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Transport of organic substances through the cytoplasmic membrane of cyanobacteria

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Cyanobacteria are mainly known to incorporate inorganic molecules like carbon dioxide and ammonia from the environment into organic material within the cell. Nevertheless cyanobacteria do import and export organic substances through the cytoplasmic membrane and these processes are essential for all cyanobacteria. In addition understanding the mechanisms of transport of organic molecules through the cytoplasmic membrane might become very important. Genetically modified strains of cyanobacteria could serve as producers and exporters of commercially important substances. In this review we attempt to present all data of transport of organic molecules through the cytoplasmic membrane of cyanobacteria that are currently available with the transported molecules ordered according to their chemical classes.

Highlights:

- Cyanobacteria are of huge ecological importance because they produce 20 – 30 % of the total oxygen in the atmosphere.
- Little is known about transport of organic molecules through the cytoplasmic membrane of cyanobacteria.
- In order to stimulate research on transport of organic molecules through the cyanobacterial cytoplasmic membrane we decided to review all available data on this topic.

Keywords: import, export, transport of carbohydrates, transport of amino acids and proteins, transport of DNA

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1. Introduction

Cyanobacteria are prokaryotes capable of oxygenic photosynthesis. As primary producers they contribute 20-30 % (Waterbury et al., 1979; Waterbury 1986) to photosynthetic productivity on earth. Some strains also exhibit large blooms, which may be dangerous for the ecology of lakes and oceans (De Figueiredo et al., 2004; Chen et al., 2016; Peng et al., 2017). Cyanobacteria are thought to be the ancestors of chloroplasts, which were formed by endosymbiosis of an initial eukaryotic and a phototrophic prokaryotic cell (Mereschkowsky, 1905).

Like all organisms cyanobacteria have a cytoplasmic membrane and as gram negative eubacteria they have an outer membrane surrounding the peptidoglycan layer. The outer membrane contains channels called porines (see e.g. Kowata et al., 2017), through which bulky molecules cannot pass. Some cyanobacteria contain an additional S-layer outside of the outer membrane (Karlsson et al., 1983; Smarda et al., 2002; McCarren et al., 2005; Trautner and Vermaas, 2013). With the exception of *Gloeobacter* all cyanobacteria additionally possess intracellular (thylakoid) membranes where photosynthetic electron transport takes place. Cyanobacteria possess photosystem I and photosystem II enabling them to perform oxygenic photosynthesis.

Cyanobacteria are capable of four growth modes (Rippka et al., 1979). For photolithoautotrophic growth light is used as energy source, water as electron source and carbon dioxide as carbon source. This is the mode of life performed by cyanobacteria if light is available. Mixotrophic growth occurs if the photolithoautotrophic growth is further enhanced in the additional presence of an organic carbon source. Photoheterotrophic growth can artificially be induced by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) mediated inactivation of photosystem II (PS II) or by deleting genes coding for essential subunits of PS II. For this mode usable exogenous organic substances serve as carbon and electron source, however, light has to be available as energy source. Chemoheterotrophic growth occurs in the dark and organic substances serve as carbon, energy and electron source.

Comparatively little is known about the import or export of organic substances through the cytoplasmic membrane of cyanobacteria. Therefore we decided to review the transport of organic substances through the cytoplasmic membrane of cyanobacteria, for which at least some information is available hoping to stimulate more research on this relevant topic. Like all organisms cyanobacterial cells have the capacity to import as well as export certain substances, which may exhibit positive or negative effects to the cell itself or to other organisms. As of November 2017 the total sequences of approximately 80 cyanobacterial strains are available (Cyanobase, 2017). This review primarly focusses on well known cyanobacteria like *Synechocystis* sp. strains PCC 6803 and PCC 6714, *Synechococcus* sp. strains PCC 6301, PCC 7942 and PCC 7002, *Anabaena* sp. strains ATCC 29413 and PCC 7120 and *Nostoc* sp. strain ATCC 29133 as most information is available for these strains. Paulsen et al. (1998) compared the percentage of genes encoding putative transporters for organic substances of *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Bacillus subtilis*, *Mycoplasma genitalium*, *Synechocystis* sp. PCC 6803 and *Methanococcus janaschii*

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and found the second lowest value for *Synechocystis* sp. PCC 6803 (2.5%) the only cyanobacterium analysed in this study.

1.1. Biochemical mechanisms of membrane transport

Four general methods for transport have been defined in living cells: free diffusion, facilitated diffusion, primary active transport and secondary active transport (Cooper, 2000; Loddish et al., 2000). Both free and facilitated diffusion occur along a concentration gradient and therefore do not need energy. Hydrophobic molecules like alkanes freely diffuse, while facilitated diffusion means that a polar molecule, which cannot pass the hydrophobic membrane by itself, is transported through a membrane protein. Whereas free diffusion is proportional to the difference of concentration between both sides, facilitated diffusion is restricted to the limited number of transport proteins within the membrane. Active transport occurs against a concentration gradient, which requires energy (e.g. light energy, redox energy or energy released by hydrolysis of ATP). While primary active transport is directly coupled to a release of energy, secondary active transport is driven by a simultaneous cotransport of another molecule along its gradient. Nevertheless this type of transport can truly be considered as active since the gradient of the other substance is permanently maintained by energy consumption. Symporters transport both molecules in the same direction, whereas transport in opposite direction occurs at antiporters.

1.2. Directions of transport in a cyanobacterial cell

According to the direction of transport we distinguish between import, export and transfer between neighbouring cells in filamentous strains, which is both import and export.

1.2.1. Import of organic substances into cyanobacteria

Imported molecules may exhibit a positive or negative effect on the strain.

1.2.1.1. Useful import of organic substances

All cyanobacteria are capable of photolithoautotrophic growth. Some cyanobacteria can grow mixotrophically (Rippka et al., 1979), of these some can grow photoheterotrophically (Rippka et al., 1979). Very few species can grow chemohetrotrophically, which are listed in table 1. For mixotrophic as well as for photoheterotrophic or chemoheterotrophic growth an external organic carbon source is necessary and the carbon source(s) used by the corresponding strains vary (see sections saccharides, amino acids and alcohols).

Organism	Substrate(s)
Anabaena sp. ATCC 29413	Fructose (Wolk and Shaffer, 1976; Haurey and Spiller, 1981)
Anabaena sp. PCC 7120	Fructose (Stebegg et al., 2012)
Anabaena sp. PCC 7120 *frtRABC*$^{+}$	Fructose (Ungerer et al., 2008)

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Anabaena sp. ?	Lactose, fructose, mannose (Sahu and Adhikary, 1981)
Nostoc sp. ATCC 29133	Glucose, fructose (Summers et al., 1995)
Nostoc sp. MAC	Glucose, fructose, sucrose (Hoare et al., 1971)
Nostoc muscorum	Glucose (Allison et al., 1937)
Calothrix marchica Lemm. Var. intermedia Rao	Glucose, fructose, sucrose, galactose, mannitol, sorbitol (Adhikary and Sahu, 1988)
Scytonema schmidlei de Toni	Glucose, fructose, sucrose, galactose, mannitol, sorbitol (Adhikary and Sahu, 1988)
Tolypothrix tenuis	Glucose (Kiyohara et al., 1960; Kiyohara et al.,1962)
Plectonema boryanum	Ribose, sucrose, mannitol, maltose, glucose, fructose, casaminoacids (White and Shilo, 1975)
Chlorogloea fritschii	Sucrose, maltose, glycine, glutamine, mannitol, glucose (Fay, 1965)
Thermosynechococcus elongates	Fructose (Zilliges and Dau, 2016)
Synechococcus sp. PCC 7942 *glf*$^{+}$	Glucose (Niederholtmeyer et al., 2010), fructose (Niederholtmeyer et al., 2010),
Synechocystis sp. PCC 6714	Glucose (Vernotte et al., 1992)
Synechocystis sp. PCC 6803 *	Glucose (Anderson and McIntosh, 1991)

* needs 5 min of illumination per 24 h

Table 1 List of cyanobacterial strains capable of chemohetrotrophic growth

1.2.1.2. Harmful import of organic substances

Whereas the majority of organic substances imported into cyanobacteria are useful for them some imported molecules can also be toxic for them. For instance cyanobacteria exhibit sensitivity towards most antibiotics. Therefore uptake mechanisms for antibiotics must exist, which are assumed to be similar to those in *E. coli*. In some cases even sugars may exhibit a harmful effect as fructose kills the *Synechocystis* sp. strains PCC 6803 (Flores and Schmetterer, 1986) and PCC 6714 (Joset et al., 1988), if it is imported by (glucose transporter) Gtr protein, the main studied sugar transporter of cyanobacteria, which interacts with glucose and to a lesser extend with fructose, too. (Joset et al., 1988; Zhang et al. 1989; Schmetterer, 1990). Some substances taken up by cyanobacteria may not necessarily be toxic

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but inhibit growth as it is the case for some amino acids like phenylalanine in *Synechococcus* sp. PCC 7002 (here called *Agmenellum quadruplicatum*, Ingram and Jensen, 1973) and *Synechocystis* sp. ATCC 29108 (Hall and Jensen, 1980), glutamate in *Anabaena* sp. ATCC 29413 (here called *Anabaena variabilis*, Chapman and Meeks, 1983) and glutamine, phenylalanine, histidine and lysine in *Synechocystis* sp. PCC 6803 (Labarre et al., 1987; Flores and Muro-Pastor, 1990). The growth of both *Synechococcus* sp. PCC 7002 and *Synechocystis* sp. ATCC 29108 is inhibited if phenylalanine is added to the growth medium, however, the effect is relieved by equimolar coaddition of tyrosine (Ingram and Jensen, 1973; Hall and Jensen, 1980). The syntheses of both phenylalanine and tyrosine partially share a common pathway and external addition of one amino acid may inhibit the synthesis of both (Jensen et al., 1967). It has not been revealed yet, whether phenylalanine and tyrosine are imported by the same transporter.

1.2.2. Export of organic substances by cyanobacteria

This review focusses on transport of organic substances through the cytoplasmic membrane. Substances exported through the cytoplasmic membrane may therefore further be distinguished whether they remain within the envelope of the cyanobacterium (periplasm, peptidoglycan layer, outer membrane) or whether they are exported to the environment.

1.2.2.1. Export of organic molecules through the cytoplasmic membrane that are part of periplasm, peptidoglycan outer membrane and S-layer

All components of the S-layer (if existing in a cyanobacterial strain), outer membrane, periplasm, peptidoglycan layer and cytoplasmic membrane are synthesized within the cytoplasm and must be exported through the cytoplasmic membrane. The outer membrane consists of lipopolysaccharids, carotens, proteins and lipids (Jürgens and Weckesser, 1985; Koebnik et al., 2000; Braun et al., 2001). No details are known about their export through the cytoplasmic membrane in cyanobacteria, however, homologues to corresponding genes in *E. coli* coding for exporters are also present in cyanobacteria. The transporters used for these substances are unknown. Periplasmic proteins include e. g. Tic22 (see below, Kouranov et al., 1998), and cytochrome *c* (see below). Peschek (1983) and Peschek (1984) demonstrated that externally supplied reduced cytochrome *c* was oxidized by the cytoplasmic membrane of intact spheroblasts of *Anacystis nidulans*. The monomeres of the peptidoglycan layer are supposed to be synthesized in the cytoplasm, exported through the cytoplasmic membrane and assembled in the periplasm as it has been demonstrated in gram negative eubacterium *E. coli* (Mengin-Lecreulx et al., 1991; van Heijenoort, 2001; van Dam et al., 2007) however, the transporter(s) for the monomers is/are unknown. In cyanobacteria possessing an S-layer like *Synechocystis* sp. PCC 6803 transport of its components through the cytolplasmic membrane has been demonstrated (Agarwal et al., 2018).

1.2.2.2. Export of organic molecules that are released to the environment

We further distinguish, between excreted organic molecules, whether they have a positive or negative effect on other organisms.

1.2.2.2.1 Export of organic substances with positive effect on other organisms

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Some organic substances are released to the environment which means they have to pass both the cytoplasmic and outer membrane. The effect of organic molecule secretion to the environment may range from beneficial to extremely toxic towards other organisms. Excretion of amino acids (Watanabe, 1951; Fogg, 1952; Stewart, 1963; Flynn and Gallon, 1990) is important for ecology in oceans as they serve as substrates for heterotrophic plankton. This effect is probably due to a loss of genes encoding transporters for hydrophobic amino acids, which are released by free diffusion and cannot be reimported anymore (Pernil et al. 2015).

1.2.2.2.2. Export of organic substances with negative effect on other organisms

Some cyanobacteria secrete cyanotoxines (e.g. microcystins) that are harmful for many organisms especially for fish by affecting their liver (Malecot et al., 2009; Marie et al., 2012) and some of these toxins like mycrocystin-LR may even affect humans (Butler et al. 2009). In 1996 some patients from a dialysis center in Caruaru (Brazil) suffered from severe illnesses and some even died from liver failure (Azevedo et al., 2002; Jochimsen et al., 1998), because the water derived from a nearby reservoir was not treated, filtered or chlorinated. Microcystins were detected in the water from both the reservoir and the dialysis center as well as in the serum and liver tissues from patients (Jochimsen et al., 1998). In 2007 a young man was poisened by a bloom of *Microcystis* spp. in Salto Grande Dam, Argentina. He suffered from fever and respiratory distress and atypical pneumonia was diagnosed. A high level of microcystin-LR was detected in water samples and the patient showed a significant increase of hepatic damage biomarkers (Gianuzzi et al., 2011).

Nodularia spumigena produces the liver toxin nodularin (Rinehart et al., 1988; Sivonen et al., 1989). Since this strain frequently forms blooms (mass proliferation) in the Baltic Sea many fish die when this happens. This is also of commercial importance because many families living along the Baltic Sea earn their living by fishing. Cyanotoxines belong to different chemical classes. While hepatotoxic microcystins are cyclopeptides, some alkaloids or some lipids are known as cyanotoxines as well (Blaha et al., 2009).

1.2.3. Transfer of organic molecules within a cyanobacterial filament

In filamentous cyanobacteria exchange of organic substances between neighbouring cells is important. Especially in heterocyst forming strains the transport of glutamine from heterocysts to neighbouring vegetative cells and from there to the other vegetative cells (Thomas et al., 1977) is essential as it is the only pathway by which vegetative cells acquire nitrogen containing organic substances. Furthermore, heterocysts have to import organic substances from the neighbouring vegetative cells because they cannot fix carbon dioxide by themselves.

2. Organic substances known to be transported through the cytoplasmic membrane

In this chapter substances that have been reported to be imported or exported through the cytoplasmic membrane will be grouped according to their chemical classes.

2.1. Saccharides

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Some cyanobacteria have developed mechanisms to import sugars in order to incorporate them into glycogen reserves. Sugars that have been tested as substrates under heterotrophic growth are glucose, fructose, sucrose, ribose (Rippka et al., 1979) and lactose (Sahu and Adhikary, 1981), whereas other sugars cannot be excluded as substrates either. Since the outer membrane probably contains saccharides a mechanism of saccharide export has to exist. However, nothing is known about it yet and only in a few cases the mechanisms of saccharide import have been revealed, which are discussed in the following paragraphs.

2.1.1. Monosaccharides

2.1.1.1. Hexoses and derivatives

Synechocystis sp. PCC 6803 can use glucose for both photoheterotrophic (Rippka et al., 1979) and light activated chemoheterotrophic growth (requiring a daily illumination of 5 min, Anderson and McIntosh, 1981). Fructose on the other hand has been known for a long time to be toxic for *Synechocystis* sp. PCC 6803 (Rippka et al., 1979). Both substances are taken up by the glucose permease Gtr (also called GlcP or Sll0771; Joset et al., 1988; Zhang et al., 1989), which is the most studied sugar transporter of cyanobacteria. The *gtr* (also called *glcP*) gene has been identified by Schmetterer (1990) and is essential for both the capacity of *Synechocystis* sp. PCC 6803 for heterotrophic growth and the toxicity of fructose for the same strain (Flores and Schmetterer, 1986). The sequence of *gtr* exhibits similarity to sugar transporters of yeast, mammals and *E. coli* (Schmetterer, 1990; Zhang et al., 1989). Since the affinity of the Gtr protein for glucose is tenfold higher than for fructose the toxic effect of fructose can be relieved by supplying glucose at the same time in a concentration of at least 10 % compared to fructose (Flores and Schmetterer, 1986). A similar effect was observed for 3-O-methylglucose (3OMG), which is a glucose analogon that cannot be metabolized. It is also imported by Gtr thereby protecting the cell against the toxicity of fructose as well as inhibiting photoheterotrophic growth on glucose (Flores and Schmetterer, 1986).

Also *Synechocystis* sp. PCC 6714, which is closely related to *Synechocystis* sp. PCC 6803, can use glucose for photoheterotrophic growth (Rippka, 1972; Rippka et al., 1979) and fructose is toxic for this strain (Astier et al., 1979). Contrary to *Synechocystis* sp. PCC 6803 *Synechocystis* sp. PCC 6714 can grow chemoheterotrophically on glucose in permanent darkness (Rippka, 1972; Astier, 1976; Rippka et al., 1979). The glucose analogon 3OMG also competes with glucose for uptake in *Synechocystis* sp. PCC 6714 (Beauclerk and Smith, 1978).

Anabaena sp. ATCC 29413 can grow on fructose in permanent darkness (Wolk and Shaffer, 1976; Haury and Spiller, 1981). In this strain the fructose transporter is encoded by the *frtABC* genes (Ungerer et al., 2008). Immediately upstream from these genes the *frtR* gene is localized, which is transcribed in the opposite direction (Ungerer et al., 2008). *frtR* encodes a putative repressor of the *frtABC* genes. This negative regulation seems to be important for fructose uptake since deletion of *frtR* alone resulted in an over expression of the *frtABC* genes which led to high sensitivity towards fructose (Ungerer et al., 2008).

Anabaena sp. PCC 7120 has been believed to be a strict photoautotroph for many years (Rippka et al., 1979), however, a few years ago the strain was discovered to grow

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mixotrophically, photoheterotrophically and chemoheterotrophically on fructose, if very high concentrations (50 – 200 mM) of fructose were supplied. The uptake of fructose by *Anabaena* sp. PCC 7120 has been demonstrated (Stebegg et al., 2012). For chemoheterotrophic growth no short time illumination as in *Synechocystis* sp. PCC 6803 was required (Stebegg et al., 2012). No genes homologous to *frtRABC* have been identified in *Anabaena* sp. PCC 7120, and when this locus from *Anabaena* sp. ATCC 29413 was introduced into *Anabaena* sp. PCC 7120 by Ungerer et al. (2008) the new transgenic strain gained the facility for mixotrophic and chemoheterotrophic growth on 5 mM fructose, which corresponds to the concentrations needed for supporting the growth of *Anabaena* sp. ATCC 29413. Glucose can be used by *Anabaena* sp. PCC 7120 for mixotrophic growth (Yu et al., 2011; Stebegg et al., 2012; Malatinszky et al., 2017), whereas even high glucose concentrations failed to support photo- or chemoheterotrophic growth (Stebegg, 2011).

Glucose uptake by *Anabaena* sp. PCC 7120 has been demonstrated by Nieves-Morion and Flores (2018). Recently two papers of E. Flores identified five genes encoding ABC glucoside transporters that are involved in sugar transport in *Anabaena* sp. PCC 7120: *alr4781* (*glsC*), *all0261* (*glsP*) (Nieves-Morion et al., 2017b), *all1823* (*glsD*), *all1916* (*glsR*) and *alr2532* (*glsQ*) (Nieves-Morion and Flores, 2018). Strains, in which one of these genes was deleted, showed reduced mixotrophic growth on glucose and fructose (Nieves-Morion and Flores, 2018). Regarding glucose dependent mixotrophy deletion of *glsC* reduced growth much more than deletion of any other of these five genes, whereas loss of *glsR* had a rather mild effect. In contrast all five single mutants reduced mixotrophic growth dependent on fructose at a similar rate. Since no single mutation completely abolished growth on fructose (Nieves-Morion and Flores, 2018) this sugar seems to be imported by more than one transporter through the cytoplasmic membrane. In view of the enormous concentrations of fructose necessary for heterotrophic growth in *Anabaena* sp. PCC 7120 (Stebegg et al., 2012) fructose might even be imported by a transporter that normally exports another molecule.

Introduction of the glucose transporter *gtr* from *Synechocystis* sp. PCC 6803 into *Anabaena* sp. PCC 7120 on a stably replicating plasmid did not confer the capacity for heterotrophic growth on glucose but on the contrary led to a strain for which even very low concentrations of glucose (5 mM) were toxic (Stebegg et al., 2012). This is probably due to the increased uptake rate of glucose by *Anabaena* sp. PCC 7120 gtr^+ compared to the glucose uptake rate by *Anabaena* sp. PCC 7120 wild type (Stebegg, 2011). *Anabaena* sp. PCC 7120 gtr^+ could grow photoheterotrophically on lower fructose concentrations (10 – 50 mM) compared to the wild type. However, 200 mM fructose were toxic for *Anabaena* sp. PCC 7120 gtr^+ both in the presence and absence of DCMU, while for the wild type this was the optimal concentration tested (Stebegg et al., 2012).

Esculin, which is a coumarin derivative of glucose, is used to trace intercellular exchange in the filaments of *Anabaena* sp. PCC 7120 (Nürnberg et al., 2015; Nieves-Morion et al. 2017a) and its transfer has been shown to be dependent on the gene products of *sepJ* (*alr2338*), *fraC* (*alr2392*) and *fraD* (*alr2393*) in *Anabaena* sp. PCC 7120 (Mullineaux et al. 2008; Merino-Puerto, 2011, Mariscal et al., 2011, Nürnberg et al., 2015). Besides esculin uptake into *Anabaena* sp. PCC 7120 is inhibited by sucrose and to a lesser extent by maltose too (Nieves-Morion et al., 2017b).

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Nostoc sp. ATCC 29133 can use glucose and fructose for dark growth (Rippka et al., 1979; Summers et al., 1995). In this strain an operon has been identified, which contains one gene encoding a major facilitator permease for glucose (GlcP, *npun_R5323*) and four genes encoding an ATP-binding cassette (ABC) type transporter for fructose (*frtA1A2BC*, which correspond to *npun_R5327*, *npun_R5326*, *npun_R5325* and *npun_R5324* according to cyanobase, 2017) (Ekman et al., 2013). These genes showed homology to the corresponding *gtr* gene in *Synechocystis* sp. PCC 6803 (Zhang et al., 1989; Schmetterer, 1990) and to *frtABC* genes in *Anabaena* sp. ATCC 29413 (Ungerer et al., 2008) respectively.

Nostoc sp. strain MAC has been reported to grow in the dark on both glucose and fructose (Hoare et al., 1971; Rippka et al. 1979) and uptake of glucose as well as of the analogon 3-O-methyl glucose has been demonstrated. These two related substances inhibited their uptake vice versa (Beauclerk and Smith, 1978) indicating an import by the same mechanism similar to PCC 6803 (see above). *Nostoc* sp. strain ATCC 29150 has also been shown to grow in the dark on fructose (Schmetterer and Flores, 1988).

White and Shilo (1975) tested several sugars as a substrate for chemoheterotrophic growth of the filamentous strain *Plectonema boryanum* and the only hexoses tested glucose and fructose both supported dark growth. Later glucose dependent dark growth was reported to occur after a phase of adaptation, which correlates with the induction of glucose incorporation (Raboy et al., 1976). Finally Raboy and Padan (1978) identified an active transporter for glucose and its analogue α-methylglucoside in this organism and the uptake of the analogue was inhibited by simultaneously added glucose. Incorporation of fructose and galactose was also demonstrated, however, coaddtion of these sugars did not influence the uptake of α-methylglucoside (Raboy and Padan, 1978).

Glucose can be imported by low light irradiance adapted *Prochlorococcus* sp. strain SS120, which leads to upregulation of genes important in glucose metabolism (Gomez-Baena et al., 2008). Zilliges and Dau (2016) investigated which substances could act as a substrate for heterotrophic growth of thermophilic cyanobacterium *Thermosynechococcus elongatus*. Glucose, galactose and especially fructose allowed photoheterotrophic growth and fructose even supported chemoheterotrophic growth in the dark. On the other hand mannose exhibited a negative effect on this strain.

Synechococcus sp. PCC 7942 normally does not import glucose or fructose, however, the transfer of the glucose carrier from *Zymomonas mobilis* (Barnell et al., 1990) into *Synechococcus* sp. PCC 7942 resulted in a strain that could both take up and excrete glucose and fructose depending on the concentration of the surrounding medium. Furthermore this strain could use glucose and fructose for chemoheterotrophic growth (Niederholtmeyer et al., 2010).

McEwen et al. (2013) investigated, whether integration of genes encoding various sugar transporters at neutral site I (GenBank accession number U30252, Golden et al., 1987; Clerico et al., 2007) into the genome of *Synechococcus* sp. PCC 7942 could confer the ability for mixotrophic growth depending on the corresponding sugars. For glucose three different genes were tested. Only the introduction of *galP* from *E. coli*, (Hernandez-Montalvo et al., 2003),

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which transports glucose as well as galactose and to a smaller extent talose and mannose in *E. coli* (McDonald et al., 1997), but neither the introduction of *gtr* (*sll0771*, here called *glcP*) from *Synechocystis* sp. PCC 6803 (Zhang et al., 1989; Schmetterer, 1990) nor of *glut1* from human erythrocytes (Mueckler et al., 1985) allowed mixotrophic on glucose.

The results of McEwen et al. may contradict the work of Zhang et al. (1998), who also integrated *gtr* into neutral site I of *Synechococcus* sp. PCC 7942, however, in Zhang's experiment the corresponding strain became highly sensitive towards glucose. The only difference between the two experiments was that Zhang et al. used continuous light, whereas McEwen et al. used diurnal conditions. In another experiment Zhang et al. (1998) also introduced an autonomously replicating plasmid containing the *gtr* gene into *Synechococcus* sp. PCC 7942, which resulted in a strain that was facultatively heterotrophic dependent on glucose, however, the plasmid could not be stably maintained. The different behaviour whether the *gtr* gene is integrated into the chromosome or located on an autonomously replicating plasmid in *Synechocystis* sp. PCC 7942 may be due to the different copy number per cell in each case.

Adhikary and Sahu (1988) discovered that the filamentous strains *Calothrix marchica* Lemm. var. *intermedia* Rao and *Scytonema schmidlei* De Toni could grow mixotrophically, photoheterotrophically and chemoheterotrophically on glucose, fructose and with less efficiency on galactose, however, nothing is known about the corresponding transporter.

Sahu and Adhikary (1981) analysed the effect of various sugars as possible heterotrophic substrates on a not exactly defined *Anabaena* sp. strain and fructose was the only tested monosaccharide that both stimulated mixotrophic growth in the light and supported chemoheterotrophic growth in the dark. Mannose reduced the photolithoautotrophic growth rate but supported with little efficiency chemoheterotrophic growth of the same strain (Sahu and Adhikary, 1981). Therefore both sugars have to enter somehow the cell but the mechanism remains unknown.

2.1.1.2 Pentoses

Xylose allowed mixotrophic growth of *Synechococcus* sp. PCC 7942 in 12h light/dark cycles (McEwen et al. 2013), however, the xylose transporter involved is unknown. McEwen et al. (2013) transferred the xylose transporter gene *xylE* derived from *E. coli* into *Synechococcus* sp. PCC 7942 and found that in the resulting strain mixotrophic growth dependent on xylose was completely repressed. The introduction of *xylE* together with *xylA* and *xylB* encoding a xylose isomerase and a xylulokinase in *E. coli* into *Synechococcus* sp. PCC 7942 led to a strain, in which mixotrophic growth was dramatically enhanced.
Sahu and Adhikary (1981) found out that xylose reduced the photolithoautotrophic growth rate of a not exactly defined *Anabaena* sp. strain. Currently it cannot be stated whether these effects are due to transport of xylose or to osmotic reasons.

Among all sugars tested by White and Shilo (1975) ribose supported the fastest dark growth of *Plectonema boryanum*. Ribose did not interfere with the glucose transporter of the same organism as α-methylglucoside uptake was not inhibited by simultaneously coadded ribose

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(Raboy and Padan, 1978).
Rippka et al., (1979) demonstrated that ribose supported photoheterotrophic growth of *Gloeocapsa* sp. PCC 7428, *Nostoc* sp. strains PCC 6302 and PCC 6310 and *Fischerella* sp. strains PCC 7521, PCC 7522 and PCC 7523 but the import of ribose into these strains has not been investigated yet.
Zilliges and Dau (2016, see above) demonstrated that arabinose and xylose reduced the photolithoautotrophic growth rate of *Thermosynechococcus elongatus*, which may be due to uptake of these sugars or to osmotic reasons.

2. 1. 2. Disaccharides

Sucrose has been demonstrated to play an important role in carbon metabolism in *Anabaena* sp. PCC 7120 under diazotrophic conditions (Curatti et al., 2002; Cumino et al., 2007). Sucrose is believed but has not been proven yet to be the carbon source transported from neighbouring vegetative cells into heterocysts (Wolk et al. 1994). Recently sucrose was discovered to serve as a substrate for mixotrophic growth of *Anabaena* sp. PCC 7120 (Malatinski et al., 2017; Nieves-Morion, 2018). This effect was strongly reduced or completely abolished if *glsC* or *glsD* was deleted (Nieves-Morion, 2018).

McEwen et al (2013) demonstrated mixotrophic growth of *Synechococcus* sp. PCC 7942 dependent on sucrose as cultivation in the presence of 5 g/l sucrose increased the growth rate three-fold compared to photolithoautotrophic conditions, however, the way by which sucrose enters *Synechococcus* sp. PCC 7942 is unknown. Further enhancement of mixotrophic growth by 40% was observed when the genes *cscB* encoding the sucrose/proton symporter and *cscK* encoding a phosphofructokinase (both from *E. coli*) were introduced into *Synechococcus* sp. PCC 7942. Introduction of *cscB* alone demonstrated that this transporter can also act as a sucrose exporter (Ducat et al., 2012). Addition of NaCl leads to the biosynthesis of intracellular sucrose to counteract osmotic stress generated by NaCl (Suzuki et al., 2010; Klahn and Hagemann, 2011). If 150mM sodium chloride is added to *Synechococcus* sp. PCC 7942 mutants containing the *E. coli* transporter cscB export of sucrose is observed (Ducat et al., 2012).

White and Shilo (1975) reported that both sucrose and maltose suported dark heterotrophic growth of *Plectonema boryanum*. Sucrose and lactose were incorporated by this strain.
Adhikary and Sahu (1988) demonstrated that sucrose supported mixotrophic, photoheterotrophic and chemoheterotrophic growth of the filamentous strains *Calothrix marchica* Lemm. Var. *intermedia* Rao and *Scytonema schmidlei* de Toni. No further experiments on sugar transport on these organisms were performed.
Sahu and Adhikary (1981) showed that lactose enhanced the growth rate in the light and supported chemoheterotrophic growth in the dark of a not exactly defined *Anabaena* sp. strain more than any other tested substrate.

2. 1. 3. Polysaccharides

Polysaccharides are exported through the cytoplasmic membrane to build up the exopolysaccarides (EPS) of the outer membrane. When the *Synechocystis* sp. PCC 6803 genes *slr0977* encoding a sugar permease, *slr0982*,encoding an ATP binding component of an ABC

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transporter and *slr1610* encoding a methyltransferase were deleted, the mutant strain exhibited flocculent phenotypes and increased adherence to glass. EPS isolated from mutant strains were altered in their composition, while the O-antigen structure and composition was unaffected compared to the wild type (Fisher et al., 2013). The molecule that is actually transported through the cytoplasmic membrane by the products of the corresponding genes has not been revealed yet.

2.2. Amino acids and peptides

2.2.1. Amino acids

Many amino acids are known to exhibit an effect on certain cyanobacterial strains. For instance some amino acids can be used as the sole nitrogen source by several cyanobacteria (Kapp et al., 1975; Neilson and Larsson, 1980; Rawson, 1985). On the other hand some amino acids are reported to have an inhibitory or even toxic effect on various strains. In both cases amino acids have to enter the cell. Although many amino acids influence cyanobacterial cells in a positive or negative manner only for glycine the mechanism of uptake has been revealed.

Bualuang and Incharoensakdi (2015) demonstrated that the photolithoautotrophic growth rate of the halotolerant cyanobacterium *Aphanothece halophytica* was increased by external glycine. Glycine even reduces the growth inhibition by high concentrations of sodium chloride. Bualuang et al. (2015) identified the *ApagcS1* gene within *Aphanothece halophytica*, whose putative product displayed high homology (58% identity) to AgcS from *Pseudomonas pseudoalcaligenes*, 33% identity to Acp of thermophilic bacterium PS3 (Kamata et al., 1992; Kanamori et al., 1999) and 32% identity to DagA of *Alteromonas haloplanktis* (MacLeod and MacLeod, 1992). AgcS, Acp and DagA and also CycA from *E. coli* (Robbins and Oxender, 1973; Ghrist and Stauffer, 1995) are all members of the alanine or glycine:cation symporter (AGCS) superfamily, members of which occur in many bacteria. *ApagcS1* from *Aphanothece halophytica* was expressed in *E. coli* mutant strain JW4166, which is deficient in glycine uptake (Bualuang et al., 2015). Transgenic strain JW4166 *ApagcS1*$^{+}$ imported glycin in a sodium dependent manner. This uptake was inhibited by simultaneously coadded asparagine and glutamine and to a lesser extent by Alanine, methionine, cysteine and serine (Bualuang et al., 2015) indicating that these amino acids also interact with ApagcS.

Neilson and Larsson (1980) investigated the effect of some amino acids on the seven cyanobacterial strains *Synechococcus* sp. PCC 6301, *Synechocystis* sp. PCC 6714, *Pseudanabaena* sp. strains B2, LPP 6402, LPP 73110 and *Anabaena* sp. PCC 7118. Glutamine could be used by *Synechococcus* sp. PCC 6301, *Synechocystis* sp. PCC 6714, B2 and LPP 73110 and PCC 7118, asparagine by *Synechocystis* sp. PCC 6714, *Pseudanabaena* sp. PCC 6903, B2 and LPP 73110, arginine by *Synechocystis* sp. PCC 6714, *Pseudanabaena* sp. PCC 6903, LPP 73110, *Anabaena* sp. PCC 7118, ornithine by LPP 73110 and *Anabaena* sp. PCC 7118 as a sole nitrogen source. None of the cyanobacterial strains tested could use glycine, glycylglycine, glutamate, aspartate, histidine, methionine, leucine, alanine, serine and proline as the sole nitrogen source (Neilson and Larsson, 1980).

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Synechocystis sp. PCC 6308 can even use arginine as carbon source (Weathers et al., 1978). Coaddition of casamino acids together with ribose further shortened the generation time of *Plectonema boryanum* when cultivated in the dark (White and Shilo, 1975), however the mechanism of uptake has not been revealed yet. On the other hand cysteine and ascorbate had a toxic effect on *Thermosynechococcus elongatus* (Zilliges and Dau, 2016).

Out of the 20 proteinogenic amino acids 13 have been checked for their capacity to serve as a sole nitrogen source in *Synechococcus* sp. PCC 7002. Alanine, arginine, asparagine, aspartate, glutamate, glutamine, glycine, histidine, phenylalanine, proline, serine and threonine were effective, while trypthophan was not (Kapp et al., 1975). However, phenylalanine was also reported to inhibit growth of *Synechococcus* sp. PCC 7002 (Ingram and Jensen, 1973). A similar inhibitory effect of phenylalanine is known for *Synechocystis* sp. ATCC 29108 (Hall and Jensen, 1980).

Anabaena sp. PCC 7122 can use alanine, arginine, asparagine, aspartate, glutamate, glutamine and serine as sources of nitrogen and the same amino acids reduced nitrogenase activity in this organism (Rawson, 1985), however, only for glutamine the uptake of the amino acid itself into *Anabaena cylindrica* has been demonstrated (Rowell et al., 1977). In *Anabaena* sp. ATCC 29413 two pathways of glutamine and glutamate transport exist, a low affinity and a high affinity system. In this strain glutamine cannot be used as sole nitrogen source and glutamate inhibited growth (Chapman and Meeks, 1983). In contrast Thiel and Leone (1986) reported that *Anabaena* sp. ATCC 29413 could grow on glutamine as nitrogen source.

Coaddition of casamino acids together with ribose further shortened the generation time of *Plectonema boryanum* when cultivated in the dark (White and Shilo, 1975), however the mechanism of uptake has not been revealed yet.

In filamentous heterocyst forming cyanobacteria vegetative cells produce glutamate, which is transported to heterocysts, where it is converted to glutamine. Heterocysts themselves cannot produce glutamate mediated by glutamate synthase (Thomas et al. 1977; Martin-Figueroa et al. 2000). Montesinos et al. (1997) investigated the uptake of various amino acids by the following different cyanobacterial strains: *Anabaena* sp. strains PCC 7120 and PCC 7937 (the latter normally called *Anabaena* sp. ATCC 29413), *Nostoc* sp. strains PCC 7413 and PCC 7107, *Calothrix* sp. strain PCC 7601, *Fischerella muscicola* UTEX 1829, *Pseudanabaena* sp. strain PCC 6903, *Synechococcus* sp. strain PCC 7942 and *Synechocystis* sp. strain PCC 6803. All of them had at least one transport system for neutral amino acids and two genes *natA* and *natB* were identified coding for components of this system. *Pseudanabaena* sp. PCC 6903 was able to take up both acidic amino acids, glutamic acid and aspartic acid, at a high rate, whereas *Synechocystis* sp. PCC 6803 was only successful for glutamic acid but not for aspartic acid uptake and the other tested strains failed for both of them. *Synechocystis*. sp. PCC 6803 and *Nostoc* sp. PCC 7413 exhibited the highest rates for uptake of the basic amino acid arginine, whereas almost no import could be detected in *Synechococcus* sp. PCC 7942 (Montesinos et al., 1997).

In *Synechocystis* sp. PCC 6803 two classes of mutants (Can1 and Aza1) have been demonstrated to be defective in uptake of amino acids (Labarre et al., 1987). While Can1

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mutants could not transport the basic amino acids arginine, histidine and lysine any more, deletion of Aza1 prevented the transport of all amino acids except for glutamate and the basic ones (Labarre et al., 1987). Flores and Muro-Pastor (1990) investigated the uptake of arginine and glutamine by *Synechocystis* sp. PCC 6803. The transport of both amino acids was inhibited by canavanine, citrulline, histidine, lysine and ornithine. Four different amino acid permeases have been identified in *Synechocystis* sp. PCC 6803: the ABC system encoded by genes *natABCDE* (*natA = slr0467*; *natB = slr0559*; *natC = sll0146*; *natD = slr0949*; *natE = slr1881*) transports neutral amino acids and histidine (Montesinos et al., 1997; Quintero et al., 2001), the ABC system encoded by the genes *bgtA* (*slr1735*) and *bgtB* (*sll1270*) transports basic amino acids and glutamine (Quintero et al., 2001) and the two transporters encoded by *gltS* (*slr1145*) and *gtrABC* (*gtrA = sll1102*, *gtrB = sll1103* and *gtrC = sll1104*) respectively are both sodium dependent glutamate transporters (Quintero et al., 2001).

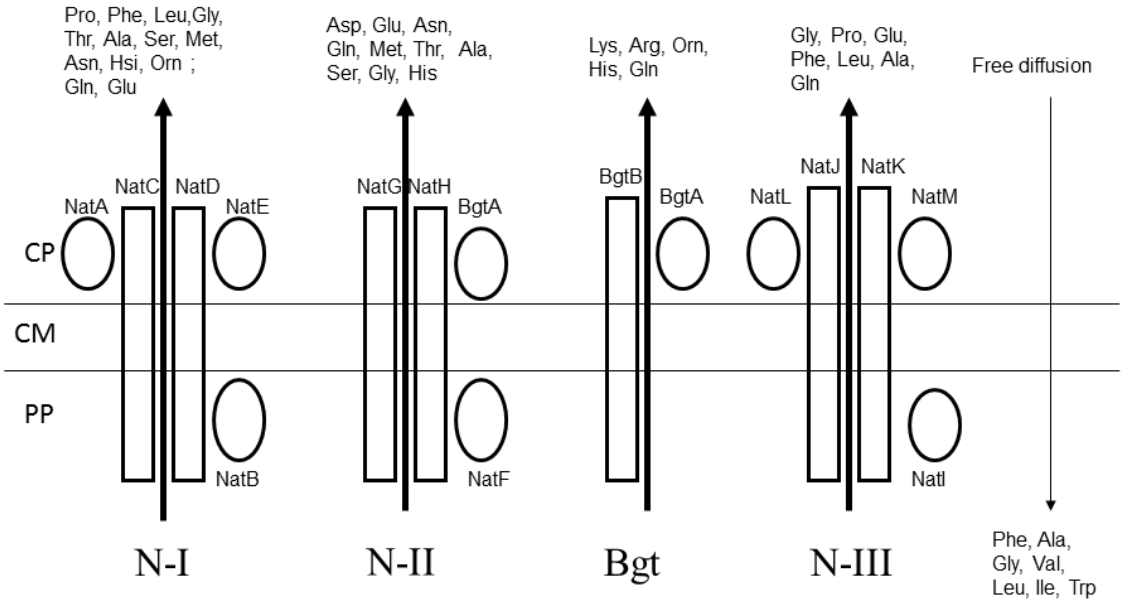

Fig. 1 Transport of amino acids through the cytoplasmic membrane of *Anabaena* sp. PCC 7120 by different transport systems. CP…..cytoplasm, CM……cytoplasmic membrane bilayer, PP….. periplasm. NatA, NatE: ATP binding subunits, NatB: periplasmatic substrate binding protein, NatC, NatD: transmembrane proteins of transport system NI; BgtA: ATP binding subunit, NatF: periplasmatic substrate binding protein, NatG, NatH: transmembrane proteins of transport system NII; BgtA: ATP binding subunit, BgtB transmembrane protein of transport system Bgt. NatI: periplasmatic substrate binding protein, NatJ, NatK: transmembrane proteins, NatL, NatM: ATP binding protein of transporter system NIII. Thick arrows indicate active transport, which generally occurs against the concentration gradient

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from the exterior to the interior, while a thin arrow demonstrates free diffusion of hydrophobic aminoacids through the cytoplasmic membrane, which takes place in the opposite direction along the gradient.

Vaishampayan (1982) investigated, which amino acids could serve as carbon or nitrogen sources for the filamentous heterocyst forming cyanobacterium *Nostoc muscorum*. The strain could use leucine, isoleucin, methionine, valine, citrulline and lysine both as nitrogen and carbon sources, histidine, glutamine, asparagine, trypthophan and serin as nitrogen but not as carbon source, arginine, proline and phenylalanine as carbon but not as nitrogen sources. Glutamate, alanine, tyrosine and cysteine exhibited a toxic effect towards *N. muscorum* (Vaishampayan, 1982).

Lee-Kaden and Simonis (1982) found out that there were different uptake mechanisms for branched chain amino acids in *Anacystis nidulans* (now *Synechococcus elongatus*): two for alanine, aminoisobutyric acid and glycine (DAG) and one for leucine, isoleucine and valine (LIV). The low affinity DAG system was dependent on Na^+ ions. The existence of neutral amino acid transport was confirmed by Montesinos et al. 1997 as *Synechococcus* sp. PCC 7942 took up alanine, leucin and phenylalanine at a higher rate than any other amino acid.

The filamentous cyanobacterium *Anabaena* sp. PCC 7120 has been known for a long time to be capable of uptake of glutamine and glutamate (Flores and Muro-Pastor, 1988), proline (Spence and Steweart, 1986) and phenylalanine (Xu and McAuley, 1990). The uptake rates of glutamine and glutamate are inhibited by the same substances indicating a common transport mechanism (Flores and Muro-Pastor, 1988), while proline inhibits dinitrogen fixation by *Anabaena* sp. PCC 7120 (Spence and Stewart, 1986). Montesinos et al. (1995) identified five amino acid transport systems. There are two transporters for basic amino acids (one high affinity and one low affinity) (Herrero and Flores, 1990), two high affinity systems for neutral amino acids and one system for acidic amino acids (Montesinos et al., 1995). Some mutants of genes encoding transporters exhibited impairment for diazotrophic growth (Montesinos et al., 1995, Picossi et al., 2005). Except for the low affinity basic amino acid transporter the other four systems are all ABC transporters (Pernil et al., 2015), and all genes encoding these transporters have been identified (see fig. 1). N-I, which mainly transports neutral hydrophobic amino acids like proline, phenylalanine and leucine is encoded by *natABCDE* (corresponding *all1046*, *alr1834*, *all1047*, *all1248*, *all2912*) (Picossi et al., 2005). N-II, which transports the acidic amino acids glutamic acid and aspartic acid but also neutral polar amino acids like glutamine and asparagine is encoded by *natFGH* (*alr4164*, *alr4165*, *alr4166*) and *bgtA* (*alr4167*) (Pernil et al., 2008). N-III, which transports mainly neutral hydrophobic aminoacids but also glutamic acid and glutamine is encoded by *natIJKLM* (*alr2535*, *alr2536*, *alr2538*, *alr2539*, *alr2541*) (Pernil et al., 2015). Bgt is encoded by *bgtAB* (*alr4167* and *alr3187*) (Pernil et al., 2008). Especially interesting is the fact that ATPase BgtA is a component of the two different transport systems N-II and Bgt (fig. 1). Deletion of the genes coding for N-I caused release of hydrophobic amino acids (Pernil et al., 2008) and supplying other amino acids interacting with N-I resulted in an increased release of α-aminoisobutyric acid to the exterior (Pernil et al., 2015).

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The uptake of alanine, glutamate, glycine, leucine and serine at extremely low concentrations by *Planktothrix rubescens* is light stimulated but saturated at light irradiances close to the photosynthetic compensation point (Walsby and Jüttner, 2006). The corresponding transporter(s) has (have) not been revealed so far.

The single cell cyanobacterium *Gloeocapsa* (*Gloeothece*) sp. CCAP 1430/3 contains a nitrogenase that also reduces acetylene even under aerobic conditions. Thomas et al. (1982) found that the amino acid derivatives azaserine, albizziine, S-carbamoyl cysteine, 7-azatryptophan, 4-fluorotryptophan, 5-methyltryptophan and 4-fluorophenylalanine inhibited acetylene reduction in this strain. No details about the entry of these substances into the cells are available.

2.2.2. Oligopeptides and proteins

In *Synechococcus* sp. PCC 6301 an ORF encoding a 351 amino acid protein shows substantial identity (43.6%) to *oppC* from *Salmonella typhimurium* encoding an oligopeptide permease membrane protein (Fujishiro et al., 1996). According to cyanobase presumptive *oppC* of *Synechococcus* sp. PCC 6301 is named *syc1073_d*. However, northern blot analysis did not detect a transcript of *oppC*. As typical –35 or –10 motifs are missing in the upstream region *oppC* might be a pseudogene or under the control of an atypical promoter (Fujishiro et al., 1996). Currently it is not known yet, whether the gene is expressed under any conditions.

Recently Agarwal et al. (2018) discovered that Sll1180, Sll1181 and Slr1270 form a type-I secretion system for S-layer protein Sll1951 in *Synechocystis* sp. PCC 6803. Mutations of both *sll1180* encoding an inner membrane ABC transporter and *sll1181* encoding a membrane fusion protein resulted in a loss of Sll1951 in the supernatant. Mutants in *sll1180* also exhibited increased sensitivity towards different antibiotics.

Most hepatotoxins produced by cyanobacteria are cyclic heptapeptides (microcystins) produced by the genera *Microcystis*, *Anabaena*, *Nostoc*, *Planktothrix*, *Anabaenopsis* and *Hapalosiphon* and cyclic pentapeptides (nodularins) produced by the genus *Nodularia* (Blaha et al., 2009). Microcystins (for a review see Fontanillo and Köhn, 2018) act as inhibitors of serine/threonine protein phosphatases (PPs) (Campos and Vascanelos, 2010). Pearson et al. (2004) identified the gene *mcyH* (*MAE_38640*) in *Microcystis aeruginosa* coding for an ABC transporter that belongs to subgroup ABC-A_1 (Saurin et al., 1999). Deletion of *mcyH* resulted in a loss not only of excretion of microcystin but even of formation of the microcystin synthesizing complex. In almost all known cyanobacterial strains genes homologous to *mcyH* exist (see table 2), regardless whether these strains are producing microcystins or not. In most cases these genes are annotated as transporters or ATP transporter binding proteins, however, especially for non microcystin transporting strains both the substrates of the transporters and the direction of transport are unknown.

Proteins are transported through the cytoplasmic membrane by Sec-, Tat- and SRP depending protein targeting pathways (Frain et al., 2016). The Tat pathway is predicted to play an important role in the transport of metalloproteins into the periplasm of 25 cyanobacterial strains (Barnett et al., 2011) and one Tat substrate in *Synechocystis* sp. PCC 6803 Sll1358, which shows similarity to an oxalate decarboxylase (Tanner et al., 2001), has been identified

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experimentally (Fulda et al., 2000; Tottey et al., 2008). The periplasmic targeting factor Tic22 is associated with cytoplasmic membrane protein Tic20 (Kouranov et al. 1998) and interacts with outer envelope biogenesis factor Omp85 (Tripp et al., 2012).

During heterocyst formation in some filamentous cyanobacteria the signal protein PatS is expressed by heterocysts und transferred to neighbouring cells to prevent heterocyst development there (Yoon and Golden, 1998; Risser and Callahan, 2009). The apocytochrome and heme portion of cytochrome *c* are transported independently through the cytoplasmic membrane into the periplasmic space, where it acts as a soluble component of the cytoplasmic membranes respiration in order to pump protons from the cytoplasm into the periplasm.The *ccs* (cytochrome *c* synthesis) proteins are responsible for attachment of heme to apolipoprotein (Sawyer and Barker, 2012; Gabilly and Hamel, 2017).

Strain	Microcystin or nodularin producing	Gene	Identity	Putative function according to cyanobase (2017)
Microcystis panniformis FACHB 1757	+	*VL20_5557*	96.8%	ABC transporter ATP binding protein
Nodularia spumigena CCY9414	+	*N9414_07671*	73.2%	ABC transporter like protein
Anabaena sp. 90	+	*ANA_C10978*	72.4%	ABC transporter ATP binding protein *mcyH*
Anabaena sp. ATCC 29413	-	*Ava1617*	65.5%	ABC transporter like protein
Microcystis aeruginosa NIES-2549	+	*MYAER_2994*	64.1%	ABC transporter ATP binding protein
Cyanothece sp. PCC 7822	-	*Cyan7822_4846*	61.3%	ABC transporter related protein

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Synechococcus sp. PCC 7942	-	*Synpcc7942_0622*	39.0%	ATPase
Synechococcus sp. UTEX 2973	-	*M744_13730*	39.0%	ABC transporter ATP-binding protein
Synechocystis sp. PCC 6803	-	*sll0182*	38.6%	ABC transporter ATP-binding protein
Synechococcus sp. PCC 6301	-	*syc0902_c*	38.6%	ABC transporter ATP binding protein
Anabaena sp. PCC 7120	-	*alr2663*	37.7%	ABC transporter ATP-binding protein

Table 2 Homologues of *mcyH* encoding the microcystin exporter of *Microcystis aeruginosa* NIES 843 in different cyanobacterial strains. Identity values were taken from cyanobase.

2.3. Carbonic acids

All biological membranes separating cells from the environment or dividing cells into different compartments consist of phospholipid bilayers. Pernil et al. (2010) discovered pyruvate uptake by *Anabaena* sp. PCC 7120 mediated by a TRAP transporter. *Synechocystis* sp. PCC 6803 has been genetically modified for the production of biofuel by introducing a mutated acyl – acyl carrier protein thioesterase from *E. coli* (Cho and Cronan, 1995) in order to produce and secrete fatty acids (Liu et al., 2011). Sahu and Adhikary (1981) discovered that sodium acetate reduced the growth rate in the light but induced at the beginning very low chemoheterotrophic growth in the dark of a not exactly defined *Anabaena* sp. strain, however, after twelve days dark growth on sodium acatate stopped. Since acetate is too hydrophilic to pass the cytoplasmic membrane there has to exist a specific transporter for it.

2.4. Alcohols

Glycerol has been demonstrated to support photoheterotrophic and mixotrophic growth of *Cyanothece* sp. ATCC 51142 (Feng et al., 2010), mixotrophic growth of *Anabaena* sp. PCC 7120 (Malatinszky et al. 2017) and can be used for heterotrophic growth of *Synechococcus* sp. PCC 7002 (Rippka et al., 1979). *Plectonema boryanum* was reported to grow in the dark dependent on mannitol (White and Shilo, 1975). Both mannitol and sorbitol could be used

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with low efficiency as a substrate for mixotrophic, photoheterotrophic and chemohetrotrophic growth by *Calothrix marchica* Lemm. Var. *intermedia* Rao and *Scytonema schmidlei* de Toni (Adhikary and Sahu, 1988), however the mode of transport has not been further investigated.

2.5. Alkaloids

Dermatoxines and cytotoxines produced by cyanobacteria include alkaloids produced by the genera *Lyngbya*, *Schizothrix* and *Oscillatoria*, while cyanobacterial neurotoxines are Tropane-related alkaloids and Carbamate alkaloids derived from *Aphanizomenon*, *Anabaena*, *Raphidiopsis*, *Oscillatoria*, *Planktothrix*, *Cylindospermum*, *Cylindospermopsis* and *Lyngbya* (Blaha et al., 2009). A well known example is Cylindrospermopsin from *Cylindrospermopsis raciborskii* (Blahova et al., 2009). All these substances are produced within the cytoplasm but excreted to the environment. In general alkaloids are too hydrophilic to be capable of passage through the cytoplasmic membrane without a specific transporter.

2.6. Organic dyes

Two fluorescin derivatives calcein and 5-carboxyfluorescin have been used to detect the intercellular exchange through the septal junctions between heterocysts and vegetative cells (Mullineaux et al., 2008; Nieves-Morion et al., 2017b). No information about the export and import systems in calcein and and 5-carboxyfluorescin transport between neighbouring cells is available.

2.7. DNA

It has been known for a long time that cyanobacteria can import DNA, which is used for genetic manipulation. There exist three ways by which DNA can pass the cyanobacterial cytoplasmic membrane: transformation, conjugation and transduction (Thiel, 1994; Koksharova and Wolk, 2002; Tandeau de Marsac and Houmard, 1987). Very few cyanobacteria have the capacity to take up pure DNA from the environment (see Table 3). The only other available list of natural transformable cyanobacteria (Johnston et al., 2014) is incorrect, because it lists *Synechococcus* sp. PCC 6301 (not transformable) and lacks *Synechococcus* sp. PCC 7942 (transformable). The latter is a highly transformable strain (Golden and Sherman, 1984) and can be transformed by DNA from *Synechococcus* sp. PCC 6301 with the same rate as by DNA derived from the same organism (Grigorieva, 1985). Additionally heterospecific transformation between the genera *Synechococcus* and *Synechocystis* has been reported (Stevens and Porter, 1986). Despite the close relationship to *Synechococcus* sp. PCC 7942 (Golden et al., 1989; Wilmotte and Stam, 1984) and contrary to previous reports (Herdman and Carr, 1971) *Synechococcus* sp. PCC 6301 cannot be naturally transformed. *Synechococcus* sp. PCC 7002 has been demonstrated to be capable of taking up both plasmid (Buzby et al., 1983) and chromosomal DNA (Essich et al., 1990). In *Synechocystis* sp. PCC 6803 only double stranded but not single stranded DNA could serve as substrate for transformation (Barten and Lill, 1995). One strand is degraded by a nuclease during uptake supplying energy for the import of the other strand. The necessity of bivalent cations for DNA uptake indicates a Ca- dependent nuclease, which is located in the cytoplasmic membrane (Barten and Lill, 1995) and homologous to EndA of *Streptococcus pneumoniae* (Puyet et al., 1990). *Synechocystis* sp. strain PCC 6714 was once naturally

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transformable (Astier and Espardellier, 1976; Joset, 1988) but has lost its competence for natural transformation over the years of cultivation. This may be due to the fact that the corresponding nuclease is now missing in *Synechocystis* sp. PCC 6714 (Barten and Lill, 1995), however blasting did not reveal a nuclease homologe to EndA in either *Synechocystis* sp. PCC 6803 or *Synechocystis* sp. PCC 6714. There exists one cyanobacterial strain *Synechocystis* sp. PCC 6308, which has to be chemically induced by $CaCl_2$ in order to be transformable (Devilly and Houghton, 1977). The mechanism of transformation is similar in all known competent strains. Double stranded DNA interacts with receptor ComEA, which transfers one strand through the transmembrane channel ComEC, while the other strand is degraded (Dubnau, 1999; Johnston et al., 2014). In *Synechocystis* sp. PCC 6803 *pilA*, *pilB*, *pilC*, *pilD*, *pilG*, *pilH*, *pilI*, *pilJ*, *pilL*, *pilM*, *pilN*, *pilO*, *pilQ*, *pilT* and *comA* have been shown to be necessary for natural transformation (Okamoto and Ohmori, 2002; Yoshihara et al., 2001; Yoshihara et al., 2002). No filamentous cyanobacteria have been discovered that are naturally transformable (see Table 3). Another form of transformation is electroporation, which does not need a special transporter. All cyanobacterial strains that have been successfully electroporated at that time are listed in Koksarova and Wolk (2002). Meanwhile *Synechocystis* sp. strains PCC 6803 (Zang et al., 2007) and PCC 6714 (Ludwig et al., 2008) could be electroporated as well.

Many cyanobacteria are able to take up plasmid DNA by conjugation from *E. coli*. This method has been developed by Wolk et al. (1984) for *Anabaena* sp. strains PCC 7120, PCC 7118 and M-131 and is assumed to function in a similar way as between two *E. coli* cells. A complete list of cyanobacterial strains that have been successfully conjugated from *E. coli* is available in Koksarova and Wolk (2002).

Finally there exists a way of DNA transfer by transduction mediated by cyanophages, which can undergo both the lytic and lysogenic cycle (Gromov et al., 1983; Mann et al., 2003; Xia et al., 2013). All known cyanophages contain double stranded DNA (Martin and Tyler, 1999; King et al., 2012; Xia et al., 2013). Host specificity differs among cyanophages as high light adapted *Prochlorococcus* is infected by highly host specific *Podoviridae*, whereas low light adapted *Prochlorococcus* and marine *Synechococcus* were infected by broad host range Myoviridae (Sullivan et al., 2003). Although several cyanophages were investigated (Hu et al., 1981; Sarma and Kaur, 1997; Rimon and Oppenheim 1975; Koz'yakov et al., 1972; Padan and Shilo, 1973; Gromov, 1983; Mendzul et al., 1985; Sherman and Brown, 1978) no cyanophage could be developed for genetical manipulation of a cyanobacterial strain.

Strain	Reference
Thermosynechococcus elongatus BP-1	Iwai et al., 2004
Synechocystis sp. PCC 6803	Grigorieva and Shestakov, 1982; Zang et al., 2007

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Synechococcus sp. PCC 7942	Shestakov and Khyen, 1970; Golden et al., 1989
Synechococcus sp. PCC 7002	Stevens and Porter, 1980

Table 3 Cyanobacterial strains that are naturally competent for DNA transformation

2. 8. others

2. 8.1. Hydrocarbons

Alkanes are hydrophobic molecules and volatile molecules like ethylene and certain terpenes can therefore be easily excreted through the membranes (Varman et al., 2013) without a specific transporter. Cyanobacteria are able to synthesize long chain alkanes (C15-C19) by two different pathways either by acyl-ACP reductases (FAR) and aldehyde deformylating oxygenases (FAD) (Schirmer et al., 2010) or by polyketide synthases (Mendez-Perez et al., 2011) thereby contributing to the hydrocarbon cycle of the ocean (Lea-Smith et al., 2015).

2.8.2. Urea

Among cyanobacteria urea is widespread as a potential nitrogen source (Kratz and Myers, 1955; Neilson and Larsson, 1980, Rawson, 1985, Kapp et al., 1975). According to Neilson and Larsson (1980) *Synechocystis* sp. PCC 6714, *Pseudanabaena* sp. strains PCC 6903 and B2, LPP 6402, LPP 73110 and *Anabaena* sp. PCC 7118 could use urea as sole nitrogen source, while *Synechococcus* sp. PCC 6301 failed to do so. Healey et al. (1977) reported that urea uptake was only slightly reduced in *Pseudoanabaena catenata* if ammonia was coadded. This may be due to the relatively short incubation time as in another cyanobacterial strain *Anabaena doliolum* urea uptake was not affected by ammonium within the first hour (Singh, 1988; Singh and Ahmad, 1989) but later urea uptake dramatically decreased (Singh and Ahmad, 1989). As increasing the concentration of coadded ammonia did not further reduce urea uptake the two substances do not seem to enter the cell by the same transporter (Healey et al., 1977). In *Synechocystis* sp. PCC 6803 the knock-out of ORF *sll0374* encoding a component of an ABC transporter resulted in a mutant strain deficient in urea uptake. In *Anabaena* sp. PCC 7120 *sll0374* homologue ORF *urtE* was identified, which belonged to an operon. Deletion of several genes of this operon abolished the capacity to import urea (Valladares et al., 2002).

2. 8. 3. Uric acid

Van Baalen (1962) and van Baalen and Marler (1963) reported that several cyanobacterial strains can use urea, uric acid and allantoin as nitrogen sources. According to Neilson and Larsson (1980) uric acid could be used by *Synechococcus* sp. PCC 6301, *Pseudoanabaena* sp. PCC 6903, LPP 6402 and LPP 73110.

2. 8. 4. Herbicides and Antibiotics

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3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Golden and Hazelkorn, 1985), 3-(4-Isopropylphenyl)-1,1-dimethylurea (isoproturon) and 4-Hydroxy-3,5-diiodbenzonitril (ioxynil) block the photosynthetic non-cyclic electron pathway of cyanobacteria. DCMU has been shown to be more effective than isoproturon and ioxynil on the cyanobacterium *Synechocystis salina* (Yotsova et al., 2017). Since all three herbicides are very hydrophobic they probably pass the cytoplasmic membrane without the use of a transporter.

Most antibiotics like kanamycin, neomycine, streptomycin, spectinomycin, gentamicin, erythromycin and chloramphenicol exhibit a toxic effect towards cyanobacteria. Aminoglycosides like kanamycin, streptomycin and gentamicin kill most gram negative bacteria and gram positive staphylococci but do not have an effect on streptococci or anaerobic bacteria, as oxidation is important for the entry of the antibiotics into the cytoplasm (Taber et al., 1987). Agarwal et al. (2018) demonstrated that mutation of sll180 encoding an inner membrane ABC transporter for S-layer protein Sll1951 resulted in an increased sensitivity towards different antibiotics indicating the role of the S-layer in antibiotic resistance. Accumulation of antibiotics in an aerobic bacteria culture occurs in three steps, an ionic binding followed by two energy dependent phases EDPI and EDPII (Andry and Bockrath, 1974; Bryan and van den Elzen, 1977; Davis, 1987; Miller et al. 1980; Taber et al., 1987). It seems that entry does not occur through a single specific transporter but rather by diffusion or a combination of various transporters. Bryan and van der Elzen (1977) found out that mutations of genes involved in aerobic energy generation in *E. coli* resulted in increased resistance towards streptomycin and gentamicin but not to spectinomycin. It is rather likely that the corresponding gene products do not act as antibiotic carriers themselves but help in energizing the actual transporters. No specific data on antibiotic uptake into cyanobacteria are currently available.

3. Future directions

This review summarizes our current knowledge on the uptake of organic molecules by cyanobacteria. In many cases the biochemical uptake mechanisms have not been clarified yet. However, understanding the transport mechanisms through the cytoplasmic membrane will be important for a detailed characterization of many metabolic processes. Besides understanding of uptake and subsequent metabolism of organic substances as a carbon source may help to grow some strains under heterotrophic conditions, which have been considered to be strict photolithoautotrophs.

The transport of amino acids through the cytoplasmic membrane of cyanobacteria has to be further investigated since no strain exists yet, where all amino acid transporters are deleted. The reason has not been revealed yet, why cyanobacteria need any transporters for amino acids, as they are able to synthesize them using ammonia or nitrate and carbon dioxide. The reason, why amino acids are imported even if they inhibit growth like phenylalanine or glutamate in certain strains needs to be investigated. Besides the metabolic fate of incorporated amino acids remains largely unknown.

The reason why some cyanobacterial strains can naturally be transformed while others cannot has not been elucidated yet. A detailed knowledge about natural transformation would allow

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to make non-transformable strains transformable, which would enormously facilitate and reduce the time for creating of new mutant strains. This would enormously facilitate and reduce the time necessary for creating mutations, e.g. in filamentous strains

Last but not least cyanobacteria have already been manipulated to produce valuable organic substances that can be used as biofuels. Since cyanobacteria only need light energy and can easily be cultivated in inorganic media they can be used as biofactories. While hydrophobic molecules like alkanes and fatty acids may leave intact cells by themselves, hydrophilic molecules will be retained inside the cells. If genes encoding specific transporter from other organisms are introduced into cyanobacteria the desired molecules can be released directly to the surounding medium without the complex purification procedures necessary if the cells are lysed. As a consequence combining natural transformation and artificial export of organic molecules has big economic potential for the future.

4. Abbriviations

DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea)
PCC	Pasteur culture collection
ATCC	American type culture collection
UTEX	University of Texas
BG11	Blue green 11
DAG	D-alanine, isobutyric acid, glycine transporter
LIV	Leucine, isoleucine, valine transporter
Gtr	Glucose transporter
GlcP	Glucose permease
ioxynil	4-Hydroxy-3,5-diiodbenzonitril
isoproturon	3-(4-Isopropylphenyl)-1,1-dimethylurea
EDPI	Energy dependent phase I
EDPII	Energy dependent phase II
ABC	ATP binding cassette
MSF	Major facilitator superfamily

5. References

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