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Roles of DevBCA-like ABC transporters in the physiology of *Anabaena* sp. PCC 7120

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ABSTRACT

The filamentous, photosynthetic cyanobacterium *Anabaena* sp. PCC 7120 can be considered as a true multicellular bacterium. Along the filament of cells, nitrogen fixation is spatially separated from the incompatible process of oxygenic photosynthesis by the formation of specialized heterocysts in a semiregular pattern. Heterocyst development involves many proteins, including a group of DevBCA-HgdD-like tripartite efflux pumps driven by ATP-binding cassette (ABC) transporters and that share similarity with MacAB or LolCDE transporters. In this minireview, we summarize the results from our studies of this group of transporters in *Anabaena* sp. PCC 7120 and discuss what remains to be elucidated.

1. Introduction

Cyanobacteria comprise an ancient, large, and morphologically diverse group of gram-negative bacteria. They were the first organisms to evolve oxygenic photosynthesis on Earth, thereby creating the oxic atmosphere (Adams and Duggan, 1999; Rippka et al., 1979). With their relatively fast growth rate and ease of genetic manipulation, cyanobacteria are promising organisms for bioengineering and for creating cell factories that use CO_2 to produce various useful chemicals, such biofuels and bioplastics (Angermayr et al., 2015; Oliver et al., 2016). However, these valuable photoautotrophs are also involved in processes with negative consequences. For instance, they sometimes form toxic blooms that influence ecosystems and consequently human health (Huisman et al., 2018).

The freshwater cyanobacterium *Anabaena* sp. PCC 7120 (hereafter *Anabaena* sp.) belongs to Section IV (filamentous, heterocystous cyanobacteria) according to the classification of (Rippka et al., 1979). This prokaryotic model organism is used for studying nitrogen fixation and cell differentiation.

Anabaena sp. forms non-branching filaments or trichomes composed of several hundred cells (Fig. 1) (Rippka et al., 1979). When grown in the presence of a combined nitrogen source, the trichomes consist mostly of vegetative cells, but when combined nitrogen is lacking, *Anabaena* sp. develops heterocysts that arise in the filaments in a semiregular pattern, every 10th to 20th cell. Heterocysts specialize in N_2 fixation and transport N-assimilation products to the neighboring vegetative cells. In turn, they obtain sugars produced by photosynthesis from the vegetative cells. Signaling molecules are transported along the filament to ensure the pattern of heterocysts and a coordinated behavior of this multicellular organism (Herrero et al., 2016). Molecule exchange occurs via cell–cell joining multiprotein complexes, called septal junctions, in the nanopores of the septal cell wall (Flores et al., 2018a, 2016; Weiss et al., 2018).

Heterocysts provide the required microoxic environment for the oxygen-sensitive N_2-fixing nitrogenase enzyme complex. This environment is formed during heterocyst development. Developing heterocysts inactivate photosystem II and degrade antenna pigments, thereby decreasing oxygenic photosynthesis (Fig. 1). In specialized honeycomb membranes at the poles of each heterocyst, oxygen-consuming respiration is enhanced. Heterocysts also build an additional envelope around the outer membrane comprised of two layers: an external heterocyst exopolysaccharide (hep) layer and an underlying heterocyst glycolipid layer (hgl). The hgl layer is made of heterocyst-specific glycolipids (HGLs) and is gas-tight to restrict oxygen infiltration into the heterocyst; the hep layer provides mechanical support for the hgl layer [reviewed in (Adams and Duggan, 1999; Herrero et al., 2016; Kumar et al., 2010; Maldener et al., 2014; Muro-Pastor and Hess, 2012; Nicolaisen et al., 2009; Wolk et al., 1994)].

The complexity of the lifestyle of *Anabaena* sp., with the ability to perform oxygenic photosynthesis, differentiate specialized cells, and fix N_2, demands a sophisticated cell envelope with various transporters for the import and export of different molecules. A search of the Cyanobase

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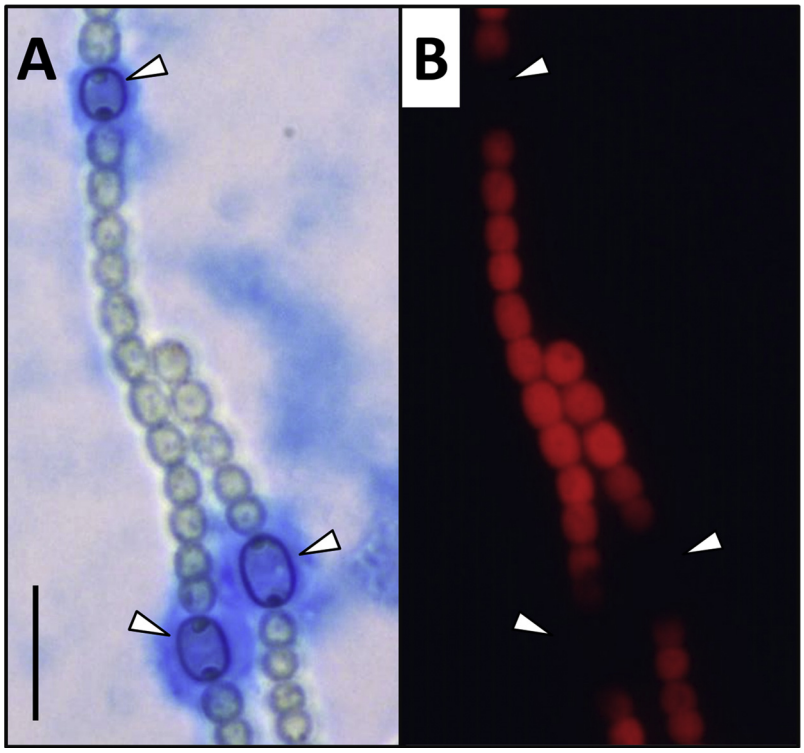

Fig. 1. (**A**) Bright-field and (**B**) fluorescent micrographs of *Anabaena* sp. PCC 7120 filaments of vegetative cells and heterocysts. (**A**) Heterocysts (white arrowheads) are stained with Alcian blue as described in Shvarev et al. (2018); (**B**) heterocysts (white arrowheads) show no red autofluorescence, whereas vegetative cells strongly autofluoresce owing to photosynthetic pigments. Bar, 10 µm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

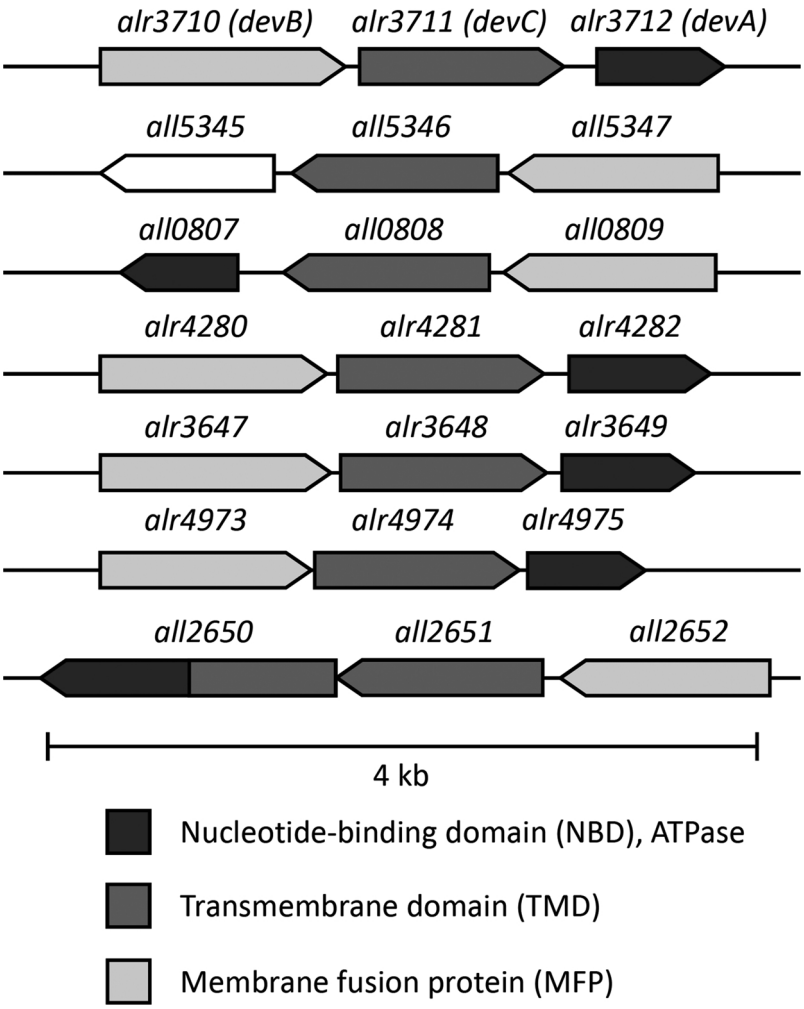

Fig. 2. Organization of gene clusters homologous to *devBCA* in the genome of *Anabaena* sp.

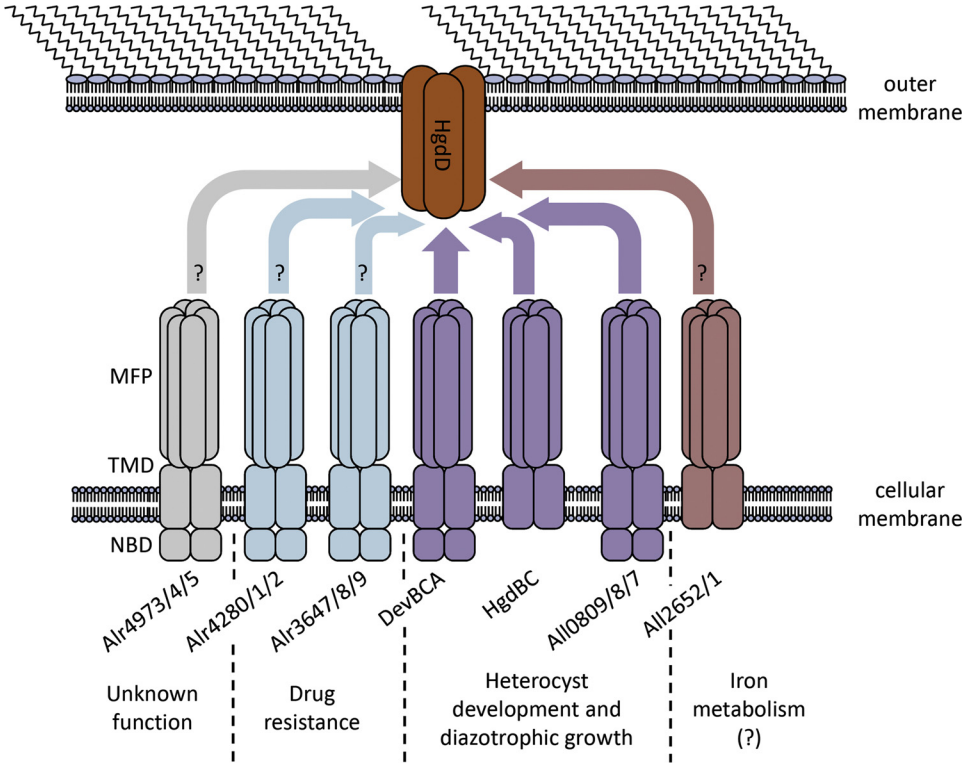

Fig. 3. Scheme showing the DevBCA-like ABC transporters in *Anabaena* sp. and their putative main functions. Blue, transporters essential for drug resistance; violet, transporters essential for heterocyst development and diazotrophic growth; red, transporter possibly involved in transfer of siderophores and iron metabolism; gray, transporter of unknown function. MFP, membrane fusion protein; TMD, transmembrane domain; NBD, nucleotide binding domain;?, interaction to HgdD has not been experimentally shown (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Sequence similarity of DevA, DevB, and DevC to the respective homologs in *Anabaena* sp. according to NCBI BLAST.

Homolog	Coverage	Identity	Length (amino acids)
DevA			
All0807	93%	63%	231
Alr4282	92%	70%	276
Alr3649	91%	70%	249
Alr4975	89%	74%	225
DevB			
All5347	95%	36%	399
All0809	95%	33%	406
Alr4280	93%	40%	436
Alr3647	90%	41%	434
Alr4973	97%	41%	399
All2652	96%	34%	396
DevC			
All5346	98%	49%	392
All0808	99%	52%	388
Alr4281	99%	52%	395
Alr3648	99%	53%	388
Alr4974	99%	57%	385
All2651	98%	41%	393

genome server (http://genome.microbedb.jp/CyanoBase) reveals around 200 genes in the genome of *Anabaena* sp. that are annotated as being transporter associated; the vast majority belong to the superfamily of ATP-binding cassette (ABC) transporters (Shvarev and Maldener, 2018). This superfamily is one of the largest protein families and has been extensively reviewed (Davidson et al., 2008; Greene et al., 2018; Higgins, 2001, 1992; Holland, 2011; Locher, 2016; Trowitzsch and Tampé, 2018; Wilkens, 2015). Most ABC transporters are homodimers, with each monomer consisting of a cytoplasmic nucleotide-binding domain (NBD) and a transmembrane domain (TMD). These domains can be located on one protein or on two separate proteins. TMDs form the route for substrate transport through the cell membrane; the conformational changes necessary for transport are enabled by ATP hydrolysis in the NBD.

In bacteria, both ABC importers and exporters exist. They fulfill various functions, e.g., nutrient uptake, secretion of cell envelope components, and drug resistance. Some ABC exporters of gram-negative bacteria interact with the outer membrane exit duct, such as the *Escherichia coli* outer membrane protein TolC, via specific periplasmic adaptor proteins, known as membrane fusion proteins (MFP), thereby forming tripartite efflux pumps (Du et al., 2018). The homotrimer TolC forms a β-barrel pore in the outer membrane and an α-barrel in the periplasm and interacts with inner membrane transporters via various MFPs. These tripartite efflux pumps provide a channel for export of different substrates across the gram-negative cell envelope, e.g., drugs and proteinaceous toxins (Du et al., 2018; Greene et al., 2018; Hinchliffe et al., 2013; Koronakis et al., 2004; Wandersman and Delepelaire, 1990). In this minireview, we will discuss the existing knowledge about a specific group of ABC transporters, namely the DevBCA-HgdD-like tripartite efflux pumps, and their roles in the physiology of *Anabaena* sp.

2. The role of the DevBCA-HgdD efflux pump in heterocyst envelope formation in *Anabaena* sp.

Septal junctions are important for diazotrophic growth and mediate molecule transport from cell to cell (Flores et al., 2018a, 2016; Weiss et al., 2018). In addition, membrane transporters of *Anabaena* sp. are also involved in heterocyst differentiation at different stages by transferring regulatory proteins and components of the heterocyst cell envelope (Flores et al., 2018a; Maldener et al., 2014; Shvarev and Maldener, 2018). In our functional characterization of the ABC-transporter-driven efflux pump DevBCA-HgdD (Fiedler et al., 1998; Maldener et al., 1994; Staron et al., 2011), we identified the genes *devB (alr3710), devC (alr3711),* and *devA (alr3712)* on one operon of the *Anabaena* sp. genome. Mutants in any one of these genes do not grow diazotrophically because they lack the hgl layer, and hence the main protection against entry of oxygen into the heterocyst. However, the mutants still synthesize the heterocyst envelope glycolipids and accumulate them in the heterocysts (Fiedler et al., 1998; Maldener et al.,

1994). The *devB* gene encodes an MFP, *devC* encodes a TMD, and *devA* encodes the NBD of an ABC transporter (see below); therefore, we speculated that these encoded proteins form an ABC transporter efflux pump that is involved in the transport of HGLs beyond the outer membrane. In line with mutant phenotypes and protein sequence similarities, the *devBCA* operon is expressed during heterocyst formation under control of the transcriptional factor NtcA, which binds to the *devBCA* promoter *in vitro* (Fiedler et al., 2001; Staron et al., 2011). This DNA binding protein is the cyanobacterial global nitrogen regulator and activates several genes that are essential for heterocyst differentiation (Flores et al., 2018b).

To transfer glycolipids through the gram-negative cell wall, DevBCA has to interact via its MFP (DevB) with an outer membrane pore. This pore is formed by the HgdD protein encoded by the gene *alr2887*; HgdD is a homolog of the *E. coli* TolC protein (Maldener et al., 2003; Moslavac et al., 2007). The *Anabaena* sp. *hgdD* mutant, like *devB, devC,* and *devA* mutants, does not form the hgl layer and hence cannot grow diazotrophically (Moslavac et al., 2007). In other bacterial tripartite efflux pumps, TolC-like proteins interact with MFPs via tip-to-tip connections between periplasm-directed loops of the TolC α-barrel and the α-helical hairpin domains of the MFP (Daury et al., 2016; Du et al., 2014; Fitzpatrick et al., 2017; Jeong et al., 2016; Xu et al., 2011). Similarly, we showed in protein–protein interaction studies that binding between the tip regions of DevB and HgdD proteins is essential for the activity of the secretion system (Staron et al., 2014). The best tested substrate for the DevBCA-HgdD transporter *in vitro* is purified HGLs, which confirms our hypothesis that DevBCA-HgdD is an HGL exporter of heterocysts (Staron et al., 2011). Many tripartite efflux pumps are also involved in multidrug resistance. As the growth of a *devA* mutant was not affected by a typical substrate of multidrug efflux pumps, i.e., ethidium bromide (Shvarev et al., 2018), DevBCA has a very particular function and an unusual substrate specificity.

To take a closer look at the structure and composition of this specific transporter complex, we searched for protein homology and predicted protein structures using the HHpred server [(Zimmermann et al., 2018), https://toolkit.tuebingen.mpg.de/#/tools/hhpred]. The sequences of the DevBCA complex subunits showed similarity to sequences of various subunits of tripartite efflux pumps. For instance, the DevB amino acid sequence [474 amino acids (aa)] is 98% homologous almost throughout its full length to sequences of various MFPs, such as that of the EmrA, MacA, AcrA, and MexA subunits of the multidrug transporters and of the MFP HlyD of the hemolysin exporter. The DevC amino acid sequence (385 aa) is highly homologous (> 98%) to that of MacB and the TMDs LolE and LolC of the lipoprotein exporter. The DevA amino acid sequence (244 aa) is highly similar to that of NBDs of ABC transporters, including LolD and MacB. The sequence of an *in silico* chimeric DevA-DevC protein is highly homologous (probability 100% according to HHpred) to that of the MacB protein, which hosts the NBD and TMD on one peptide (Greene et al., 2018). The homology to these various secretion systems also reflects the unique and specific role of DevBCA-HgdD, which does not belong to one specific export system for drugs, peptides, or lipoproteins, but instead to a new class of glycolipid transporters. Even though DevBCA-HgdD is similar to tripartite multidrug efflux pumps, it is not involved in drug resistance.

The heterocyst-specific efflux pump of *Anabaena* sp. is noteworthy also because of the size of its MFP (DevB); the periplasm-directed α-helical hairpin domain of ca. 230 aa [according to the prediction of the sequence-based trans-membrane domain prediction MINNOU online tool (Cao et al., 2006), http://minnou.cchmc.org/] is considerably longer than that of *E. coli* MFPs (70–190 aa according to MINNOU). This longer length could be explained by the much larger distance between the outer and cytoplasmic membranes of ca. 46 nm and the thickness of the peptidoglycan layer of ca. 14 nm in *Anabaena* sp. (Hoiczyk and Hansel, 2000; Wilk et al., 2011), compared to the ca. 15–20 nm distance and ca. 7 nm thickness in *E. coli* (Hobot et al., 1984; Matias et al., 2003). The periplasmic part of DevB of *Anabaena* sp. must traverse this distance to connect to the tip of the HgdD protein, and the longer length might also be required to cross the heterocyst cell wall.

Interestingly, among all cyanobacterial genomes examined, only the *hgdD* gene product of heterocyst-forming cyanobacteria has an amino-terminal extension of approximately 300 amino acids compared to TolC from *E. coli*. According to the HHpred server, this extension shares similarity with the AMIN domain [PF11741; (de Souza et al., 2008)], which is present in several bacterial periplasmic proteins. The AMIN-like domain could mediate targeting of HgdD to a specific region in the cell envelope. Like TolC in *E. coli*, HgdD is a versatile protein. Except for its role in heterocyst development, it participates in multidrug resistance and in protein and siderophore transport (Hahn et al., 2015, 2013, 2012; Nicolaisen et al., 2010). HgdD homologs in other cyanobacteria also have similar functions (Gonçalves et al., 2018; Maeda et al., 2018; Oliveira et al., 2016).

3. Function of DevBCA homologs in *Anabaena* sp.

According to the Cyanobase server, *Anabaena* sp. carries six additional gene clusters coding for transporters homologous to DevBCA (Figs. 2 and 3; Table 1). This number differs in other cyanobacterial species. For instance, the filamentous *Nostoc* sp. PCC 7524 has nine such clusters, and the unicellular *Synechocystis* sp. PCC 6803 has only one such gene cluster.

One of the transporters of *Anabaena* sp. that is similar to DevBCA is composed of the proteins All0809, All0808, and All0807 (Table 1, Fig. 2) (Staron and Maldener, 2012). Mutants in any of the genes of the cluster cannot grow diazotrophically, even though they form mature heterocysts. *in vitro* experiments have shown that the HGLs are not a substrate of this pump, and the specific substrate has not yet been identified. Since the MFP All0809 interacts with HgdD and All0808/All0807 *in vitro*, this transporter probably forms an efflux pump similar to DevBCA-HgdD (Staron and Maldener, 2012). However, its precise function is unknown, and the genes of this cluster are down-regulated soon after the removal of a combined nitrogen source. The pump might function in the periplasmic space of the vegetative cells of the filaments.

Another DevB-like MFP and a DevC-like TMD are encoded by the clustered genes *hgdB* (*all5346*) and *hgdC* (*all5347*), respectively (Table 1). Interestingly, the ATPase gene (*devA* homolog) is replaced by the gene *hgdA,* which encodes a UDP-galactose 4-epimerase. HgdB, HgdC, and HgdA are essential for the diazotrophic growth of *Anabaena* sp. (Fan et al., 2005; Shvarev et al., 2019, 2018). The *hgdB* and *hgdA* genes are up-regulated after removal of combined nitrogen in the late stage of heterocyst differentiation; moreover, proteins HgdC and HgdA are localized specifically to heterocysts (to the cell envelope and cytoplasm, respectively). *hgdB* and *hgdA* mutants cannot grow without a combined nitrogen source because they form heterocysts with an aberrant hgl layer that does not protect nitrogenase from oxygen (Fan et al., 2005; Shvarev et al., 2019, 2018). The ratio between two major forms of HGLs in this heterocyst envelope significantly differs from that of the wild type, which indicates that a correct proportion of the two components is essential for the proper function as an oxygen barrier (Shvarev et al., 2019, 2018). Moreover, the DevB-homolog HgdB interacts with HgdD; therefore, HgdBC-HgdD is probably another tripartite efflux pump driven by an ATPase encoded by a gene outside the cluster (Shvarev et al., 2018). This ABC exporter plays a role in a later stage of heterocyst differentiation and hgl layer formation and is presumably involved in fine-tuning the composition of the hgl layer. The substrate of the HgdBC transporter has not yet been identified, but it could be a protein involved in laminated layer formation or one specific HGL. The epimerase HgdA might play a role in the synthesis of different HGL forms, which then are transported by HgdBC (Shvarev et al., 2019).

To date, little is known about the function of the other four gene clusters homologous to *devBCA* in *Anabaena* sp., namely *alr3647/alr3648/alr3649*, *alr4280/alr4281/alr4282*, *all2652/all2651/all2650*,

and *alr4973/alr4974/alr4975* (Table 1). These gene clusters are not important for diazotrophic growth of *Anabaena* sp. (Staron, 2012). There is evidence that the gene clusters *alr3647/alr3648/alr3649* and *alr4280/alr4281/alr4282* contribute to antibiotic resistance and represent multidrug efflux pumps (Shvarev and Maldener, unpublished).

The gene cluster *all2652/all2651/all2650* is located close to a group of genes responsible for the synthesis of siderophores (Jeanjean et al., 2008). Therefore, it might be involved in siderophore transport. In addition to the TMD All2651, a second TMD is fused to the ATPase domain on All2650. As predicted, All2650 forms six transmembrane helices, while the DevC-homologs form only four. It is still not clear whether the All2652/All2651/All2650 proteins comprise a single transporter, or whether All2652/All2651 recruits another NBD, while All2650 fulfills a separate function.

4. Conclusions

The complex physiology and multicellular lifestyle of *Anabaena* sp. PCC 7120 requires a number of sophisticated transport machineries of different types, with a prevalence of ABC transporters. ABC-transporter-driven tripartite efflux pumps play crucial roles in the *Anabaena* sp. life cycle. These pumps are essential for heterocyst development, antibiotic resistance, and probably other aspects of growth (Fig. 3). Despite their very similar predicted topology, their functions and substrates differ and are specific. Further investigation of these efflux pumps will elucidate unknown details of the functions and biology of ABC transporters. Such a basic understanding is of great importance considering the various clinical problems related to ABC transporters, such as the multiple drug resistance of pathogenic bacteria and cancer cells.

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Conflict of interest

None declared.

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References

Adams, D.G., Duggan, P.S., 1999. Tansley Review No. 107 Heterocyst and akinete differentiation in cyanobacteria. New Phytol. 144, 3–33. https://doi.org/10.1046/j.1469-8137.1999.00505.x.

Angermayr, S.A., Rovira, A.G., Hellingwerf, K.J., 2015. Metabolic engineering of cyanobacteria for the synthesis of commodity products. Trends Biotechnol. 33, 352–361. https://doi.org/10.1016/j.tibtech.2015.03.009.

Cao, B., Porollo, A., Adamczak, R., Jarrell, M., Meller, J., 2006. Enhanced recognition of protein transmembrane domains with prediction-based structural profiles. Bioinformatics 22, 303–309. https://doi.org/10.1093/bioinformatics/bti784.

Daury, L., Orange, F., Taveau, J.C., Verchère, A., Monlezun, L., Gounou, C., Marreddy, R.K.R., Picard, M., Broutin, I., Pos, K.M., Lambert, O., 2016. Tripartite assembly of RND multidrug efflux pumps. Nat. Commun. 7, 1–8. https://doi.org/10.1038/ncomms10731.

Davidson, A.L., Dassa, E., Orelle, C., Chen, J., 2008. Structure, function, and evolution of bacterial ATP-Binding cassette systems. Microbiol. Mol. Biol. Rev. 72, 317–364. https://doi.org/10.1128/MMBR.00031-07.

de Souza, R.F., Anantharaman, V., de Souza, S.J., Aravind, L., Gueiros-Filho, F.J., 2008. AMIN domains have a predicted role in localization of diverse periplasmic protein complexes. Bioinformatics 24, 2423–2426. https://doi.org/10.1093/bioinformatics/btn449.

Du, D., Wang, Z., James, N.R., Voss, J.E., Klimont, E., Ohene-Agyei, T., Venter, H., Chiu, W., Luisi, B.F., 2014. Structure of the AcrAB-TolC multidrug efflux pump. Nature 509, 512–515. https://doi.org/10.1038/nature13205.

Du, D., Wang-Kan, X., Neuberger, A., van Veen, H.W., Pos, K.M., Piddock, L.J.V., Luisi, B.F., 2018. Multidrug efflux pumps: structure, function and regulation. Nat. Rev. Microbiol. https://doi.org/10.1038/s41579-018-0048-6.

Fan, Q., Huang, G., Lechno-Yossef, S., Wolk, C.P., Kaneko, T., Tabata, S., 2005. Clustered genes required for synthesis and deposition of envelope glycolipids in *Anabaena* sp. Strain PCC 7120. Mol. Microbiol. 58, 227–243. https://doi.org/10.1111/j.1365-2958.2005.04818.x.

Fiedler, G., Arnold, M., Hannus, S., Maldener, I., 1998. The DevBCA exporter is essential for envelope formation in heterocysts of the cyanobacterium *Anabaena* sp. Strain PCC 7120. Mol. Microbiol. 27, 1193–1202. https://doi.org/10.1046/j.1365-2958.1998.00762.x.

Fiedler, G., Muro-Pastor, A.M., Flores, E., Maldener, I., 2001. NtcA-dependent expression of the *devBCA* operon, encoding a heterocyst-specific ATP-binding cassette transporter in *Anabaena* spp. J. Bacteriol. 183, 3795–3799. https://doi.org/10.1128/JB.183.12.3795-3799.2001.

Fitzpatrick, A.W.P., Llabrés, S., Neuberger, A., Blaza, J.N., Bai, X.C., Okada, U., Murakami, S., Van Veen, H.W., Zachariae, U., Scheres, S.H.W., Luisi, B.F., Du, D., 2017. Structure of the MacAB-TolC ABC-type tripartite multidrug efflux pump. Nat. Microbiol. 2. https://doi.org/10.1038/nmicrobiol.2017.70.

Flores, E., Herrero, A., Forchhammer, K., Maldener, I., 2016. Septal junctions in filamentous heterocyst-forming Cyanobacteria. Trends Microbiol. 24, 79–82. https://doi.org/10.1016/j.tim.2015.11.011.

Flores, E., Nieves-Morión, M., Mullineaux, C., 2018a. Cyanobacterial septal junctions: properties and regulation. Life 9. https://doi.org/10.3390/life9010001.

Flores, E., Picossi, S., Valladares, A., Herrero, A., 2018b. Transcriptional regulation of development in heterocyst-forming cyanobacteria. Biochim. Biophys. Acta - Gene Regul. Mech. https://doi.org/10.1016/j.bbagrm.2018.04.006.

Gonçalves, C.F., Pacheco, C.C., Tamagnini, P., Oliveira, P., 2018. Identification of inner membrane translocase components of TolC-mediated secretion in the cyanobacterium *Synechocystis* sp. PCC 6803. Environ. Microbiol. https://doi.org/10.1111/1462-2920.14095.

Greene, N.P., Kaplan, E., Crow, A., Koronakis, V., 2018. Antibiotic resistance mediated by the MacB ABC transporter family: a structural and functional perspective. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00950.

Hahn, A., Stevanovic, M., Mirus, O., Schleiff, E., 2012. The TolC-like protein HgdD of the cyanobacterium *Anabaena* sp. PCC 7120 is involved in secondary metabolite export and antibiotic resistance. J. Biol. Chem. 287, 41126–41138. https://doi.org/10.1074/jbc.M112.396010.

Hahn, A., Stevanovic, M., Mirus, O., Lytvynenko, I., Pos, K.M., Schleiff, E., 2013. The outer membrane TolC-like channel HgdD is part of tripartite resistance-nodulation-cell division (RND) efflux systems conferring multiple-drug resistance in the cyanobacterium *Anabaena* sp. PCC 7120. J. Biol. Chem. 288, 31192–31205. https://doi.org/10.1074/jbc.M113.495598.

Hahn, A., Stevanovic, M., Brouwer, E., Bublak, D., Tripp, J., Schorge, T., Karas, M., Schleiff, E., 2015. Secretome analysis of *Anabaena* sp. PCC 7120 and the involvement of the TolC-homologue HgdD in protein secretion. Environ. Microbiol. 17, 767–780. https://doi.org/10.1111/1462-2920.12516.

Herrero, A., Stavans, J., Flores, E., 2016. The multicellular nature of filamentous heterocyst-forming cyanobacteria. FEMS Microbiol. Rev. 40, 831–854. https://doi.org/10.1093/femsre/fuw029.

Higgins, C.F., 1992. ABC TRANSPORTERS : from microorganisms to man. Annu. Rev. Cell Biol. 8, 67–113. https://doi.org/10.1146/annurev.cb.08.110192.000435.

Higgins, C.F., 2001. ABC transporters: physiology, structure and mechanism - an overview. Res. Microbiol. 152, 205–210. https://doi.org/10.1016/S0923-2508(01)01193-7.

Hinchliffe, P., Symmons, M.F., Hughes, C., Koronakis, V., 2013. Structure and operation of bacterial tripartite pumps. Annu. Rev. Microbiol. 67, 221–242. https://doi.org/10.1146/annurev-micro-092412-155718.

Hobot, J.A., Carlemalm, E., Villiger, W., Kellenberger, E., 1984. Periplasmic gel: new concept resulting from the reinvestigation of bacterial cell envelope ultrastructure by new methods. J. Bacteriol. 160, 143–152.

Hoiczyk, E., Hansel, A., 2000. Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J. Bacteriol. 182, 1191–1199. https://doi.org/10.1128/JB.182.5.1191-1199.2000.

Holland, I.B., 2011. ABC transporters, mechanisms and biology: an overview. Essays Biochem. 50, 1–17. https://doi.org/10.1042/bse0500001.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16, 471–483. https://doi.org/10.1038/s41579-018-0040-1.

Jeanjean, R., Talla, E., Latifi, A., Havaux, M., Janicki, A., Zhang, C.C., 2008. A large gene cluster encoding peptide synthetases and polyketide synthases is involved in production of siderophores and oxidative stress response in the cyanobacterium*Anabaena* sp. strain PCC 7120. Environ. Microbiol. 10, 2574–2585. https://doi.org/10.1111/j.1462-2920.2008.01680.x.

Jeong, H., Kim, J.S., Song, S., Shigematsu, H., Yokoyama, T., Hyun, J., Ha, N.C., 2016. Pseudoatomic structure of the tripartite multidrug efflux pump AcrAB-TolC reveals the intermeshing cogwheel-like interaction between AcrA and TolC. Structure 24, 272–276. https://doi.org/10.1016/j.str.2015.12.007.

Koronakis, V., Eswaran, J., Hughes, C., 2004. Structure and function of TolC: the bacterial exit duct for proteins and drugs. Annu. Rev. Biochem. 73, 467–489. https://doi.org/10.1146/annurev.biochem.73.011303.074104.

Kumar, K., Mella-Herrera, R.A., Golden, J.W., 2010. Cyanobacterial heterocysts. Cold Spring Harb. Perspect. Biol. 2, a000315. https://doi.org/10.1101/cshperspect.a000315.

Locher, K.P., 2016. Mechanistic diversity in ATP-binding cassette (ABC) transporters. Nat. Struct. Mol. Biol. 23, 487–493. https://doi.org/10.1038/nsmb.3216.

Maeda, K., Tamura, J., Okuda, Y., Narikawa, R., Midorikawa, T., Ikeuchi, M., 2018.

Genetic identification of factors for extracellular cellulose accumulation in the thermophilic cyanobacterium *Thermosynechococcus vulcanus*: proposal of a novel tripartite secretion system. Mol. Microbiol. 109, 121–134. https://doi.org/10.1111/mmi.13977.

Maldener, I., Fiedler, G., Ernst, A., Fernández-Piñas, F., Wolk, C.P., 1994. Characterization of *devA*, a gene required for the maturation of proheterocysts in the cyanobacterium *Anabaena* sp. strain PCC 7120. J. Bacteriol. 176, 7543–7549. https://doi.org/10.1128/jb.176.24.7543-7549.1994.

Maldener, I., Hannus, S., Kammerer, M., 2003. Description of five mutants of the cyanobacterium *Anabaena* sp. Strain PCC 7120 affected in heterocyst differentiation and identification of the transposon-tagged genes. FEMS Microbiol. Lett. 224, 205–213. https://doi.org/10.1016/S0378-1097(03)00444-0.

Maldener, I., Summers, M.L., Sukenik, A., 2014. Cellular differentiation in filamentous cyanobacteria. In: Flores, E., Herrero, A. (Eds.), The Cell Biology of Cyanobacteria. Caister Academic Press, Norfolk, UK, pp. 263–291.

Matias, V.R.F., Al-Amoudi, A., Dubochet, J., Beveridge, T.J., 2003. Cryo-transmission Electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. J. Bacteriol. 185, 6112–6118. https://doi.org/10.1128/JB.185.20.6112-6118.2003.

Moslavac, S., Nicolaisen, K., Mirus, O., Al Dehni, F., Pernil, R., Flores, E., Maldener, I., Schleiff, E., 2007. A TolC-like protein is required for heterocyst development in *Anabaena* sp. Strain PCC 7120. J. Bacteriol. 189, 7887–7895. https://doi.org/10.1128/JB.00750-07.

Muro-Pastor, A.M., Hess, W.R., 2012. Heterocyst differentiation: from single mutants to global approaches. Trends Microbiol. 20, 548–557. https://doi.org/10.1016/j.tim.2012.07.005.

Nicolaisen, K., Hahn, A., Schleiff, E., 2009. The cell wall in heterocyst formation by *Anabaena* sp. PCC 7120. J. Basic Microbiol. 49, 5–24. https://doi.org/10.1002/jobm.200800300.

Nicolaisen, K., Hahn, A., Valdebenito, M., Moslavac, S., Samborski, A., Maldener, I., Wilken, C., Valladares, A., Flores, E., Hantke, K., Schleiff, E., 2010. The interplay between siderophore secretion and coupled iron and copper transport in the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120. Biochim. Biophys. Acta Biomembr. 1798, 2131–2140. https://doi.org/10.1016/j.bbamem.2010.07.008.

Oliveira, P., Martins, N.M., Santos, M., Pinto, F., Büttel, Z., Couto, N.A.S., Wright, P.C., Tamagnini, P., 2016. The versatile TolC-like Slr1270 in the cyanobacterium *Synechocystis* sp. PCC 6803. Environ. Microbiol. 18, 486–502. https://doi.org/10.1111/1462-2920.13172.

Oliver, N.J., Rabinovitch-Deere, C.A., Carroll, A.L., Nozzi, N.E., Case, A.E., Atsumi, S., 2016. Cyanobacterial metabolic engineering for biofuel and chemical production. Curr. Opin. Chem. Biol. 35, 43–50. https://doi.org/10.1016/j.cbpa.2016.08.023.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of Cyanobacteria. Microbiology 111, 1–61. https://doi.org/10.1099/00221287-111-1-1.

Shvarev, D., Maldener, I., 2018. ATP-binding cassette transporters of the multicellular cyanobacterium *Anabaena* sp. PCC 7120: a wide variety for a complex lifestyle. FEMS Microbiol. Lett. 365, 1–6. https://doi.org/10.1093/femsle/fny012.

Shvarev, D., Nishi, C., Wörmer, L., Maldener, I., 2018. The ABC transporter components HgdB and HgdC are important for glycolipid layer composition and function of heterocysts in *Anabaena* sp. PCC 7120. Life 8, 26. https://doi.org/10.3390/life8030026.

Shvarev, D., Nishi, C.N., Maldener, I., 2019. Glycolipid composition of the heterocyst envelope of *Anabaena* sp. PCC 7120 is crucial for diazotrophic growth and relies on the UDP-galactose 4-epimerase HgdA. Microbiologyopen. https://doi.org/10.1002/mbo3.811.

Staron, P., 2012. Structural and Functional Characterization of the ATP-Driven Glycolipid-efflux Pump DevBCA-TolC and Its Homologues in the Filamentous Cyanobacterium *Anabaena* sp. PCC 7120. PhD Thesis. Eberhard-Karls Universität Tübingen, Tübingen.

Staron, P., Maldener, I., 2012. All0809/8/7 is a DevBCA-like ABC-type efflux pump required for diazotrophic growth in *Anabaena* sp. PCC 7120. Microbiology 158, 2537–2545. https://doi.org/10.1099/mic.0.058909-0.

Staron, P., Forchhammer, K., Maldener, I., 2011. Novel ATP-driven pathway of glycolipid export involving TolC protein. J. Biol. Chem. 286, 38202–38210. https://doi.org/10.1074/jbc.M111.269332.

Staron, P., Forchhammer, K., Maldener, I., 2014. Structure-function analysis of the ATP-driven glycolipid efflux pump DevBCA reveals complex organization with TolC/HgdD. FEBS Lett. 588, 395–400. https://doi.org/10.1016/j.febslet.2013.12.004.

Trowitzsch, S., Tampé, R., 2018. ABC transporters in dynamic macromolecular assemblies. J. Mol. Biol. 430, 4481–4495. https://doi.org/10.1016/j.jmb.2018.07.028.

Wandersman, C., Delepelaire, P., 1990. TolC, an *Escherichia coli* outer membrane protein required for hemolysin secretion. Proc. Natl. Acad. Sci. U. S. A. 87, 4776–4780. https://doi.org/10.1073/pnas.87.12.4776.

Weiss, G.L., Kieninger, A., Maldener, I., Forchhammer, K., Pilhofer, M., 2018. Structure and function of a bacterial gap junction analog. bioRxiv.

Wilk, L., Strauss, M., Rudolf, M., Nicolaisen, K., Flores, E., Kühlbrandt, W., 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. https://doi.org/10.1111/j.1462-5822.2011.01655.x.

Wilkens, S., 2015. Structure and mechanism of ABC transporters. F1000Prime Rep. 7, 1–9. https://doi.org/10.12703/P7-14.

Wolk, C.P., Ernst, A., Elhai, J., 1994. Heterocyst metabolism and development. The Molecular Biology of Cyanobacteria. Springer, Netherlands, pp. 769–823.

Xu, Y., Song, S., Moeller, A., Kim, N., Piao, S., Sim, S.H., Kang, M., Yu, W., Cho, H.S., Chang, I., Lee, K., Ha, N.C., 2011. Functional implications of an intermeshing cogwheel-like interaction between TolC and MacA in the action of macrolide-specific efflux pump MacAB-TolC. J. Biol. Chem. 286, 13541–13549. https://doi.org/10.1074/jbc.M110.202598.

Zimmermann, L., Stephens, A., Nam, S.Z., Rau, D., Kübler, J., Lozajic, M., Gabler, F., Söding, J., Lupas, A.N., Alva, V., 2018. A completely reimplemented MPI bioinformatics toolkit with a new HHpred server at its core. J. Mol. Biol. 430, 2237–2243. https://doi.org/10.1016/j.jmb.2017.12.007.