Multiple ABC glucoside transporters mediate sugar-stimulated growth in the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120

Mercedes Nieves-Morión and Enrique Flores*

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Américo Vespucio 49, E-41092 Seville, Spain

*For correspondence. E-mail: eflores@ibvf.csic.es; Tel: +34 954489523

Running title: ABC glucoside transporters in *Anabaena*

Originality-Significance Statement

This work illustrates the capability of the heterocyst-forming cyanobacterium *Anabaena* for mixotrophic growth and identifies ABC glucoside uptake transporters essential for this physiological behavior. This emphasizes an aspect of cyanobacterial physiology –mixotrophy– that should be considered for a full understanding of the wide distribution of cyanobacteria in nature.

Summary

Cyanobacteria are generally capable of photoautotrophic growth and are widely distributed on Earth. The model filamentous, heterocyst-forming strain *Anabaena* sp. PCC 7120 has been long considered a strict photoautotroph but is now known to be able to assimilate fructose. We have previously described two components of ABC glucoside uptake transporters from *Anabaena* that are involved in uptake of the sucrose analog esculin: GlsC (a nucleotide-binding domain subunit [NBD]) and GlsP (a transmembrane component [TMD]). Here we created *Anabaena* mutants of genes encoding three further ABC transporter components needed for esculin uptake: GlsD (NBD), GlsQ (TMD) and GlsR (periplasmic substrate-binding protein). Phototrophic growth of *Anabaena* was significantly stimulated by sucrose, fructose and glucose. Whereas the *glsC* and *glsD* mutants were drastically hampered in sucrose-stimulated growth, the different *gls* mutants were generally impaired in sugar-dependent growth. Our results suggest the participation of Gls and other ABC transporters encoded in the *Anabaena* genome in sugar-stimulated growth. Additionally, Gls transporter components influence the function of septal junctions in the *Anabaena* filament. We suggest that mixotrophic growth is important in cyanobacterial physiology and may be relevant for the wide success of these organisms in diverse environments.

Introduction

Cyanobacteria are an ecologically important group of organisms that significantly impact the carbon and nitrogen cycles in the biosphere (Whitton and Potts, 2000). They are characterized by their ability to perform oxygenic photosynthesis, and they are generally capable of photoautotrophic growth (Rippka *et al.*, 1979). Although many cyanobacteria are strict photoautotrophs, some are capable of photoheterotrophic growth assimilating sugars (Rippka *et al.*, 1979). Additionally, some heterocyst-forming cyanobacteria are capable of sugar-dependent chemoheterotrophic growth in the dark (Wolk and Shaffer, 1976; Bottomley and van Baalen, 1978; Schmetterer and Flores, 1988). Consistently, some sugar transporters that mediate sugar uptake supporting some kind of heterotrophic growth have been identified in cyanobacteria. Well-known examples include a major facilitator superfamily (MFS) glucose transporter, GlcP, that has been characterized in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803 (Zhang *et al.*, 1989; Schmetterer, 1990) and in the heterocyst-forming cyanobacterium *Nostoc punctiforme* (Ekman *et al.*, 2013), and an ABC fructose transporter, Frt, that has been characterized in the heterocyst-forming cyanobacteria *Anabaena variabilis* (Ungerer *et al.*, 2008) and *N. punctiforme* (Ekman *et al.*, 2013).

Heterocyst-forming cyanobacteria are filamentous organisms that, in the absence of a source of combined nitrogen, contain two cell types: vegetative cells that fix CO_2 performing oxygenic photosynthesis and heterocysts that are specialized for the fixation of N_2 (Flores and Herrero, 2010). In the diazotrophic filament, an exchange of nutrients takes place that results in the transfer of reduced carbon from vegetative cells to heterocysts and of fixed nitrogen from heterocysts to vegetative cells (Wolk, 1968; Wolk *et al.*, 1974; Jüttner, 1983). Intercellular molecular exchange has been traced with fluorescent markers (including calcein and 5-carboxyfluorescein) and shown to take place by simple diffusion (Mullineaux *et al.*, 2008; Nieves-Morión *et al.*, 2017a). The cyanobacterial filament consists of individual cells surrounded by their cytoplasmic membrane and peptidoglycan layer(s) but sharing the outer membrane, which is continuous along the filament determining the presence of a continuous periplasmic space, and the cells in the filament are joined by proteinaceous structures termed septal junctions (Wilk *et al.*, 2011; Flores *et al.*, 2016; Herrero *et al.*, 2016). Proteins SepJ (also known as FraG), FraC and FraD that are located at the intercellular septa have been identified as putative components of the septal junctions (reviewed in Flores *et al.*, 2016; Herrero *et al.*, 2016).

In the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120 (hereafter *Anabaena*), sucrose appears to be a quantitatively important metabolite

transferred from vegetative cells to heterocysts (Curatti *et al.*, 2002; Cumino *et al.*, 2007), where it is hydrolyzed by invertase InvB producing fructose and glucose to support heterocyst metabolism (López-Igual *et al.*, 2010; Vargas *et al.*, 2011). A fluorescent analogue of sucrose, esculin (Gora *et al.*, 2012), has also been used as a marker to trace intercellular molecular transfer, and it has additionally been used to test uptake from the outer medium into *Anabaena* cells (Nürnberg *et al.*, 2015). Three glucoside transporters (or components of transporters) that are involved in uptake of esculin have been identified in *Anabaena* (Nieves-Morión *et al.*, 2017b). HepP (*Anabaena* ORF product All1711) is a MFS transporter that was previously shown to be needed for deposition of the polysaccharide layer of the heterocyst envelope (López-Igual *et al.*, 2012), GlsC (Alr4781) is a nucleotide-binding subunit of an ABC transporter, and GlsP (All0261) is a transmembrane (permease) subunit of an ABC transporter. In addition to mediating esculin uptake, these proteins were shown to influence the septal junctions. Whereas GlsC is needed for proper localization of SepJ at the intercellular septa, HepP and GlsP influence septal function in a process that may involve interactions with septal protein SepJ (Nieves-Morión *et al.*, 2017b).

Anabaena is an important model in studies of N_2 fixation, heterocyst differentiation and bacterial multicellularity that has been considered for a long time to be a strict photoautotroph. Recent work has shown however that *Anabaena* can grow heterotrophically using fructose as long as this sugar is provided at relatively high concentrations (≥ 50 mM) (Stebegg *et al.*, 2012). Incorporation of the genes encoding the Frt transporter from *A. variabilis* into the *Anabaena* genome permits growth of *Anabaena* dependent on lower concentrations of fructose (5 mM; Ungerer *et al.*, 2008). Hence, *Anabaena* has the metabolic capability to use fructose as a carbon and energy source but lacks a high affinity transporter for this sugar. On the other hand, *Anabaena* has been recently reported to grow mixotrophically using a number of carbon sources, including some sugars (fructose, glucose, maltose, sucrose), amino acids (glutamate, glutamine, proline) and other simple organic compounds (glycerol, pyruvate) (Malatinszky *et al.*, 2017). Whereas ABC transporters for amino acids (Pernil *et al.*, 2015) and a TRAP transporter that can take up pyruvate (Pernil *et al.*, 2010) are known to be expressed in *Anabaena*, transporters that mediate the uptake of sugars are less known. In this work, we addressed the identification of further components of the ABC transporters that mediate esculin uptake and the possible role of those transporters in sugar assimilation as well as in other aspects of the physiology of *Anabaena*.

Results

Identification and inactivation of further components of glucoside transporters

ABC uptake transporters typically comprise one periplasmic solute-binding protein (SBP), two integral membrane proteins (transmembrane domains [TMD]) and two nucleotide-binding domains (NBD) that hydrolyze ATP in the cytoplasm (Cui and Davidson, 2011). We were interested in the identification of transporter partners of GlsC (NBD) and GlsP (TMD). The genome of *Anabaena* contains 12 ORFs that encode proteins that are most similar to components of ABC uptake transporters for sugars (Table S1). These genes are spread in the *Anabaena* genome precluding the possibility of predicting their association in specific ABC transport complexes. Orthologues of *glsC* have no neighbors encoding ABC transporter components in any of the cyanobacterial genomes whose sequence is available (https://img.jgi.doe.gov/cgi-bin/m/main.cgi). Orthologues of *glsP* are however frequently accompanied by another ABC TMD-encoding gene in the genomes of heterocyst-forming cyanobacteria (Fig. S1). The *Anabaena* gene most similar to this gene is *alr2532*. In some heterocyst-forming and non-heterocyst-forming cyanobacteria, as well as in some other bacteria, a gene encoding a periplasmic SBP is clustered together with those two TMD-encoding genes (Fig. S1). The *Anabaena* gene most similar to this gene is *all1916*. We therefore constructed *Anabaena* mutants bearing inactivated versions of *all1916* or *alr2532*. No gene encoding an ABC transporter NBD is however clustered together with these genes in any available cyanobacterial genomic sequence. Because in *Anabaena* there is only one gene other than *glsC* that encodes a predicted ABC sugar transporter NBD protein, *all1823* (Table S1), we constructed an *Anabaena* mutant of this gene as well.

The genes were inactivated by insertion of pCSL145, a plasmid that cannot replicate in *Anabaena* and bears the *npt* gene encoding neomycin/kanamycin phosphotransferase. Internal fragments of each of the genes were inserted in pCSL145 to serve as platforms for integration into the *Anabaena* chromosome by homologous recombination, the constructs were transferred to *Anabaena* by conjugation, and exconjugants were selected as neomycin-resistant clones (Fig. S2). Clones that were homozygous for chromosomes containing the inactivated construct were identified by PCR analysis and named CSMN17 (*all1823*::pCSL145), CSMN18 (*all1916*::pCSL145) and CSMN19 (*alr2532*::pCSL145).

To investigate the possible role of the inactivated proteins in glucoside transport, uptake of esculin was studied with filaments of wild-type *Anabaena* and the three mutants grown in BG11 medium (containing nitrate) or grown in BG11 medium and

incubated for 18 h in $BG11_0$ medium (lacking any source of combined nitrogen). Strain CSMN17 showed a low esculin uptake activity in either BG11 or $BG11_0$ medium, strain CSMN18 showed about half of the wild-type esculin uptake activity in either medium, and strain CSMN19 was affected in esculin uptake in $BG11_0$ medium but not significantly in BG11 medium (Table 1). Thus, the three genes encode components of transporters that participate in glucoside uptake. We name All1823 as GlsD (NBD), All1916 as GlsR (periplasmic SBP) and Alr2532 as GlsQ (TMD).

Sugar-stimulated growth

We then investigated whether the ABC esculin transporters identified in this work and those described previously (Nieves-Morión *et al.*, 2017b) could mediate a growth response to sugars. Attempts of growth tests in the dark or in the light in the presence of 10 µM DCMU with sugars gave inconsistent results, and growth tests in shaken cultures failed to show any positive effect of sucrose. However, 100 mM sucrose, fructose or glucose stimulated phototrophic growth of *Anabaena* on plates, specifically in $BG11_0$ medium (Fig. 1). We then tested the effect of the three sugars, added at 50 mM, on growth in standing liquid cultures (in microtiter plates) in the light. Sucrose, fructose and glucose significantly increased the yield of *Anabaena* in both BG11 and $BG11_0$ media (Student's *t* test $p \leq 0.011$; see WT in Table S2A) suggesting mixotrophic growth. Fructose and glucose stimulated growth more than sucrose (Table S2 and Fig. 2). On the other hand, maltose had only a marginal positive effect on the growth of *Anabaena* (not shown).

Sucrose-stimulated growth in BG11 and $BG11_0$ media was drastically hampered by inactivation of the NBD proteins GlsC and GlsD, and it was impaired by inactivation of the TMD proteins GlsP and GlsQ and of the SBP protein GlsR (Fig. 2; see whole set of data in Table S2). Fructose- and glucose-stimulated growth was also impaired in all the mutants, and the effect of the inactivation of *glsC* on glucose-stimulated growth was especially significant. These results show that the identified ABC uptake transporter components mediate a positive growth response of *Anabaena* to sucrose, fructose and glucose.

Whereas direct uptake of radiolabeled sucrose (Nicolaisen *et al.*, 2009b; López-Igual *et al.*, 2012) and fructose (Stebegg *et al.*, 2012) has been previously shown for *Anabaena*, to the best of our knowledge uptake of glucose has not been reported. We therefore tested uptake of [^{14}C]glucose in filaments grown in BG11 medium and filaments incubated for 18 h in $BG11_0$ medium. *Anabaena* filaments could take up glucose, at

higher levels after incubation in $BG11_0$ medium, but they did so significantly only at relatively high sugar concentrations indicating a very low affinity for the sugar (Fig. S3).

Analysis of septal junction function

Inactivation of *glsC* and *glsP* in *Anabaena* impairs diazotrophic growth likely because of effects on the septal junctions (Nieves-Morión *et al.*, 2017b). We tested growth of the *glsD*, *glsQ* and *glsR* mutants in liquid and solid BG11 or $BG11_0$ medium. Growth was not significantly impaired in any of the mutants in the liquid media (Table S2, no sugar added). On the other hand, whereas none of the mutants showed a deficit in growth on solid BG11 medium, the *glsR* and *glsQ* mutants were found to be impaired in growth on solid $BG11_0$ medium (Fig. 3).

Septal junctions mediate intercellular molecular transfer that can be studied by Fluorescence Recovery After Photobleaching (FRAP) analysis with fluorescent markers including calcein and 5-carboxyfluorescein (5-CF) (Mullineaux *et al.*, 2008; Merino-Puerto *et al.*, 2011). Intercellular transfer of these markers, quantified as the recovery constant *R*, was tested in the *glsD*, *glsQ* and *glsR* mutants. Whereas the *glsQ* mutant showed *R* values that were consistently lower than the wild-type figures for both calcein and 5-CF, the *glsD* and *glsR* mutants were impaired in calcein transfer but not in 5-CF transfer (Table 2). Hence, the *glsQ* mutant appears to be the most affected in intercellular transfer of fluorescence markers.

Localization of SepJ and protein-protein interactions

Specific glucoside transporters have been found to influence septal junctions by either affecting localization of the septal protein SepJ or by possible protein-protein interactions with SepJ (Nieves-Morión *et al.*, 2017b). We investigated the subcellular localization of SepJ in the mutants generated in this work by immunofluorescence analysis performed with antibodies raised against the coiled-coil domain of SepJ (Ramos-León *et al.*, 2015). SepJ was found localized at the intercellular septa of filaments of the *glsD*, *glsQ* and *glsR* mutants in a similar way as in the wild type (Fig. S4).

To investigate possible interactions of GlsD and GlsQ with SepJ and to assess whether some of the identified components of ABC transporters could be partners in specific transporters, we performed Bacterial Adenylate Cyclase Two Hybrid analysis (BACTH). In this analysis, adenylate cyclase activity is reconstituted from two fragments, T25 and T18, of adenylate cyclase from *Bordetella pertussis* brought together by interacting proteins fused to each of those fragments (Karimova *et al.*, 1998).

Reconstituted adenylate cyclase in *E. coli* produces cAMP that promotes induction of *lacZ* encoding β-galactosidase. Because cAMP has to be produced in the cytoplasm, the periplasmic SBP GlsR could not be included in this analysis. T25 and T18 fusions to GlsC and GlsP have been described previously (Nieves-Morión *et al.*, 2017b). We have now constructed N-terminal and C-terminal fusions of both T25 and T18 to each of the newly identified proteins GlsD and GlsQ. GlsD is a cytoplasmic protein and GlsQ is an integral membrane protein with its N- and C-termini predicted to be cytoplasmic. Of the tested combinations, GlsD interacting with itself produced high levels of β-galactosidase activity indicating self-interaction (Fig. 4; see complete set of data in Table S3). Previous work showed self-interaction also for GlsC (Nieves-Morión *et al.*, 2017b; included in Fig. 4 for comparison). Interestingly, GlsD interacted also with GlsC. Additionally, GlsD was found to interact with GlsP, GlsC with GlsQ and GlsP with GlsQ. Although these latter interactions were relatively weak, it should be noted that they were statistically significant (see Table S3). Finally, GlsQ was found to interact with SepJ, resembling the GlsP-SepJ interaction described previously (Nieves-Morión *et al.*, 2017b; included in Fig. 4 for comparison). This interaction may constitute the basis for the requirement of GlsQ for full intercellular molecular transfer and diazotrophic growth on plates.

Discussion

Results in this work indicate that GlsD (NBD) has a quantitatively important role in esculin uptake and in sucrose-stimulated growth in *Anabaena*, suggesting that GlsD is an essential component of transporters involved in the uptake of sucrose. Our results additionally show that GlsC (NBD) is also needed to assimilate sucrose, suggesting that GlsC and GlsD work together in the ABC glucoside uptake complexes of *Anabaena*. Consistently, GlsC and GlsD were observed to interact in BACTH analysis. Nonetheless, each of these proteins is also able to interact with itself suggesting that they can act as homodimers in some ABC transporter complexes. A transporter containing specifically GlsC appears to be especially relevant for glucose uptake. NBD proteins serving different TMD complexes in ABC transporters –the so-called multitask ABC ATPases– are well known, and classical examples are MalK and similar proteins that energize di- and oligo-saccharide uptake in several bacteria (Schlösser *et al.*, 1997; Webb *et al.*, 2008; Ferreira and de Sá-Nogueira, 2010).

The GlsP and GlsQ TMDs are also needed for full esculin uptake and sugar-stimulated growth, they interact with each other in BACTH analysis, and the genes encoding their orthologues are clustered together in the genomes of many heterocyst-

forming cyanobacteria. These observations together suggest that GlsP and GlsQ are partners in ABC transporter complexes. The periplasmic SBP GlsR is also needed for full esculin uptake and sugar-stimulated growth, and a gene encoding a GlsR orthologue is clustered together with genes encoding GlsP and GlsQ orthologues in the genomes of some cyanobacteria. Therefore, GlsR may be a partner of GlsP and GlsQ. On the other hand, because the *glsP*, *glsQ* and *glsR* mutants still show substantial activity of esculin uptake and sucrose, fructose- and glucose-stimulated growth, additional SBPs and TMDs should be involved in the uptake of these sugars.

The 12 possible components of ABC sugar uptake transporters encoded in the *Anabaena* genome (Table S1) belong to the Transporter Classification Database (TCDB) family 3.A.1.1 (Saier, 2000; for the most recent discussion of the TCDB see Saier *et al.*, 2016), which transport disaccharides and other complex saccharides and for which the MalEFGK transporter of *Escherichia coli* (MalE, SBP; MalF and MalG, TMDs; MalK, NBD) is a well-known representative (Nikaido, 1994). The predicted *Anabaena* proteins are four periplasmic SBP, six TMDs (three most similar to MalF and three to MalG), and two NBDs (Table S1). This data together with our results discussed above suggest the presence in *Anabaena* of at least three ABC glucoside transporters, one of which may be constituted by GlsR (SBP), GlsP-GlsQ (TMDs) and GlsC-GlsD (NBDs). The membrane complex of this transporter may use additional SBP(s), which is common in ABC transporters (Davidson *et al.*, 2008), and the NBD proteins appear to be shared by the three glucoside transporters as discussed above.

The Gls proteins have a role not only in sucrose-stimulated growth but also in fructose- and glucose-stimulated growth. These results indicate that fructose and glucose can be incorporated into *Anabaena* at least in part by the ABC glucoside uptake transporters. Transport of fructose and glucose appears to take place, however, with low affinity. Thus, our results of direct uptake of [^{14}C]glucose by *Anabaena* permit to estimate a relatively high K_s of at least 20 mM, and the analysis of uptake of [^{14}C]fructose reported by Stebegg *et al.* (2012) also suggest low affinity. In contrast, the K_s of *Anabaena* for sucrose (determined in fragmented filaments) is 4.9 µM (Nicolaisen *et al.*, 2009b) and for esculin 150 µM (in BG11 medium) or 119 µM (in $BG11_0$ medium) (Nieves-Morión *et al.*, 2017b). Hence, *Anabaena* expresses high-affinity cytoplasmic membrane transporters for sucrose but not for fructose or glucose (see also Ungerer *et al.*, 2008). Indeed, no ORF evidently encoding a fructose or glucose transporter is found in the *Anabaena* genome. Why, then, is growth stimulated more by fructose or glucose than by sucrose? We have previously reported that *Anabaena* appears to lack any sucrose porin (Nicolaisen *et al.*, 2009b), but the *Anabaena* genome contains genes encoding homologs to porin OprB (Nicolaisen *et al.*, 2009a). OprB porins mediate the movement through the

outer membrane of glucose and other monosaccharides (Wylie and Worobec, 1995; van den Berg, 2012), and an OprB-like porin in *Nostoc punctiforme* is involved in glucose and fructose uptake (Ekman *et al.*, 2013). Facilitated movement through the outer membrane may be a key factor to permit assimilation of the sugars by *Anabaena*. Once in the periplasm, fructose and glucose can be transported into the cytoplasm, albeit with low affinity, by cytoplasmic membrane glucoside transporters. In contrast, sucrose hardly passes the outer membrane, but the sucrose molecules that reach the periplasm can be transported into the cytoplasm with high affinity. Sugar concentrations in freshwater and terrestrial environments are normally in the µM range, but there are reports of up to 4.5 mM (Hobbie and Hobbie, 2013), implying that the *Anabaena* sugar transporters might be useful in such environments.

The ABC transporter Gls appears to be needed also for normal function of septal junctions in the *Anabaena* filament. As mentioned earlier, GlsC (NBD) is required for normal subcellular localization of SepJ and septal maturation (Nieves-Morión *et al.*, 2017b). The NBD protein GlsD studied here is however not needed for SepJ localization indicating that this is a specific role of GlsC likely acting independently of GlsD. On the other hand, inactivation of GlsQ (TMD) has an effect similar to that of inactivation of GlsP (TMD) on the intercellular transfer of calcein and 5-CF (compare to data in Nieves-Morión *et al.*, 2017b). The requirement of the ABC glucoside transporter Gls for the normal function of the septal junctions could be based on protein-protein interactions between the TMD subunits and SepJ (as shown by BACTH analysis), and it can account, at least partly, for the growth defect of the mutants.

Our work has identified an ABC glucoside transporter (Gls) from *Anabaena* that can be probed with the fluorescent analog esculin and is involved in the uptake of sucrose, fructose and glucose. In addition to its influence on the septal junctions, a putative function of this transporter is to mediate sugar assimilation. It should be noted, however, that we have consistently observed sugar-stimulated growth of *Anabaena* mainly in standing liquid cultures. Different relationships of gases (O_2, CO_2) in different incubation conditions (growth on a surface or in shaken or standing liquid cultures) may affect the growth response to sugars. The presence in the *Anabaena* genome of numerous genes encoding organic substrate transporters is consistent with the idea that microorganisms are prepared to take up extensively substrates that become available (Hobbie and Hobbie, 2013). Whereas the wide distribution of cyanobacteria in our planet is likely based on their photoautotrophic lifestyle, the capability of mixotrophic growth could enhance fitness in many ecological niches.

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Conflict of interest

The authors have no conflict of interest to declare.

References

Bottomley, P.J., and van Baalen, C. (1978) Dark hexose metabolism by photoautotrophically and heterotrophically grown cells of the blue-green alga (Cyanobacterium) *Nostoc* sp. strain Mac. *J Bacteriol* **135:** 888-894.

Cui, J., and Davidson, A.L. (2011) ABC solute importers in bacteria. *Essays Biochem* **50:** 85-99.

Cumino, A.C., Marcozzi, C., Barreiro, R., and Salerno, G.L. (2007) Carbon cycling in *Anabaena* sp. PCC 7120. Sucrose synthesis in the heterocysts and possible role in nitrogen fixation. *Plant Physiol* **143:** 1385-1397.

Curatti, L., Flores, E., and Salerno, G. (2002) Sucrose is involved in the diazotrophic metabolism of the heterocyst-forming cyanobacterium *Anabaena* sp. *FEBS Lett* **513**: 175-178.

Davidson, A.L., Dassa, E., Orelle, C., and Chen, J. (2008) Structure, function, and evolution of bacterial ATP-binding cassette systems. *Microbiol Mol Biol Rev* **72:** 317-364.

Ekman, M., Picossi, S., Campbell, E.L., Meeks, J.C., and Flores, E. (2013) A *Nostoc punctiforme* sugar transporter necessary to establish a cyanobacterium-plant symbiosis. *Plant Physiol* 161: 1984-1992.

Ferreira, M.J., and de Sá-Nogueira, I. (2010) A multitask ATPase serving different ABC-type sugar importers in *Bacillus subtilis*. *J Bacteriol* **192:** 5312-5318.

Flores, E., and Herrero, A. (2010) Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat Rev Microbiol* 8: 39-50.

Flores, E., Herrero, A., Forchhammer, K., and Maldener, I. (2016) Septal junctions in filamentous heterocyst-forming cyanobacteria. *Trends Microbiol* **24:** 79-82.

Gora, P.J., Reinders, A., and Ward, J.M. (2012) A novel fluorescent assay for sucrose transporters. *Plant Methods* **8:**13.

Herrero, A., Stavans, J., and Flores, E. (2016) The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiol Rev* **40:** 831-854.

Hobbie, J.E., and Hobbie, E.A. (2013) Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. *Front Microbiol* 4:324.

Jüttner, F. (1983) ^{14}C-labeled metabolites in heterocysts and vegetative cells of *Anabaena cylindrica* filaments and their presumptive function as transport vehicles of organic carbon and nitrogen. *J Bacteriol* **155:** 628-633.

Karimova, G., Pidoux, J., Ullmann, A., and Ladant, D. (1998) A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc Natl Acad Sci USA* **95:** 5752-5756.

Kolman, M.A., Nishi, C.N., Perez-Cenci. M., and Salerno, G.L. (2015) Sucrose in cyanobacteria: from a salt-response molecule to play a key role in nitrogen fixation. *Life (Basel)* **5:** 102-126.

López-Igual, R., Flores, E., and Herrero, A. (2010) Inactivation of a heterocyst-specific invertase indicates a principal role of sucrose catabolism in the heterocysts of *Anabaena* sp. *J Bacteriol* **192**: 5526–5533.

López-Igual, R., Lechno-Yossef, S., Fan, Q., Herrero, A., Flores, E., and Wolk, C.P. (2012) A major facilitator superfamily protein, HepP, is involved in formation of the heterocyst envelope polysaccharide in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* **194:** 4677-4687.

Malatinszky, D., Steuer, R., and Jones, P.R. (2017) A comprehensively curated genome-scale two-cell model for the heterocystous cyanobacterium *Anabaena* sp. PCC 7120. *Plant Physiol* **173:** 509-523.

Merino-Puerto, V., Schwarz, H., Maldener, I., Mariscal, V., Mullineaux, C.W., Herrero, A., and Flores, E. (2011) FraC/FraD-dependent intercellular molecular exchange in the filaments of a heterocyst-forming cyanobacterium, *Anabaena* sp. *Mol Microbiol* **82:** 87-98.

Mullineaux, C.W., Mariscal, V., Nenninger, A., Khanum, H., Herrero, A., Flores, E., and Adams, D.G. (2008) Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. *EMBO J* **27**: 1299-1308.

Nikaido, H. (1994) Maltose transport system of *Escherichia coli*: an ABC-type transporter. *FEBS Lett* **346:** 55-58.

Nicolaisen, K., Hahn, A., and Schleiff, E. (2009a) The cell wall in heterocyst formation by Anabaena sp. PCC 7120. *J Basic Microbiol* **49:** 5-24.

Nicolaisen, N., Mariscal, V., Bredemeier, R., Pernil, R., Moslavac, S., López-Igual, R., Maldener, I., Herrero, A., Schleiff, E., and Flores, E. (2009b) The outer membrane of a heterocyst-forming cyanobacterium is a permeability barrier for uptake of metabolites that are exchanged between cells. *Mol. Microbiol.* 74:58-70.

Nieves-Morión, M., Mullineaux, C.W., and Flores, E. (2017a) Molecular diffusion through cyanobacterial septal junctions. *mBio* **8:** e01756-16.

Nieves-Morión, M., Lechno-Yossef, S., López-Igual, R., Frías, J.E., Mariscal, V., Nürnberg, D.J., Mullineaux, C.W., Wolk, C.P., and Flores, E. (2017b) Specific glucoside transporters influence septal structure and function in the filamentous, heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* **199:** e00876-16.

Nürnberg, D.J., Mariscal, V., Bornikoel, J., Nieves-Morión, M., Krauß, N., Herrero, A., Maldener, I., Flores, E., and Mullineaux, C.W. (2015) Intercellular diffusion of a fluorescent sucrose analog via the septal junctions in a filamentous cyanobacterium. *mBio* **6(2):** e02109.

Pernil, R., Herrero, A., and Flores, E. (2010) A TRAP transporter for pyruvate and other monocarboxylate 2-oxoacids in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* **192:** 6089-6092.

Pernil, R., Picossi, S., Herrero, A., Flores, E., and Mariscal, V. (2015) Amino acid transporters and release of hydrophobic amino acids in the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *Life (Basel)* **5:** 1282-1300.

Ramos-León, F., Mariscal, V., Frías, J.E., Flores, E., and Herrero, A. (2015) Divisome-dependent subcellular localization of cell-cell joining protein SepJ in the filamentous cyanobacterium *Anabaena*. *Mol Microbiol* **96:** 566-580.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Stanier, R.Y. (1979) Generic assignments, strain stories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* **111**: 1-61.

Saier, M.H., Jr. (2000) Families of transmembrane sugar transport proteins. *Mol Microbiol* **35:** 699-710.

Saier, M.H., Jr., Reddy, V.S., Tsu, B.V., Ahmed, M.S., Li, C., and Moreno-Hagelsieb, G. (2016) The Transporter Classification Database (TCDB): recent advances. *Nucleic Acids Res* **44(D1):** D372-9.

Schlösser, A., Kampers, T., and Schrempf, H. (1997) The *Streptomyces* ATP-binding component MsiK assists in cellobiose and maltose transport. *J Bacteriol* **179:** 2092-2095.

Schmetterer, G.R. (1990) Sequence conservation among the glucose transporter from the cyanobacterium *Synechocystis* sp. PCC 6803 and mammalian glucose transporters. *Plant Mol Biol* **14:** 697-706.

Schmetterer, G, and Flores, E. (1988) Uptake of fructose by the cyanobacterium *Nostoc* sp. ATCC 29150. *Biochim Biophys Acta* 942: 33-37.

Stebegg, R., Wurzinger, B., Mikulic, M., and Schmetterer, G. (2012) Chemoheterotrophic growth of the cyanobacterium *Anabaena* sp. strain PCC 7120 dependent on a functional cytochrome c oxidase. *J Bacteriol* **194:** 4601-4607.

Ungerer, J.L., Pratte, B.S., and Thiel, T. (2008) Regulation of fructose transport and its effect on fructose toxicity in *Anabaena* spp. *J Bacteriol* **190:** 8115-8125.

van den Berg, B. (2012) Structural basis for outer membrane sugar uptake in pseudomonads. *J Biol Chem* **287:** 41044-41052.

Vargas, W.A., Nishi, C.N., Giarrocco, L.E., and Salerno, G.L. (2011) Differential roles of alkaline/neutral invertases in *Nostoc* sp. PCC 7120: Inv-B isoform is essential for diazotrophic growth. *Planta* **233**: 153-162.

Webb, A.J., Homer, K.A., and Hosie, A.H. (2008) Two closely related ABC transporters in *Streptococcus mutans* are involved in disaccharide and/or oligosaccharide uptake. *J Bacteriol* **190:**168-178.

Whitton, B.A., and Potts, M., Eds. (2000) The Ecology of Cyanobacteria. Their Diversity in Time and Space. Kluwer Academic Press.

Wilk, L., Strauss, M., Rudolf, M., Nicolaisen, K., Flores, E., Kühlbrandt, W., and Schleiff, E. (2011) Outer membrane continuity and septosome formation between vegetative cells in the filaments of *Anabaena* sp PCC 7120. *Cell Microbiol* **13:** 1744-1754

Wolk, C.P. (1968) Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica*. *J Bacteriol* **96:** 2138-2143.

Wolk, C.P., and Shaffer, P.W. (1976) Heterotrophic micro- and macrocultures of a nitrogen-fixing cyanobacterium. *Arch Microbiol* **110:** 145-147.

Wolk, C.P., Austin, S.M., Bortins, J., and Galonsky, A. (1974) Autoradiographic localization of ^{13}N after fixation of ^{13}N-labeled nitrogen gas by a heterocyst-forming blue-green alga. *J Cell Biol* **61:** 440-453.

Wylie, J.L., and Worobec, E.A. (1995) The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*. *J Bacteriol* **177:** 3021-3026.

Zhang, C.C., Durand, M.C., Jeanjean, R., and Joset, F. (1989) Molecular and genetical analysis of the fructose-glucose transport system in the cyanobacterium *Synechocystis* PCC6803. *Mol Microbiol* **3:** 1221-1229.

Table 1. Esculin uptake in *Anabaena* and some mutant strains.

Strain	Genotype	Product of the mutated gene	Esculin uptake (nmol [mg Chl]$^{-1}$ min^{-1})			
			BG11		$BG11_0$	
			Mean ± SEM (n)	% of WT (*p*)	Mean ± SEM (n)	% of WT (*p*)
PCC 7120	Wild type (WT)		0.159 ± 0.010 (30)		0.282 ± 0.018 (24)	
CSMN17	*all1823*::pCSL145	NBD (GlsD)	0.037 ± 0.010 (5)	23.3 % (<0.001)	0.020 ± 0.007 (5)	7.1 % (<0.001)
CSMN18	*all1916*::pCSL145	SBP (GlsR)	0.091 ± 0.010 (5)	57.2 % (0.013)	0.134 ± 0.012 (5)	53.9 % (0.003)
CSMN19	*alr2532*::pCSL145	TMD (GlsQ)	0.143 ± 0.023 (6)	89.9 % (0.536)	0.132 ± 0.016 (5)	46.8 % (0.001)

Filaments grown in BG11 medium (in the presence of 5 µg neomycin sulfate [Nm] mL^{-1} for the mutants) were washed and resuspended in BG11 or $BG11_0$ (BG11 medium lacking $NaNO_3$) media without Nm and incubated for 18 h under culture conditions. Filaments were then resuspended in the same media supplemented with 10 mM HEPES-NaOH (pH 7) and used in assays of uptake of 100 µM esculin as described in Suppl. Experimental procedures. Data are mean and SEM of the results from the indicated number of assays performed with independent cultures. Significance of the difference between each mutant and the wild type was assessed by the Student's *t* test; *p* is indicated in each case. NBD, nucleotide-binding domain; SBP, substrate-binding protein; TMD, transmembrane domain.

Table 2. Transfer of calcein and 5-CF between nitrate-grown vegetative cells in *Anabaena* and ABC glucoside transporter mutant strains[a].

Strain (mutated genes)	**Calcein transfer (*R*, s^{-1})**		**5-CF transfer (*R*, s^{-1})**	
	Mean ± SEM (n)	% of WT (*p*)	Mean ± SEM (n)	% of WT (*p*)
PCC 7120 (WT)	0.068 ± 0.006 (64)		0.090 ± 0.004 (160)	
CSMN17 (*glsD*)	0.047 ± 0.007 (34)	69 % (0.029)	0.110 ± 0.005 (33)	122 % (0.015)
CSMN18 (*glsR*)	0.040 ± 0.006 (32)	59 % (0.004)	0.085 ± 0.011 (25)	94 % (0.651)
CSMN19 (*glsQ*)	0.029 ± 0.011 (22)	43 % (0.002)	0.064 ± 0.006 (44)	71 % (0.001)

[a] Filaments of the wild type and the indicated mutants grown in BG11 medium (with Nm for the mutants) and incubated in BG11 medium without Nm for 18 to 24 h were used in FRAP analysis as described in Suppl. Experimental procedures. Data (*R*, recovery constant) are the mean and SEM of the results obtained with the indicated number of filaments (n) subjected to FRAP analysis. Filaments from eight cultures of the WT or three cultures from each mutant were used for calcein, and filaments from 12 cultures of the WT or two cultures from each mutant were used for 5-CF. Student's *t* test (mutant vs. wild type) *p* is indicated in each case.

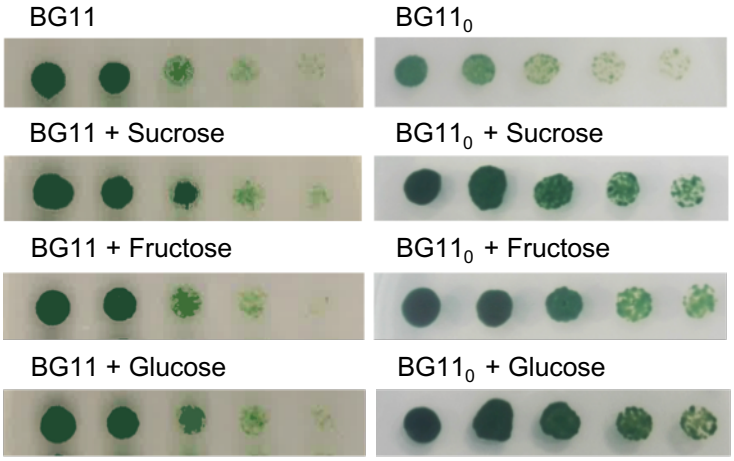

Fig. 1. Growth of *Anabaena* on solid BG11 or BG11$_0$ medium. Media were solidified with 1 % Bacto agar and supplemented with 10 mM TES-NaOH (pH 7.5) buffer and, when indicated, 100 mM sucrose, fructose or glucose. Filaments grown in BG11 medium were collected, washed with BG11$_0$ medium and spotted on plates as shown (successively diluted spots contained 10, 5, 2.5, 1.25, 0.625 ng chlorophyll *a*). The plates were incubated for 6 days (BG11 medium) or 10 days (BG11$_0$ medium) at 30 ºC in the light (ca. 25 µmol photons m^{-2} s^{-1}) in an air atmosphere and photographed.

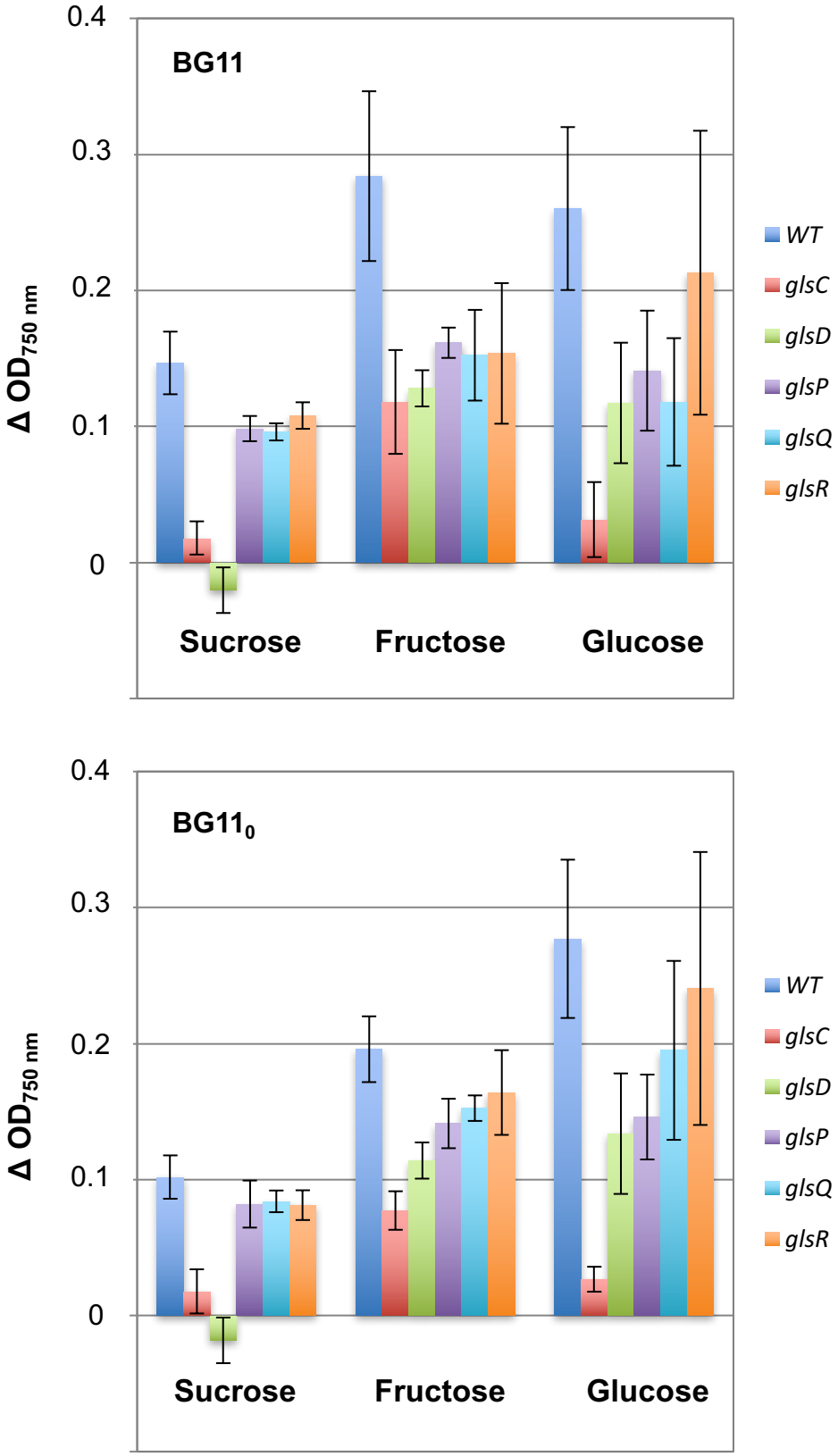

Fig. 2. Sugar-stimulated growth in *Anabaena* and some ABC glucoside uptake transporter mutants. The increase in growth yield in standing liquid cultures in response to the addition of 50 mM of the indicated sugar (i.e., $OD_{750\ nm}$ with sugar less $OD_{750\ nm}$ without sugar) is presented as the mean and SEM. See Table S2 for the complete set of data.

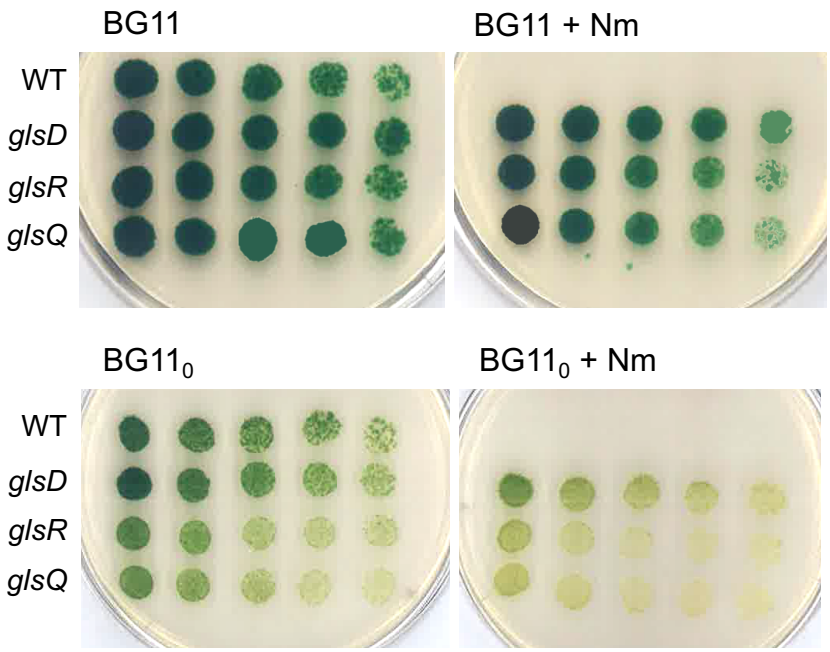

Fig. 3. Growth of strains CSMN17 (*glsD*::pCSL145), CSMN18 (*glsR*::pCSL145) and CSMN19 (*glsQ*::pCSL145) on solid BG11 or $BG11_0$ medium. Filaments grown in BG11 medium (in the presence of Nm for the mutants) were collected, washed with $BG11_0$ medium and spotted on plates as shown (successively diluted spots contained 10, 5, 2.5, 1.25, 0.625 ng chlorophyll *a*). The plates were incubated for 8 days (BG11 medium) or 14 days ($BG11_0$ medium) and photographed. The growth medium in the left panels was not supplemented with Nm to allow comparison with wild-type *Anabaena* (WT). Addition of Nm (right panels) inhibited growth of the wild type as expected.

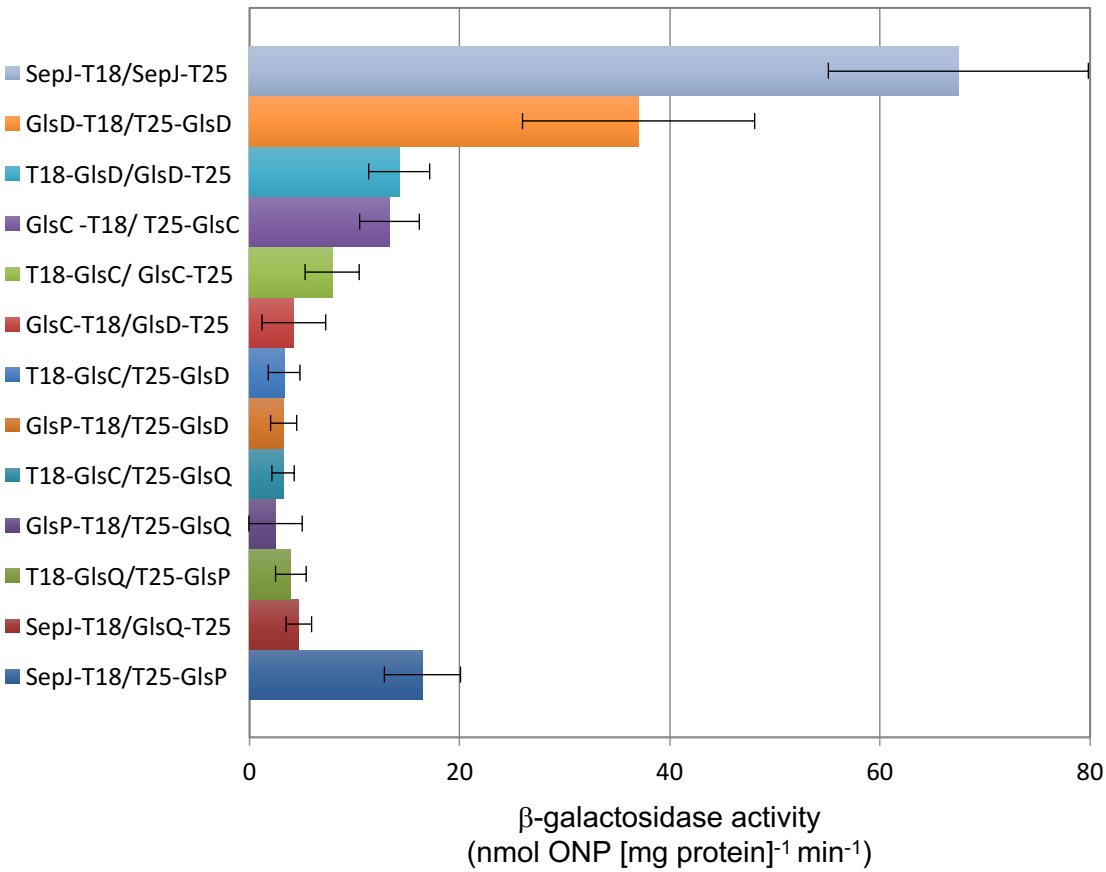

Figure 4. Bacterial two-hybrid analysis of interactions between Gls proteins and between Gls proteins and SepJ. Interactions of T25- and T18-fusion proteins produced in *E. coli* were measured as β-galactosidase activity in liquid cultures. The protein fused to the N- or the C-terminus of T18 or T25 is indicated in each case (N-terminus, protein-T18 or protein-T25; C-terminus, T18-protein or T25-protein). Data are mean and SEM of the activity with the indicated pair of fusion proteins less the activity of the T25/T18 negative control. Only interactions that were significant at $p < 0.05$ are shown. See Table S3 for the complete set of data.

Supplementary experimental procedures

Strains and growth conditions

Anabaena sp. strain PCC 7120 (also known as *Nostoc* sp.) and derivative strains were grown in BG11 medium modified to contain ferric citrate instead of ferric ammonium citrate (Rippka *et al.*, 1979) or $BG11_0$ medium (BG11 further modified by omission of $NaNO_3$) at 30 ºC in the light (ca. 25 µmol photons m^{-2} s^{-1}), in shaken (100 r.p.m.) liquid cultures or in plates in medium solidified with 1 % Bacto agar. When indicated, BG11 or $BG11_0$ liquid medium was supplemented with 50 mM sucrose, fructose, glucose or maltose and 10 mM TES-NaOH buffer (pH 7.5), all sterilized by filtration, and production of biomass was determined as OD_{750nm} after 7 days of incubation without shaking. When appropriate, neomycin sulfate (Nm) was added to the cyanobacterial cultures at the following concentrations: 5 µg ml^{-1} (liquid cultures) or 30-40 µg ml^{-1} (solid media). Chlorophyll *a* content of cultures was determined by the method of Mackinney (1941).

Escherichia coli strains were grown in LB medium, supplemented when appropriate with antibiotics at standard concentrations (Ausubel *et al.*, 2014). *E. coli* strain DH5α was used for plasmid constructions. This strain, bearing a conjugative plasmid, and strain HB101 bearing a methylase-encoding helper plasmid and one cargo plasmid were used for conjugation with *Anabaena* (Elhai *et al.*, 1997).

Construction of mutant strains

For inactivation of *all1823*, *all1916*, and *alr2532*, internal fragments of 738 bp, 708 bp and 564 bp, respectively, were amplified by PCR using primers all1823-1/all1823-2, all1916-1/all1916-2, alr2532-1/alr2532-2, respectively. All primers bore SacI sites in their 5' ends and are described in Table S4. Genomic DNA from *Anabaena* was used as a template. The amplified fragments were cloned into vector pSpark I producing pCSMN36, pCSMN37 and pCSMN38, and were transferred as SacI-fragments to SacI-digested pCSL145 (mobilizable plasmid containing cassette C.K1 [Nm^R]; Elhai and Wolk, 1988) producing pCSMN39, pCSMN40 and pCSMN41, which were verified by sequencing. These plasmids were transformed into *E. coli* HB101 [pRL623] and transferred to *Anabaena* by conjugation with selection for Nm^R. Clones that had incorporated pCSL145 by single recombination were selected for further study and named strain CSMN17, CSMN18 and CSMN19, respectively. The genetic structure of selected clones was studied by PCR to test recombination in the correct genomic location and test for segregation with the primer pairs indicated in Fig. S12.

Physiological parameters

To test uptake of esculin, *Anabaena* cultures grown in BG11 medium —with antibiotics for the mutants— were harvested by centrifugation, washed with BG11 or $BG11_0$ medium without antibiotics and incubated for 18 h in the same medium under culture conditions. Cells were harvested, washed and resuspended in growth medium supplemented with 10 mM HEPES-NaOH buffer (pH 7). Assays of uptake were started by addition of esculin hydrate (Sygma-Aldrich) at 100 µM, and cell suspensions were withdrawn and filtered. Cells on the filters were washed with 10 mM HEPES-NaOH buffer (pH 7) were resuspended in 2 ml of the same buffer. Fluorescence of the resulting cell suspension was measured in a Varian Cary Eclipse Fluorescence Spectrophotometer (excitation 360 ± 10 nm; emission 462 ± 10 nm). Esculin solutions in the same buffer were used as standards.

For calcein and 5-CF transfer assays (FRAP analysis), calcein and 5-CF staining were performed as previously reported (Mullineaux *et al.*, 2008; Merino-Puerto *et al.*, 2011). Cell suspensions were spotted onto agar and placed in a temperature-controlled sample holder with a glass cover slip on top. All measurements were carried out at 30 °C. For both calcein and 5-

CF, cells were imaged with a Leica HCX Plan Apo 63X, 1.4-NA oil immersion objective attached to a Leica TCS SP5 confocal laser-scanning microscope with a 488-nm line argon laser as the excitation source. Fluorescent emission was monitored by collection across windows of 500 to 520 nm and a 150-µm pinhole. After an initial image was recorded, the bleach was carried out by an automated FRAP routine as previously reported (Mullineaux *et al.*, 2008). For FRAP data analysis, kinetics of transfer of the fluorescent tracer to a cell somewhere in the middle of a filament (i.e., with two cell junctions) was quantified. The recovery rate constant, *R*, was calculated from the formula $C_B = C_0 + C_R (1 - e^{-2Rt})$, where C_B is fluorescence in the bleached cell, C_0 is fluorescence immediately after the bleach and tending towards ($C_0 + C_R$) after fluorescence recovery, t is time and *R* is the recovery rate constant due to transfer of the tracer from one neighbor cell (Nieves-Morion *et al.*, 2017).

For ^{14}C-labeled glucose uptake assays, filaments were grown in BG11 and $BG11_0$ media, harvested at room temperature, washed three times with BG11 or $BG11_0$ media and resuspended in the same media supplemented with 10 mM HEPES-NaOH (pH 7), to give a cell density corresponding to 10 µg chlorophyll *a* ml^{-1}. The assay was started by adding 0.2 ml of a sugar solution containing 10, 100 or 500 mM glucose and a small amount of [^{14}C]glucose (300 Ci mol^{-1}; American Radiolabeled Chemicals) to a 1.8-ml suspension. The filament suspensions were incubated at 30 ºC in the light (85 µmol m^{-2} s^{-1}), and 0.6-ml samples (taken at 20, 40 and 60 min) were filtered using 0.45-mm pore size Millipore HA filters. After washing with 10 mM HEPES-NaOH (pH 7) to remove excess labeled sugar, the filters were placed in a scintillation cocktail and their radioactivity was measured. Nonspecific retention of radioactivity was determined by using boiled cells.

Immunolocalization of SepJ

Cells from 1.5 ml of liquid cultures were collected by centrifugation, placed atop a poly-L-lysine-precoated microscope slide, and covered with a 45-µm-pore-size Millipore filter. The filter was removed and the slide was left to dry at room temperature, immersed in 70% ethanol at -20 ºC for 30 min, and dried for 15 min at room temperature. The cells were washed twice (2 min each time, room temperature) by covering the slide with PBS-T (Phosphate Buffered Saline supplemented with 0.05% Tween 20). Subsequently, the slides were treated with a blocking buffer (5% milk powder in PBS-T) for 15 min. Cells on the slides were then incubated for 90 min with anti-SepJ-CC antibodies (Mariscal *et al.*, 2011) diluted in blocking buffer (1:250), washed three times with PBS-T, incubated for 45 min in the dark with anti-rabbit antibody conjugated to FITC (1:500 dilution in PBS-T; Sigma), and washed three times with PBS-T. After dried, several drops of FluorSave (Calbiochem) were added atop, covered with a coverslip, and sealed with nail lacquer. Fluorescence was imaged using a Leica DM6000B fluorescence microscope and an ORCA-ER camera (Hamamatsu). Fluorescence was monitored using a FITC L5 filter (excitation, band-pass [BP] 480/40 filter; emission, BP 527/30 filter), and images were analyzed with ImageJ software (http://imagej.nih.gov/ij).

BACTH strain construction and assays

The possible interaction between different glucoside transporter components and between them and SepJ was tested using Bacterial Adenylate Cyclase Two Hybrid (BACTH) analysis. All tested genes were amplified using *Anabaena* DNA as template. The following primers were used: all1823-5 and all1823-6 to amplify *glsD*, and alr2532-4 and alr2532-5 to amplify *glsQ*. Other constructs were previously described (Ramos-León *et al.*, 2015; Nieves-Morión *et al.*, 2017). The PCR products were transferred as XbaI- and KpnI-digested fragments to pUT18, pUT18C, pKNT25, and pKT25 (Battesti *et al.*, 2012), producing fusions to the 5' and 3' ends of the genes encoding the adenylate cyclase T18 and T25 fragments, respectively. The resulting plasmids (pCSMN59: *all1823*::pUT18; pCSMN60: *all1823*::pUT18C; pCSMN61: *all1823*::pKT25; pCSMN62: *all1823*::pKTN25; pCSMN63: *alr2532*::pUT18; pCSMN64: *alr2532*::pUT18C;

pCSMN65: *alr2532*::pKT25; pCSMN66: *alr2532*::pKTN25) were transformed into *E. coli* XL1-Blue for amplification. Isolated plasmids were cotransformed into *E. coli* BTH101 (cya-99). Transformants were plated onto LB medium containing selective antibiotics and 1% glucose. Efficiencies of interactions between different hybrid proteins were quantified in cells from liquid cultures by measuring β-galactosidase activity as previously described (Nieves-Morión *et al.*, 2017). The amount of *o*-nitrophenol produced from *o*-nitrophenol-β-galactoside (ONPG) was determined and referred to the amount of protein determined by a modified Lowry procedure (Bailey, 1967).

References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (2014) Current Protocols in Molecular Biology. New York: *Greene Publishing and Wiley-Interscience.*

Bailey, J.L. (1967) Techniques in protein chemistry, 2nd ed., p. 340. Elsevier, Amsterdam.

Battesti, A., and Bouveret, E. (2012) The bacterial two-hybrid system based on adenylate cyclase reconstitution in *Escherichia coli*. *Methods* **58:** 325-334.

Elhai, J., and Wolk, C.P. (1988) A versatile class of positive-selection vectors based on the nonviability of palindrome-containing plasmids that allows cloning into long polylinkers. *Gene* **68:** 119-138.

Elhai, J., Vepritskiy, A., Muro-Pastor, A.M., Flores, E., and Wolk, C.P. (1997) Reduction of conjugal transfer efficiency by three restriction activities of *Anabaena* sp. strain PCC 7120. *J Bacteriol* **179:** 1998-2005.

Mackinney, G. (1941) Absorption of light by chlorophyll solutions. *J Biol Chem* **140**: 315-322.

Mariscal, V., Herrero, A., Nenninger, A., Mullineaux, C.W., and Flores, E. (2011) Functional dissection of the three-domain SepJ protein joining the cells in cyanobacterial trichomes. *Mol Microbiol* **79:** 1077-1088.

Merino-Puerto, V., Schwarz, H., Maldener, I., Mariscal, V., Mullineaux, C.W., Herrero, A., and Flores, E. (2011) FraC/FraD-dependent intercellular molecular exchange in the filaments of a heterocyst-forming cyanobacterium, *Anabaena* sp. *Mol Microbiol* **82:** 87-98.

Mullineaux, C.W., Mariscal, V., Nenninger, A., Khanum, H., Herrero, A., Flores, E., and Adams, D.G. (2008) Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. *EMBO J* **27**: 1299-1308.

Nieves-Morión, M., Lechno-Yossef, S., López-Igual, R., Frías, J.E., Mariscal, V., Nürnberg, D.J., Mullineaux, C.W., Wolk, C.P., and Flores, E. (2017) Specific glucoside transporters influence septal structure and function in the filamentous, heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* **199:** e00876-16.

Ramos-León, F., Mariscal, V., Frías, J.E., Flores, E., and Herrero, A. (2015) Divisome-dependent subcellular localization of cell-cell joining protein SepJ in the filamentous cyanobacterium *Anabaena*. *Mol Microbiol* **96:** 566-580.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* **111:** 1-61.

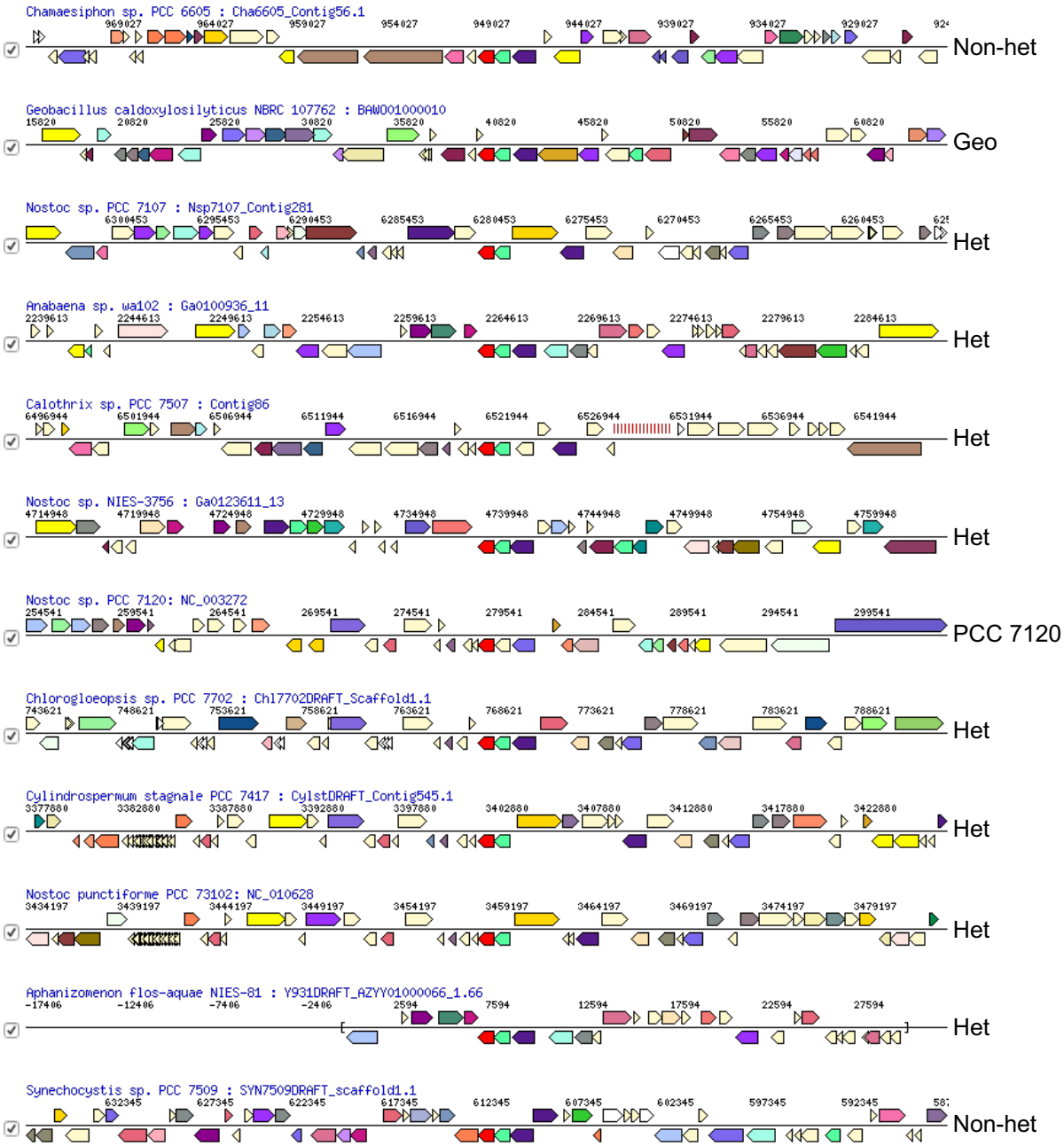

Figure S1. Genomic region of *Anabaena* (*Nostoc*) sp. strain PCC 7120 ORF *all0261* (*glsP*) and some examples of genomic regions containing an *all0261* orthologue. Whereas *all0261* is not accompanied by any ABC component-encoding gene, the *all0261* orthologues shown are accompanied by another TMD-encoding gene (colored green) or by TMD- and SBP-encoding genes (SBP gene colored dark purple). Het, heterocyst-forming cyanobacterium; Non-het, non-heterocyst-forming cyanobacterium. This gene cluster is also present in some bacteria other than cyanobacteria, and it is common in *Geobacillus* spp. (one strain shown [Geo]). Genomic regions retrieved from: https://img.jgi.doe.gov/cgi-bin/m/main.cgi.

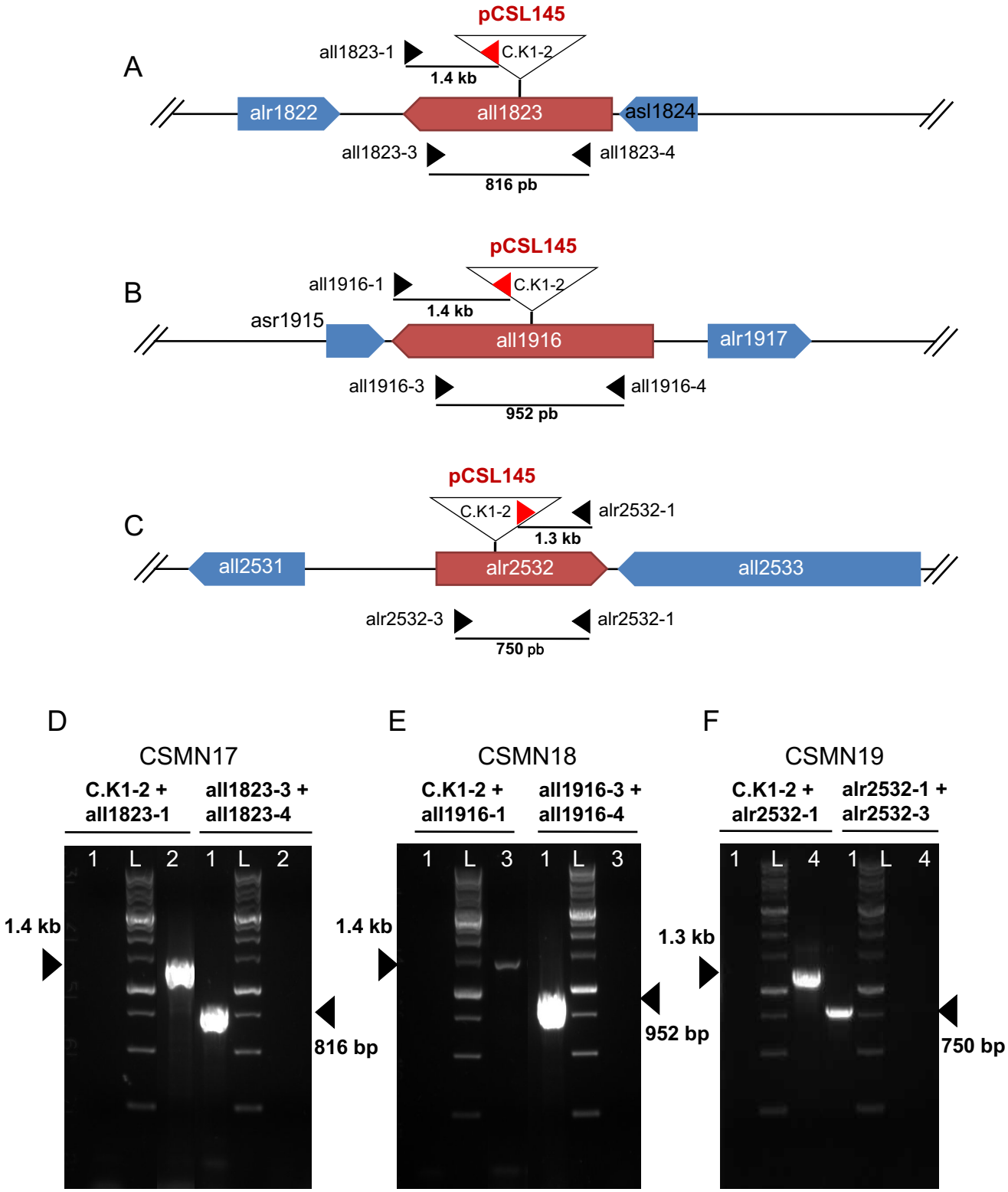

Figure S2. Construction and verification of mutants of genes *glsD*, *glsR* and *glsQ* in *Anabaena*. Schematic of the insertional mutation of genes (A) *all1823* (*glsD*), (B) *all1916* (*glsR*) and (C) *alr2532* (*glsQ*), with indication of their genomic regions, the inserted plasmid, and primers used in PCR analysis (black triangles, primers corresponding to the *Anabaena* genes; red triangle, primer corresponding to cassette C.K1 present in the inserted plasmid). (D, E, F) Verification of strains by colony PCR. L, 1-kb DNA ladder. Primer pairs are indicated on top. Templates: 1, wild-type DNA; 2, DNA from mutant CSMN17 (*glsD*; panel D); 3, DNA from mutant CSMN18 (*glsR*; panel E); 4, DNA from mutant CSMN19 (*glsQ;* panel F).

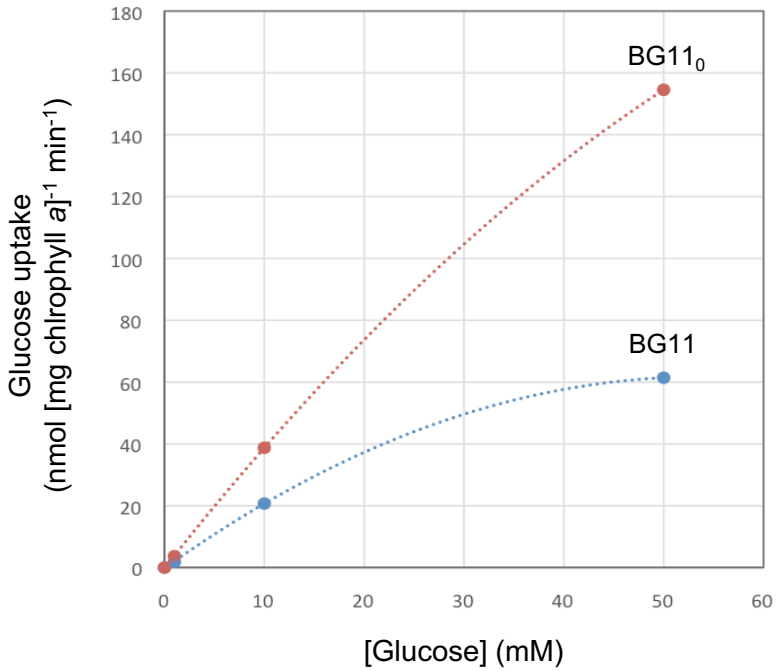

Figure S3. Uptake rates of [^{14}C]glucose in wild-type *Anabaena*. Filaments grown in BG11 medium or grown in BG11 medium and incubated for 18 h in BG11$_0$ medium were used in uptake assays with 1, 10 or 50 mM [^{14}C]glucose as described in Suppl. Experimental procedures.

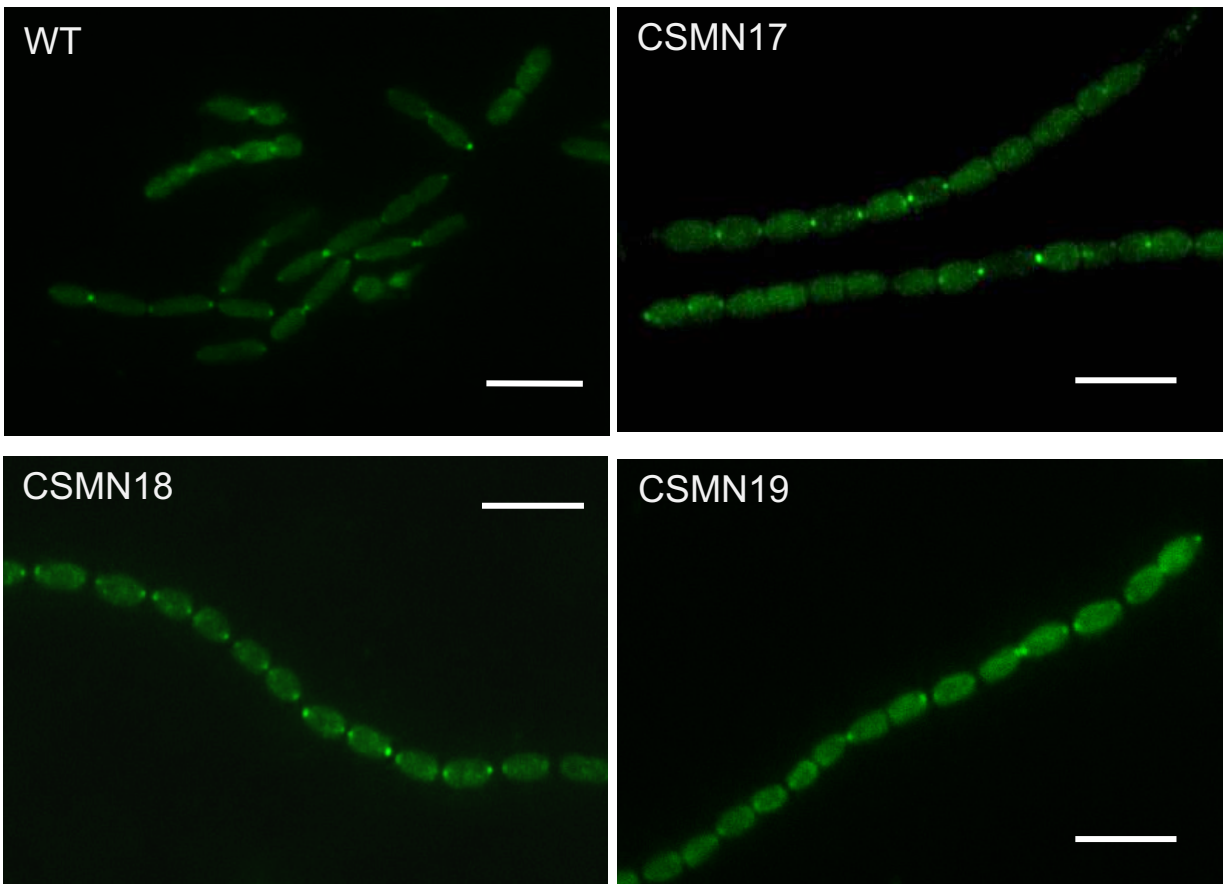

Figure S4. Immunofluorescence localization of SepJ in wild-type *Anabaena* (WT) and *glsD* mutant CSMN17 (*all1823*::pCSL145), *glsR* mutant CSMN18 (*all1916*::pCSL145) and *glsQ* mutant CSMN19 (*alr2532*::pCSL145). Filaments were grown in BG11 medium (with Nm for the mutants) and subjected to immunofluorescence analysis with anti-SepJ coiled coil antibodies. Antibody green fluorescence is shown. Brightness and contrast were adjusted to improve visibility. Size bars, 10 μm.

Table S1. ORFs from the *Anabaena* genome predicted to encode components of ABC sugar uptake transporters.

ORF	Type of subunit	TCDB family	*p*	Substrate	Protein
all1027	SBP	3.A.1.1.40	10^{-64}	Chitobiose	
all1916	SBP	3.A.1.1.20	10^{-25}	Fructo-oligosaccharide	GlsR
alr2722	SBP	3.A.1.1.7	10^{-21}	Trehalose/maltose	
alr4277	SBP	3.A.1.1.20	10^{-32}	Fructo-oligosaccharide	
all0261	TMD^{G}	3.A.1.1.34	10^{-59}	Arabino-saccharide	GlsP
alr0738	TMD^{F}	3.A.1.1.20	10^{-61}	Fructo-oligosaccharide	
alr0789	TMD^{F}	3.A.1.1.4	10^{-55}	Lactose	
alr2532	TMD^{F}	3.A.1.1.20	10^{-60}	Fructo-oligosaccharide	GlsQ
all4824	TMD^{G}	3.A.1.1.34	10^{-53}	Arabino-saccharide	
all5282	TMD^{G}	3.A.1.1.41	10^{-51}	Trehalose/maltose/sucrose	
all1823	NBD	3.A.1.1.25	10^{-119}	Trehalose/maltose/sucrose	GlsD
alr4781	NBD	3.A.1.1.25	10^{-111}	Trehalose/maltose/sucrose	GlsC

The type of subunit (transmembrane domain, TMD; periplasmic solute-binding protein, SBP; nucleotide-binding protein or ATPase domain, NBD) and TCDB family (http://www.tcdb.org) to which the *Anabaena* ORF products are most similar, along with the expect probability (*p*) in the BLAST analysis, are indicated. The substrates that have been described for those transporter families are also shown. Note that all these transport proteins belong to the 3.A.1.1 family (The Carbohydrate Uptake Transporter-1 [CUT1] Family), for which a well-known representative is the malto-oligosaccharide MalEFGK transporter of *Escherichia coli* (MalE, SBP; MalF and MalG, TMDs; MalK, NBD). Among the indicated permeases encoded in the *Anabaena* genome, three are most similar to MalF and three to MalG (marked as superscripts). The right-hand column includes the names of the products of the genes that we have inactivated. Four additional ORFs in *Anabaena*, *alr5361, alr5362, alr5367* and *alr5368*, may encode a periplasmic SBP, an NBD and two TMDs, respectively, of an ABC nucleoside transporter.

Table S2. Sugar-stimulated growth in *Anabaena* and some ABC glucoside uptake transporter mutant strains.

A			**$OD_{750\ nm}$**							
Strain	Genotype	Prot.	**BG11 medium, sugar added:**				**$BG11_0$ medium, sugar added:**			
			None	Sucrose	Fructose	Glucose	None	Sucrose	Fructose	Glucose
WT	Wild type (WT)		0.185 ± 0.020 (7)	0.330 ± 0.046 (6) **[0.011]**	0.471 ± 0.089 (5) **[0.004]**	0.449 ± 0.080 (6) **[0.005]**	0.173 ± 0.013 (7)	0.275 ± 0.012 (7) **[<0.001]**	0.372 ± 0.027 (6) **[<0.001]**	0.453 ± 0.069 (6) **[0.001]**
DR3912a	*alr4781*::C.S3	GlsC	0.123 ± 0.012 (5)	0.141 ± 0.019 (5) [0.442]	0.237 ± 0.048 (4) **[0.036]**	0.151 ± 0.039 (4) [0.479]	0.124 ± 0.012 (5)	0.142 ± 0.022 (5) [0.494]	0.197 ± 0.027 (4) **[0.031]**	0.147 ± 0.022 (4) [0.365]
CSMN17	*all1823*::pCSL145	GlsD	0.165 ± 0.029 (5)	0.144 ± 0.018 (5) [0.564]	0.297 ± 0.069 (4) [0.095]	0.286 ± 0.086 (4) [0.211]	0.159 ± 0.026 (5)	0.141 ± 0.013 (5) [0.548]	0.278 ± 0.039 (4) **[0.033]**	0.298 ± 0.074 (4) [0.093]
DR3915	*all0261*::C.S3	GlsP	0.198 ± 0.020 (5)	0.296 ± 0.013 (5) **[0.003]**	0.360 ± 0.035 (4) [0.127]	0.340 ± 0.064 (4) [0.060]	0.118 ± 0.010 (5)	0.200 ± 0.026 (5) **[0.020]**	0.264 ± 0.029 (4) **[0.001]**	0.269 ± 0.026 (4) **[0.001]**
CSMN19	*alr2532*::pCSL145	GlsQ	0.185 ± 0.020 (5)	0.263 ± 0.011 (4) **[0.017]**	0.318 ± 0.039 (3) **[0.014]**	0.307 ± 0.066 (4) [0.092]	0.150 ± 0.016 (5)	0.234 ± 0.017 (5) **[0.007]**	0.299 ± 0.012 (4) **[<0.001]**	0.341 ± 0.058 (4) **[0.010]**
CSMN18	*all1916*::pCSL145	GlsR	0.174 ± 0.021 (5)	0.269 ± 0.029 (4) **[0.031]**	0.315 ± 0.081 (3) [0.074]	0.391 ± 0.131 (4) [0.107]	0.158 ± 0.017 (5)	0.239 ± 0.012 (5) **[0.005]**	0.325 ± 0.043 (4) **[0.006]**	0.402 ± 0.116 (4) [0.052]

B			**Δ OD_{750nm} (sugar-no sugar)**					
Strain	Genotype	Prot.	**BG11 medium, sugar added:**			**$BG11_0$ medium, sugar added:**		
			Sucrose	Fructose	Glucose	Sucrose	Fructose	Glucose
WT	Wild type (WT)		0.147 ± 0.023 (6)	0.284 ± 0.062 (5)	0.260 ± 0.060 (6)	0.102 ± 0.016 (7)	0.196 ± 0.024 (6)	0.277 ± 0.058 (6)
DR3912a	*alr4781*::C.S3	GlsC	0.018 ± 0.012 (5) **[0.001]**	0.118 ± 0.038 (4) [0.072]	0.032 ± 0.028 (4) **[0.019]**	0.018 ± 0.016 (5) **[0.005]**	0.077 ± 0.014 (4) **[0.006]**	0.027 ± 0.009 (4) **[0.009]**
CSMN17	*all1823*::pCSL145	GlsD	-0.020 ± 0.014 (5) **[<0.001]**	0.128 ± 0.033 (4) [0.081]	0.117 ± 0.051 (4) [0.131]	-0.018 ± 0.017 (5) **[<0.001]**	0.114 ± 0.013 (4) **[0.034]**	0.134 ± 0.044 (4) [0.114]
DR3915	*all0261*::C.S3	GlsP	0.098 ± 0.009 (5) [0.105]	0.162 ± 0.011 (4) [0.130]	0.141 ± 0.044 (4) [0.187]	0.082 ± 0.017 (5) [0.425]	0.141 ± 0.018 (4) [0.142]	0.146 ± 0.031 (4) [0.127]
CSMN19	*alr2532*::pCSL145	GlsQ	0.096 ± 0.006 (4) [0.121]	0.152 ± 0.033 (3) [0.180]	0.118 ± 0.047 (4) [0.127]	0.084 ± 0.008 (5) [0.398]	0.153 ± 0.009 (4) [0.201]	0.195 ± 0.066 (4) [0.386]
CSMN18	*all1916*::pCSL145	GlsR	0.108 ± 0.010 (4) [0.231]	0.154 ± 0.052 (3) [0.204]	0.213 ± 0.104 (4) [0.683]	0.081 ± 0.011 (5) [0.352]	0.164 ± 0.031 (4) [0.438]	0.241 ± 0.100 (4) [0.743]

Filaments grown in BG11 medium (in the presence of Nm for the mutants) were washed with $BG11_0$ medium without Nm and inoculated in 2 mL of BG11 or $BG11_0$ medium supplemented with 10 mM TES-NaOH (pH 7.5). The suspensions, in microtiter plates, were incubated under air in the light (25 µmol photons m^{-2} s^{-1}) at 30 ºC for 1 week, and their $OD_{750\ nm}$ was determined after careful homogenization. Axenicity of the cultures was corroborated by microscopic analysis and plating in LB medium and further incubation at 30 ºC. (A) Data are the mean and SEM of the results from the number of assays (performed with independent cultures) indicated in parenthesis. Significance of the difference of each strain with and without a sugar added was assessed by the Student's *t* test; *p* indicated in each case in brackets; bold face, $p < 0.05$. (B) The increase in growth yield in response to addition of a sugar (i.e., $OD_{750\ nm}$ with sugar less $OD_{750\ nm}$ without sugar, data from part A of the table) is presented. The difference between each mutant and the wild type for each sugar was assessed by the Student's *t* test; *p* indicated in each case in brackets; bold face, $p < 0.05$.

Table S3. Bacterial two-hybrid analysis of protein-protein interactions.

	T18	SepJ-T18	GlsC-T18	T18-GlsC	GlsP-T18	T18-GlsP	GlsD-T18	T18-GlsD	GlsQ-T18	T18-GlsQ
T25	12.49 ± 0.53 (21)	*10.88 ± 0.43 (6)*	*13.01 ± 1.74 (4)*	*12.34 ± 0.40 (4)*	*12.11 ± 2.05 (4)*	*10.95 ± 1.68 (4)*	13.14 ± 1.41 (3)	10.91 ± 1.11 (3)	10.08 ± 1.58 (2)	6.20 (1)
SepJ-T25	*11.22 ± 2.04 (2)*	*80.12 ± 12.34 (9) [<0.001]*	*13.25 ± 1.30 (5) [0.951]*	*15.75 ± 6.53 (3) [0.494]*	*11.74 ± 1.65 (5) [0.394]*	*14.66 ± 1.06 (4) [0.298]*	11.21 ± 1.47 (3) [0.404]	11.23 ± 1.77 (3) [0.423]	12.03 ± 1.84 (3) [0.778]	14.58 ± 3.43 (4) [0.266]
GlsC-T25	Nd	*11.66 ± 0.81 (6) [0.221]*	*12.52 ± 1.66 (4) [0.695]*	*20.52 ± 2.45 (4) [0.003]*	*12.36 ± 0.55 (3) [0.587]*	*13.00 ± 3.30 (3) [0.944]*	13.51 ± 1.13 (3) [0.498]	11.80 ± 1.33 (3) [0.647]	11.68 ± 1.79 (3) [0.604]	14.27 ± 1.83 (3) [0.263]
T25-GlsC	Nd	*13.09 ± 1.25 (6) [0.963]*	*25.98 ± 2.73 (4) [<0.001]*	*15.93 ± 2.59 (6) [0.247]*	*12,72 ± 1.55 (4) [0.783]*	*12.79 ± 2.15 (3) [0.842]*	11.74 ± 2.25 (3) [0.646]	12.32 ± 0.87 (3) [0.908]	12.03 ± 1.84 (3) [0.770]	9.20 ± 0.20 (2) [0.075]
GlsP-T25	Nd	*14.34 ± 0.84 (7) [0.324]*	*12.32 ± 1.39 (5) [0.579]*	*14.19 ± 3.72 (4) [0.702]*	*12.16 ± 1.06 (4) [0.484]*	*11.64 ± 2.35 (3) [0.431]*	11.84 ± 2.11 (3) [0.687]	10.96 ± 0.70 (3) [0.302]	9.30 ± 0.50 (2) [0.084]	9.21 ± 0.09 (2) [0.076]
T25-GlsP	Nd	*29.12 ± 3.54 (7) [<0.001]*	*11.80 ± 1.02 (5) [0.314]*	*15.48 ± 4.14 (4) [0.435]*	*11.85 ± 0.32 (3) [0.376]*	*12.04 ± 3.52 (3) [0.638]*	11.33 ± 2.31 (3) [0.480]	12.24 ± 1.74 (3) [0.872]	10.76 ± 0.47 (3) [0.241]	**16.59 ± 1.36 (4) [0.006]**
GlsD-T25	13.1 (1)	14.4 ± 1.51 (6) [0.136]	**16.9 ± 2.99 (4) [0.017]**	14.6 ± 1.29 (6) [0.090]	13.8 ± 1.22 (5) [0.309]	11.9 ± 0.68 (3) [0.710]	12.42 ± 1.20 (3) [0.961]	**26.90 ± 2.85 (3) [2.23 10^{-8}]**	11.37 ± 3.17 (3) [0.528]	14.86 ± 1.84 (6) [0.097]
T25-GlsD	14.5 (1)	13.1 ± 0.87 (4) [0.639]	11.6 ± 0.50 (4) [0.472]	**16 ± 1.41 (4) [0.018]**	**15.9 ± 1.12 (5) [0.009]**	14 ± 0.58 (6) [0.171]	**49.69 ± 11.04 (3) [2.08 10^{-9}]**	11.23 ± 1.17 (3) [0.4041]	10.60 ± 1.79 (3) [0.233]	10.80 ± 1.50 (2) [0.355]
GlsQ-T25	15.2 (1)	**17.4 ± 1.09 (4) [0.001]**	13.1 ± 0.87 (3) [0.687]	14.7 ± 1.94 (4) [0.141]	13.3 ± 1.28 (5) [0.514]	11.5 ± 0.17 (3) [0.516]	10.50 ± 1.17 (3) [0.191]	10.54 ± 1.72 (3) [0.217]	9.40 ± 1.40 (3) [0.051]	14.42 ± 1.07 (6) [0.104]
T25-GlsQ	8.8 (1)	12.5 ± 0.58 (4) [0.966]	11.9 ± 2.51 (3) [0.732]	**15.8 ± 0.92 (5) [0.009]**	**15.9 ± 2.48 (6) [0.042]**	14.1 ± 1.72 (3) [0.300]	10.98 ± 0.65 (3) [0.307]	11.32 ± 2.01 (3) [0.464]	10.10 ± 1.71 (3) [0.133]	14.78 ± 0.99 (4) [0.091]

Interactions of T25- and T18-fusion proteins produced in *E. coli* were measured as β-galactosidase activity in liquid cultures. Activity corresponds to nmol *o*-nitrophenol produced (mg protein)$^{-1}$ min^{-1}. The protein fused to the N- or the C-terminus of T18 or T25 is indicated in each case (N-terminus, protein-T18 or protein-T25; C-terminus, T18-protein or T25-protein). The mean and SEM of the results obtained with the indicated number of independent transformants (n) is presented. The difference between each fusion protein combination and the T18/T25 pair was assessed by the Student's *t* test; bold type denotes significant differences (* $p \leq 0.05$; ** $p \leq 0.01$). All other combinations gave activities not significantly different from the T25/T18 control. Nd, not determined. Italics, data from Nieves-Morión *et al.* (2017).

Table S4. Oligodeoxynucleotide primers used in this work. Introduced restriction sites are underlined.

Primer name	**Sequence (5´ to 3´)**
all1823-1	AGAGAGCTCGATGTGGCGATGGTGTTC
all1823-2	TACGAGCTCATCGGGTAAACTCACACG
all1823-3	TGGTAAATAACATCCCCGCAAGAG
all1823-4	GATGTAGACGCACGTTTCCACT
all1823-5	TCTCTAGAAAAAGTTCGTTTAGAAGATATAAA
all1823-6	ACTGGTACCTCCTGGGGTGATATTTTA
all1916-1	TCTGAGCTCTATATGGATGTGATCAAAACC
all1916-2	TTCGAGCTCATGCTGTGATTGCTTACTCAT
all1916-3	ATCGCGATCGCCATTGTTG
all1916-4	GCATTCCGGCTTTACCTGTGA
all2532-1	ATCGAGCTCAGCTTGTTCGGATATGTATTCATG
alr2532-2	CTGGAGCTCTGCACCATCTAATTCTGC
alr2532-3	ACCATTTGATAAATCAGCAGCA
alr2532-4	GACTAAACTCTAGAGTTGCGAATCAGAAGGT
alr2532-5	ATAGGTACCTCACCAGCAAAAACTCG
C.K1-2	GGGATCTCATGCTGGAGT