sp^r	This study
DR825.848	*all4109*::C.K3*t4*, $P_{all4110/4109}$-*all4109*, Nm^r, Sm^r, Sp^r	This study
CSVT2.SR834	Δ*fraD* (*alr2393*), *all4109*-5xGS-*sfgfp*, Sm^r, Sp^r	This study
***Escherichia coli* strains**		
NEB 10β	Δ*(ara-leu) 7697 araD139 fhuA* Δ*lacX74 galK16 galE15 e14- Φ80*d*lacZ*Δ*M15 recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1* Δ*(mrr-hsdRMS-mcrBC)*	NEB *biolabs*
HB101	F–, *thi*-1, *hsd*S20 (r_B^-, m_B^-), *sup*E44, *rec*A13, *ara*-14, *leu*B6, *pro*A2, *lac*Y1, *gal*K2, *rps*L20 (str^r), *xyl*-5, *mtl*-1	Sambrook *et al*., 1989
J53 (RP-4)	R+, *met*, *pro* (RP-4: *Ap*, *Tc*, *Km*, Tra^+, *IncP*)	Wolk *et al*., 1984
Plasmids		
pIM800	P_{fraCDE}-*gfpmut2* in pRL1049, Sm^r, Sp^r	This study
pIM825	C.K3*t4* cassette flanked by upstream and C-terminal fragment of *all4109* in pRL277, Sm^r, Sp^r, Km^r	This study
pIM834	C-terminal *all4109*-fragment fused to 5xGS-linker and *sfgpf* in pRL277, Sm^r, Sp^r	This study
pIM848	$P_{all4110/4109}$-*all4109* in pRL1049, Sm^r, Sp^r	This study
pIM779	P_{fraCDE}-*gfpmut2-fraD* in pRL1049, Sm^r, Sp^r	Weiss *et al*., 2019
pIM660.2	C-terminal *alr3353*-fragment fused to 5xGS-linker and *sfgfp* in pRL277, Sm^r, Sp^r	Bornikoel, unpublished
pRL1049	Self-replicating plasmid for *Anabaena* sp., Sm^r, Sp^r	Black and Wolk, 1994
pRL277	Non-replicating, mobilizable vector containing *sacB*, Sm^r, Sp^r	Black *et al*., 1993
pRL528	Helper plasmid for mobilization used in triparental mating, Cm^r	Wolk *et al*., 1984

Sm: Streptomycin, Sp: Spectinomycin, Km: Kanamycin, Nm: Neomycin, Cm: Chloramphenicol

Supplement Table 2: Oligonucleotides used in this work.

Oligo #	Sequence (5' → 3')
1383	TCTAGAGGATCTCAATGAATA
1384	ATGCTTGTAAACCGTTTTG
1444	TAGTGGATCCGGTAGTGGATCCGGTAGCGCATCAAAAGGTGAAGAATTATTTAC
1445	GCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCATTATTTATATAATTCATCCATACCATG
1998	GATATCCCGCAAGAGGCCCTTTCGTCTTCAAGAATTCTGCCGTTCCTTGTCATCTG
2235	CCACAACGGTTTCCCTCTACCGGGATCCGGTTATTTGTATAGTTCATCCATGCCATGTG
2397	ATTCATTGAGATCCTCTAGATGGTCAGTACTCCTAGTC
2398	CAAAACGGTTTACAAGCATATTGGACTTATGCCCTACC
2399	ATGGCAGAAATTCGATATCTAGATCTCGAGTGCTCGATGCGATTATTG
2400	TAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGTTGTCAGTTGCCAGTTG
2446	GCTTTGCAGGCGTGAG
2447	TCGCACTGGACGTTATC
2450	ATGGCAGAAATTCGATATCTAGATCTCGATTGGTGTATCTTATATATT
2451	ACCGGATCCACTACCGGATCCACTACCCTTTTTATTCCAGGGTAGG
2511	AGAGGCCCTTTCGTCTTCAAGAATTTTGCCGTCAGGCTTAG
2512	TGGTCAGTACTCCTAGTCATGCAGTTATACCCGAAACTT
2513	ATGACTAGGAGTACTGACC
2514	GACCACAACGGTTTCCCTCTACCGGTTATTTCTTTTTATTCCAGGGTAG

Supplement Table 3: Performed co-IPs with respective controls.

Antibody	Co-IP	Sample	Control
α-GFP	1	CSVT2.779	CSVT2.779
		CSVT2.779	empty beads
	2	CSVT2.779	7120.800
		CSVT2.779	
	3	CSVT2.779	CSVT2.779 empty beads
α-FraD	1	WT	WT pre-immunserum
	2		CSVT2
	3		

- glutaraldehyde
+ glutarladehyde

CSVT2.779: Δ*fraD* + p(*gfpmut2-fraD*); 7120.800: WT + p(*gfpmut2*); CSVT2: Δ*fraD*.

References:

Black TA, Cai Y, Wolk CP 1993. Spatial expression and autoregulation of *hetR*, a gene involved in the control of heterocyst development in *Anabaena*. *Mol. Microbiol.* 9, 77–84.

Black TA, Wolk CP 1994. Analysis of a Het- mutation in *Anabaena* sp. strain PCC 7120 implicates a secondary metabolite in the regulation of heterocyst spacing. *J. Bacteriol.* 176, 2282–2292.

Merino-Puerto V, Mariscal V, Mullineaux CW, Herrero A, Flores E. 2010. Fra proteins influencing filament integrity, diazotrophy and localization of septal protein SepJ in the heterocyst-forming cyanobacterium *Anabaena* sp. *Molecular microbiology* 75: 1159-70

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. 1979. Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. *Microbiology (Reading, England)* 111: 1-61

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. xxxviii + 1546 pp. pp.

Weiss GL, Kieninger A-K, Maldener I, Forchhammer K, Pilhofer M, (2019). Structure and Function of a Bacterial Gap Junction Analog. *Cell* 178:2, 374-84.e15.

Wolk CP, Vonshak A, Kehoe P, Elhai J. 1984. Construction of shuttle vectors capable of conjugative transfer from Escherichia coli to nitrogen-fixing filamentous cyanobacteria. *Proceedings of the National Academy of Sciences* 81: 1561-65