Compartmentalized function through cell differentiation in filamentous cyanobacteria

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Abstract | Within the wide biodiversity that is found in the bacterial world, Cyanobacteria represents a unique phylogenetic group that is responsible for a key metabolic process in the biosphere — oxygenic photosynthesis — and that includes representatives exhibiting complex morphologies. Many cyanobacteria are multicellular, growing as filaments of cells in which some cells can differentiate to carry out specialized functions. These differentiated cells include resistance and dispersal forms as well as a metabolically specialized form that is devoted to N_2 fixation, known as the heterocyst. In this Review we address cyanobacterial intercellular communication, the supracellular structure of the cyanobacterial filament and the basic principles that govern the process of heterocyst differentiation.

Oxygenic photosynthesis
A biological process that converts light energy into chemical energy and splits water to release oxygen.

Hypha
A branching cellular filament that forms the mycelium of actinobacteria and fungi.

Fruiting body
A cell aggregate where spores are formed in some myxobacteria and fungi.

Primary productivity
The production of organic compounds from CO_2.

Heterocyst
A terminally differentiated N_2-fixing cell found in some filamentous cyanobacteria.

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The bacterial world contains much metabolic biodiversity. If life was present on Earth at least 3.5 billion years ago, bacteria had notably diversified by around 2.3 billion years ago, by which time an astonishing range of metabolic capabilities, including oxygenic photosynthesis, had developed[1]. Since then, bacteria have kept a genetic reservoir of the key redox-chemistry metabolic capabilities of the biosphere[2]. Although commonly viewed as single-celled organisms, many bacteria are multicellular. Morphologically complex bacteria were probably present on Earth in the Archean eon (that is, before 2.5 billion years ago)[1,3]. Multicellular forms are found in several different bacterial phyla, such as Actinobacteria, Cyanobacteria and Myxococcales. Actinobacteria develop hyphae, myxobacteria can construct a fruiting body and both produce spores. Cyanobacteria, however, exhibit a wider range of developmental forms.

As the first organisms to use oxygenic photosynthesis, cyanobacteria were key players in the early evolution of life on Earth. Oxygenic photosynthesis requires the coordinated action of two photosystems (PSI and PSII) that, working in series, generate the electrochemical potential needed to extract electrons from water, releasing O_2. The oxygenic photosynthetic apparatus probably evolved from a functional pairing of the two different types of photosystem that already existed at the time, in either the same or different organisms, along with the addition of the Mn_4Ca oxygen-evolving complex[4]. The production of oxygen by cyanobacteria was responsible for the oxidation of Earth's atmosphere around 2.4 to 2.2 billion years ago; cyanobacteria also contributed substantially to primary productivity in the Proterozoic eon and are now important players in the carbon and nitrogen cycles of the biosphere[5]. Some cyanobacteria can grow heterotrophically, but most are characterized by their reliance on photosynthesis, which produces the 'assimilatory power' (ATP and reducing equivalents in the form of NADPH or reduced ferredoxin) that is used in the fixation of CO_2 (a process termed photoautotrophic metabolism) and the assimilation of other nutrients including nitrogen.

The cyanobacteria are a phylogenetically coherent group of organisms that also include chloroplasts[6,7]. However, cyanobacteria have diversified through evolution, producing both unicellular and multicellular forms. The multicellular forms consist of trichomes (FIG. 1) that, in some strains, can contain hundreds of cells[8]. Most cyanobacteria divide by binary fission, but some exhibit alternatives to the standard mode of bacterial division, reproducing by budding or by multiple fission of enlarged cells[8,9]. In some filamentous cyanobacteria, vegetative cells can differentiate into heterocysts (FIG. 1a), which are specialized for the fixation of N_2 gas[10]; a subset of these cyanobacteria can also form spores, which are known as akinetes[8] (FIG. 1c). In the filaments of most heterocyst-forming cyanobacteria, such as *Anabaena* spp. and *Nostoc* spp., the cells divide in only one plane, but in the filaments of some other species, such as *Fischerella* spp., the cells can divide in more than one plane, producing branched filaments[8] (FIG. 1b). Some filamentous

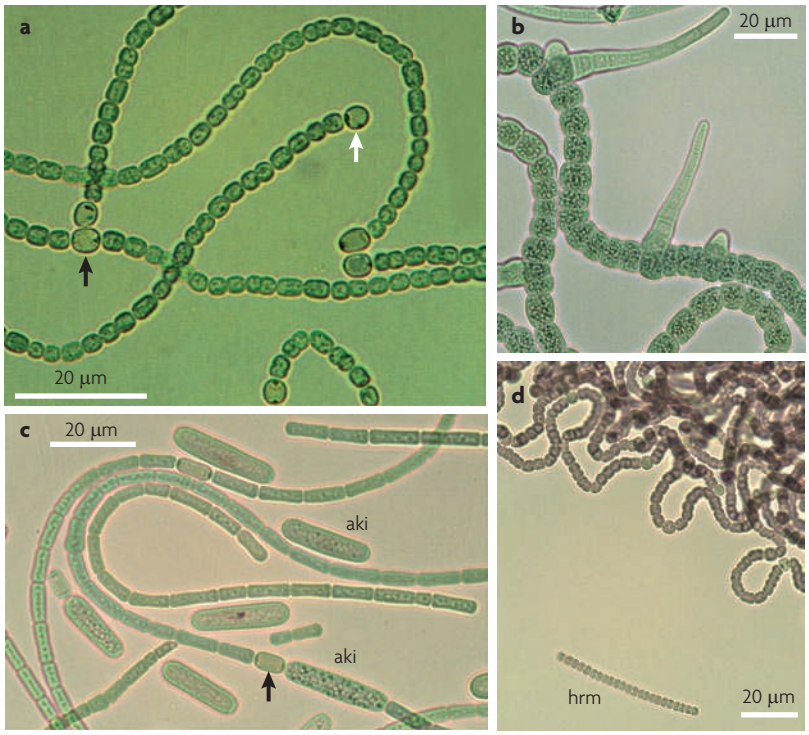


Figure 1 | **Cell types in heterocyst-forming cyanobacteria. a** | N_2-grown filaments of *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120) showing terminal heterocysts (white arrow; note the presence of only one polar granule) and intercalary heterocysts (black arrow; note the presence of two polar granules) in addition to vegetative cells. **b** | Filaments of *Fischerella muscicola* showing true filament branches that result from cell division in more than one plane. **c** | N_2-grown filaments of *Anabaena cylindrica*, with heterocysts (black arrow) and akinetes (aki) in addition to vegetative cells; akinetes adjacent to hererocysts in the filament and isolated akinetes (already released from the filament) can be observed. **d** | N_2-grown *Nostoc* sp. PCC 9203 showing mature filaments of vegetative cells and heterocysts and one hormogonium (hrm; note the small size of its cells) that has moved away from the colony. Images courtesy of José E. Frías, CSIC and Universidad de Sevilla, Spain.

Akinete
A cyanobacterial spore that tolerates cold and desiccation but not heat.

Hormogonium
A short filament made of small cells that serve a dispersal function in some filamentous cyanobacteria.

Murein sacculus
Also known as peptidoglycan. A large polymer surrounding the bacterial cell as a mesh and consisting of amino sugars and amino acids.

Lipopolysaccharide
A major component of the outer membrane of Gram-negative bacteria, consisting of a lipid covalently bound to a long polysaccharide that faces the extracellular medium.

cyanobacteria also produce hormogonia, which are small motile filaments that can serve a dispersal function in some benthic species[8] (FIG. 1d). Therefore, four different cell types can be found in some cyanobacteria: the vegetative cells of the mature filaments and the differentiated akinetes, heterocysts and hormogonial cells (FIG. 2).

Multicellularity involves at least three well-defined processes: cell–cell adhesion, intercellular communication and cell differentiation. In this Review, we reflect on some of the features of the filamentous, heterocyst-forming cyanobacteria that are relevant for multicellularity. These features include a special supracellular structure and the developmental process that produces a filament with two metabolically interdependent cell types, the photosynthetic vegetative cells and the N_2-fixing heterocysts. *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120) and *Nostoc punctiforme* PCC 73102 are becoming model organisms in this field (BOX 1). Whereas *Anabaena* sp. PCC 7120 is an obligate autotroph in which some cells can differentiate into heterocysts, *N. punctiforme* PCC 73102 is a facultative heterotroph with a complex life cycle and exhibits all of the developmental possibilities described above.

Filament structure

Cyanobacteria bear a Gram-negative type of cell envelope containing an outer membrane that is external to the cytoplasmic membrane and to the murein sacculus, which is thicker than in most other well-characterized Gram-negative bacteria[11,12]. Although evident in the early electron microscopy-based cytological literature[13,14], it has been realized only recently that the outer membrane of heterocyst-forming cyanobacteria does not enter the septum between adjacent cells in the filament, even though these cells are surrounded by their cytoplasmic membrane and peptidoglycan layers[13–16] (FIG. 3C). Staining the outer membrane and cytoplasmic membrane of *Fischerella* spp. with a fluorescent dye also reveals that the outer membrane is continuous along the entire filament[17], which might help to keep cells together in the filament.

The bacterial outer membrane is asymmetrical, bearing an inner leaflet that is rich in phospholipids and an outer leaflet that is rich in lipopolysaccharide (LPS)[18]. It also contains proteins including porins, which usually take the form of β-barrels with a central pore that allows the passage of small ions and molecules, making the outer membrane permeable to small molecules[19]. Porins have been noted in the outer-membrane proteome of *Anabaena* sp. PCC 7120 (REF. 20), but inspection of the genomic sequence of *Anabaena* sp. PCC 7120. fails to identify orthologues of the best characterized proteobacterial porins[15,21]. In addition, in some cyanobacteria, including an *Anabaena* species, LPS lacks the typical components of the enterobacterial LPS, such as phosphate, 3-deoxy-D-manno-octulosonic acid and heptose[12,22]. An *Anabaena* sp. PCC 7120 mutant that apparently does not produce LPS has been isolated[23], but the effect of the mutation on filament structure has not been reported. A better characterization of the cyanobacterial outer membrane would contribute to our understanding of its role in filament structure and physiology.

A continuous periplasm. The presence of a continuous outer membrane suggests that the periplasmic space, which lies between the cytoplasmic membrane and outer membrane, is also continuous[15]. Importantly, this seems to be the case not only along the string of vegetative cells but also between vegetative cells and heterocysts[24]. The periplasm might therefore allow movement of molecules along the filament. Accordingly, a soluble, periplasmic GFP that is produced in differentiating *Anabaena* sp. PCC 7120 heterocysts can be found at a distance from the producing cells, suggesting that the GFP moves through the periplasm; by contrast, if the GFP is produced as a cytoplasmic membrane-anchored protein, it is found only in the producing cells[25] (FIG. 3a). However, barriers to diffusion of the 27 kDa GFP do seem to exist[25,26]. The nature of these barriers is currently unknown, but they could correspond to the peptidoglycan entering the intercellular septa, which might have a size exclusion limit of 25 kDa to 50 kDa[25]. Whether such barriers might also affect the diffusion of smaller molecules is unknown.

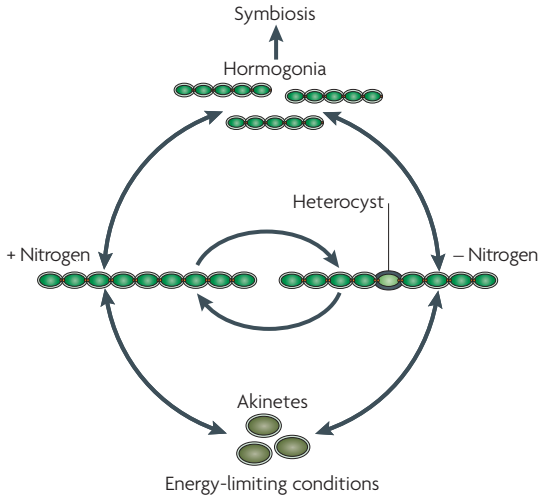


Figure 2 | **The complex life cycles of some heterocyst-forming cyanobacteria.** Some filamentous cyanobacteria, such as *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120), form heterocysts in response to deprivation of combined nitrogen; when filaments are re-supplied with combined nitrogen, heterocysts become spaced apart as intervening vegetative cells grow and divide. In other heterocyst-forming strains, some vegetative cells differentiate into akinetes in response to energy-limiting conditions; akinetes germinate under favourable conditions, producing growing filaments with or without heterocysts, depending on the availability of combined nitrogen. Some filamentous cyanobacteria, such as *Nostoc punctiforme* PCC 73102, also produce hormogonia, which are dispersal forms that can also function as infection units for the establishment of symbiotic associations with plants. The differentiation of hormogonia can be stimulated by some environmental conditions, such as nitrogen deprivation, and by a plant hormogonium-inducing factor[60]; when hormogonia resume growth, they produce filaments with or without heterocysts, depending on the availability of combined nitrogen.

Cell-to-cell joining structures. *Anabaena* sp. PCC 7120 mutants showing filament fragmentation have been isolated, suggesting the existence of genes encoding proteins that are specifically involved in cell–cell adhesion[27–29]. Two of these genes, *sepJ* (also known as *alr2338*) and the filament integrity protein gene (*fraC*), are predicted to encode integral cytoplasmic-membrane proteins[29–31]. SepJ is also predicted to carry a long extracytoplasmic segment that bears coiled-coil domains[31], which are known to participate in protein–protein interactions. A SepJ–GFP fusion protein localizes to the cell poles in the intercellular septa of the *Anabaena* sp. PCC 7120 filaments[31] (FIG. 3b). The GFP part of this fusion protein is expected to reside on the cytoplasmic face of the cytoplasmic membrane; therefore, the fact that a single fluorescent spot is seen between adjacent vegetative cells suggests that the SepJ molecules from two adjacent cells are closely clustered together. However, the SepJ–GFP units seem to be more separated in the septa between vegetative cells and heterocysts, where separate fluorescent spots are observed[31] (FIG. 3b). SepJ could be a component of cell-to-cell joining structures in the heterocyst-forming cyanobacteria. Whether other proteins, such as FraC, contribute to such structures is unknown.

The SepJ-containing cell-to-cell joining structures could correspond to the thin structures that are perpendicular to the cytoplasmic membrane in the intercellular septa of the filament, as observed by electron microscopy (FIG. 3c) (see REF. 14 for an example in the early literature), which have been termed 'microplasmodesmata' (REF. 32). These structures, seen by freeze-fracture electron microscopy as pits and protrusions in the different leaflets of the cytoplasmic membranes of adjacent cells, are around five times as abundant in septa between vegetative cells as they are in septa between vegetative cells and heterocysts[33]. Although this is a controversial issue, it should be noted that, because of their small size (less than 20 nm in outside diameter), these structures are unlikely to correspond to true plasmodesmata, which are cytoplasmic bridges delimited by cytoplasmic membrane[33]. In addition, the lack of transfer of the cytoplasmic membrane-anchored GFP from heterocysts to adjacent vegetative cells (FIG. 3a) supports the idea that there is no cytoplasmic-membrane continuity between the two types of cells[25].

Differentiated cells

Heterocysts. N_2 fixation is carried out exclusively by prokaryotes (Bacteria or Archaea) in either an anoxic environment or under intracellular micro-oxic conditions that are appropriate for the expression and function of the N_2-fixing enzyme, nitrogenase. This enzyme is encoded by the *nifHDK* operon and is extremely sensitive to oxygen. In a photosynthetic, oxygen-evolving cyanobacterial filament, the heterocyst provides a micro-oxic site for N_2 fixation. Therefore, in these organisms, compartmentalization of oxygenic photosynthesis and N_2 fixation in different cell types is the solution to the problem of incompatibility between the two processes.

The heterocyst (FIG. 4) carries a distinctive envelope consisting of a glycolipid laminated layer that is a permeability barrier for gases and, external to it, a thicker homogeneous layer of polysaccharide that apparently protects the glycolipid layer from physical damage[34]. Consistent with the recent identification of a TolC-like protein, HgdD, needed for deposition of the glycolipid layer[35], these layers are found outside the outer membrane[15]. TolC family proteins are trimeric, pore-forming, outer-membrane β-barrel proteins that work in concert with cytoplasmic-membrane transporters to export materials out of Gram-negative bacteria. Cells with mutations in the *devBCA* genes[36], which encode a heterocyst-specific ABC transporter, or in *hgdD*[35] have similar phenotypes, suggesting that DevBCA could be the companion to HgdD. Many of the genes encoding proteins that may be involved in the biosynthesis of the heterocyst-specific glycolipid and polysaccharide layers are grouped in gene islands in the genome of *Anabaena* sp. PCC 7120 (REFS 37,38).

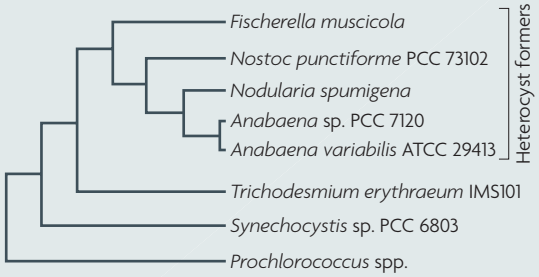
The organization of the intracytoplasmic membranes (thylakoids) is different in the heterocysts and in the vegetative cells, probably reflecting the particular bioenergetics

β-Barrel
A protein domain made of β-strands that forms a closed structure with an internal opening.

Anoxic
Containing no oxygen.

Micro-oxic
Containing very low amounts of oxygen.

Box 1 | **Genomes and genetics of heterocyst-forming cyanobacteria**



The availability of an experimental system for DNA transfer that permits genetic analysis of heterocyst-forming cyanobacteria has been essential for the current understanding of these organisms, along with substantial genomic information, most of which can be found on CyanoBase and the US Department of Energy Joint Genome Institute website. The complete genome sequence is currently available for around 50 cyanobacterial strains, including 3 heterocyst formers: *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120) (which has a genome of 7.21 Mb and 6,223 predicted genes), *Anabaena variabilis* ATCC 29413 (which has a genome of 7.07 Mb and 5,724 predicted genes), and *Nostoc punctiforme* PCC 73102 (which has a genome of 9.06 Mb and 6,794 predicted genes). *Anabaena* sp. PCC 7120 and *Anabaena variabilis* ATCC 29413 are very closely related, but *N. punctiforme* PCC 73102 is more distant (see the figure; phylogenetic distances between strains are not depicted). In addition, the genome sequences of heterocyst-forming *Nodularia* and *Calothrix* strains are being completed by the Gordon and Betty Moore Foundation Microbial Genome Sequencing Project. The genome sizes of these organisms are substantially larger than those of most sequenced unicellular cyanobacteria, which are in the range of about 1.7 Mb for the marine *Prochlorococcus* spp. strains to 3.97 Mb for the facultative photoheterotroph *Synechocystis* sp. PCC 6803. However, the sequenced cyanobacterial strain that is most closely related to the heterocyst formers, the marine filamentous *Trichodesmium erythraeum* IMS101, has a genome size of 7.75 Mb. A trend of increasing genome size is consistent with the notion that larger genomes are needed to support a multicellular organism than are needed to support a related single-celled organism[104].

Wolk and colleagues developed an experimental system to transfer DNA into heterocyst-forming cyanobacteria that was optimized for *Anabaena* sp. PCC 7120 (REF. 105). It is based on the transfer of plasmids from *Escherichia coli* to the cyanobacterium by means of conjugation. To increase efficiency, protection of the transferred DNA against resident restriction endonucleases is achieved by pre-methylation of the plasmid to be transferred, carried out in *E. coli*[106]. The transferred plasmid can replicate in the cyanobacterium if it carries an appropriate origin of replication, but it can also integrate into the recipient's genome through homologous recombination if it carries homologous DNA. The different possible fates of the transferred DNA give multiple possibilities of genetic manipulation, from complementation of mutants to targeted mutagenesis and construction of strains carrying reporter gene fusions[107]. This genetic system is also functional with *N. punctiforme* PCC 73102 and *A. variabilis* ATCC 29413[107].

Data for the figure is from published molecular sequence phylogenetic analyses[5–7,63].

of the heterocysts. The water-splitting activity of PSII is lost during heterocyst differentiation, favouring the establishment of an intracellular micro-oxic environment[39], but some oxygen will still enter the heterocyst (along with N_2), probably through its poles[40]. The 'neck'-like structures at the heterocyst poles might diminish the surface of contact with the adjacent vegetative cells to decrease the amount of gas entering the heterocyst[40]. Respiration consumes the oxygen that enters the heterocysts[39], and two heterocyst-specific terminal respiratory oxidases have been identified in *Anabaena* sp. PCC 7120 (REF. 41). Additionally, PSI-dependent photophosphorylation is important in heterocyst bioenergetics[39].

The heterocyst lacks ribulose 1,5-bisphosphate carboxylase–oxygenase and does not photosynthetically fix CO_2, relying instead on fixed carbon that is received from the neighbouring vegetative cells[42]. Reduced compounds are transferred to heterocysts, probably in the form of a sugar such as sucrose[39,43], and the oxidative pentose-phosphate pathway is important for sugar catabolism in the heterocyst[44]. Ammonium is produced by the fixation reaction that is carried out by nitrogenase, but in N_2-fixing filaments it is immediately incorporated into glutamine and other amino acids[45]. Heterocysts contain high levels of glutamine synthetase (which catalyses the ATP-dependent amidation of glutamate, producing glutamine) and lack glutamine-2-oxoglutarate amidotransferase (GOGAT) (which catalyses the reductive transamidation of 2-oxoglutarate (2-OG), producing two glutamate molecules)[39,46]. Isolated heterocysts that can fix N_2 therefore produce glutamine when incubated in the presence of glutamate[47]. These observations suggest that there is a glutamate–glutamine exchange between vegetative cells and heterocysts that results in a net export of nitrogen from the heterocysts[45,47]. In the heterocyst poles, at the cytoplasmic side of the heterocyst 'necks', granules of cyanophycin (multi-L-arginyl-poly-(L-aspartic acid)) accumulate[48]. Interestingly, in terminal heterocysts the 'neck' and cyanophycin granule are formed only at the heterocyst pole close to the adjacent vegetative cell (FIGS 1,4), another morphogenetic effect. The heterocyst's cyanophycin has been suggested to serve as a nitrogen buffer, transiently storing fixed nitrogen[49], although the accumulation of cyanophycin is not required for diazotrophic growth[50,51].

Akinetes. Akinetes are spore-like cells that are frequently larger than vegetative cells (FIG. 1) and that differentiate from these cells in response to environmental conditions such as light limitation or phosphate deprivation[52], which probably result in a shortage of cellular energy. Consistently, in a glucose-6-phosphate 1-dehydrogenase (*zwf*) mutant of *N. punctiforme* PCC 73102, which is impaired in heterotrophy, akinetes differentiate when the filaments are placed in the dark in the presence of fructose[53]. In N_2-fixing filaments, the position of

akinetes in relation to heterocysts is strain-specific[52]. For instance, in *Anabaena cylindrica* akinetes differentiate adjacent to heterocysts (FIG. 1). Akinetes show a reduced but existent metabolic activity[52], accumulate carbon (glycogen) and nitrogen (cyanophycin) reserves[52], and bear a thickened cell wall that shares some components with the heterocyst envelope[54–56]. Genes that are induced during akinete differentiation have now been identified[53,57], and a global gene expression analysis carried out with *N. punctiforme* PCC 73102 has identified 497 genes (around 7% of the genome) that change their expression levels in differentiating akinetes[58]. Many of the upregulated genes encode unassigned proteins, suggesting that they have novel functions, and many downregulated genes encode central metabolism proteins, which is consistent with the akinetes being in a non-growth state[58]. Akinete germination takes place under conditions that are permissive for growth and frequently involves the release of a short-filament germling through a pore in the akinete envelope[52].

Hormogonia. Hormogonia are short filaments that are made of small cells and show gliding motility (involving the action of pili or the production of slime) or gas vacuole-mediated buoyancy[59,60]. They can be seen both at the edge of and at a distance from *Nostoc* spp. colonies grown on solid growth media (FIG. 1). No unique nutritional or physical factor is known to trigger hormogonium differentiation, although it can be stimulated by changes in multiple factors[59,60]. The differentiation of hormogonia involves cell division without an increase in biomass, resulting in their characteristic small cells, and requires a distinct transcriptional programme[58,59]. In differentiating *N. punctiforme* PCC 73102 hormogonia, the expression levels of 1,827 genes (around 25% of the genome) have been found to differ from the expression levels in vegetative cells[58]. Half of the downregulated genes are predicted to encode central metabolism proteins, around half of the upregulated genes encode unassigned proteins and 22% of the upregulated genes encode proteins that are involved in adaptation functions, including signal transduction, taxis and motility[58]. These data illustrate the role of hormogonia as dispersal units that, once formed, are released by fragmentation of the producing vegetative filament. After a few days hormogonia become sessile and start to grow, producing a new vegetative filament that may or may not bear heterocysts, depending on the availability of fixed nitrogen.

Hormogonia are the infection units in the establishment of symbiosis between heterocyst-forming cyanobacteria and plants[59,60], and some plant factors can stimulate hormogonia differentiation[60]. The transcriptional programme of differentiation that is triggered by a bryophyte-derived hormogonium-inducing factor and differentiation that is induced by nitrogen deprivation only partially overlap in *N. punctiforme* PCC 73102 (REF. 61). In the hormogonia of this cyanobacterium, pili are important for plant infection, as shown by inactivation of *pil*-like genes[62].

Monophyletic
Concerning a group of organisms that have evolved from a common ancestor.

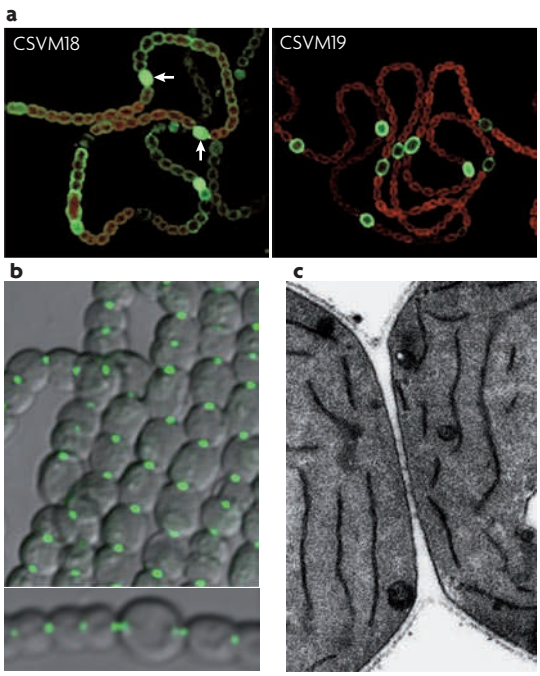


Figure 3 | **Putative structures for intercellular communication. a** | Periplasmic continuity between the heterocyst and the adjacent vegetative cells. The left micrograph shows *Anabaena* sp. strain CSVM18, the differentiating heterocysts (green cells indicated by white arrows) of which express GFP that is soluble in the periplasm (see REF. 25 for details). The right micrograph shows *Anabaena* sp. strain CSVM19, which expresses a periplasmic GFP that is anchored to the cytoplasmic membrane of the producing cells (green cells). Whereas the cytoplasmic membrane-anchored GFP remains in the producing cell, the soluble GFP is seen not only in producing cells but also in the periphery of non-producing cells, suggesting that the GFP moves through the periplasm. The micrographs are overlays of the cyanobacterial red autofluorescence and the GFP green fluorescence, captured by confocal microscopy, of filaments from colonies grown on solid medium without a source of combined nitrogen. **b** | Cell-to-cell joining structures. The top panel shows nitrate-grown filaments of *Anabaena* sp. strain CSAM137 expressing a protein consisting of SepJ (also known as Alr2338) fused to GFP; this fusion protein localizes to the cell poles in the intercellular septa[31]. The bottom panel shows a portion of a N_2-grown filament of the same strain with a heterocyst, revealing double fluorescence spots at the heterocyst–vegetative cell junctions. The figures are overlays of bright field microscopy and GFP green fluorescence, captured by confocal microscopy. **c** | A transmission electron micrograph showing an intercellular septum in a filament of wild-type *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120), in which thin structures perpendicular to the cytoplasmic membranes of the two adjacent cells can be observed. Parts **a** and **b** courtesy of Vicente Mariscal, CSIC and Universidad de Sevilla, Spain. Part **c** courtesy of Iris Maldener, Universität Tübingen, Germany.

Evolutionary relationships. Heterocyst-forming cyanobacteria are monophyletic, suggesting that the heterocyst evolved only once. Akinetes have been preserved in old sedimentary rocks, providing evidence for the presence

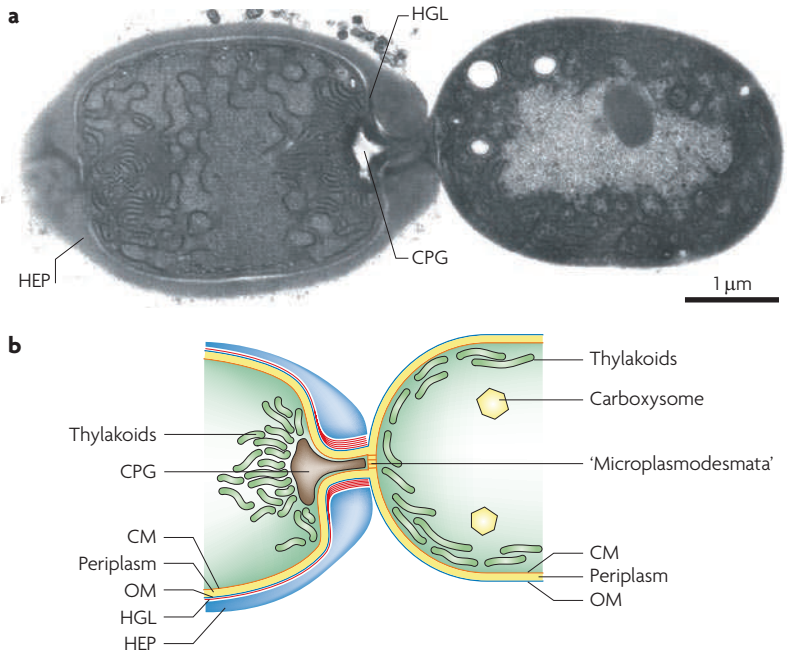


Figure 4 | **The heterocyst and the heterocyst-vegetative cell septum. a** | A transmission electron micrograph showing a terminal heterocyst (left) and its adjacent vegetative cell (right) from a filament of *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120). In the preparation of samples for electron microscopy, the cyanophycin granule (CPG) is frequently lost, leaving a white, empty space in the micrographs. **b** | Schematic of portions of a heterocyst and an adjacent vegetative cell. A continuous periplasm that is delimited by the cytoplasmic membrane (CM) and outer membrane (OM) and contains the peptidoglycan runs along the filament. In the septum between the two cells, as well as in the septa between vegetative cells along the filament, thin structures that have been called 'microplasmodesmata' are present. In the heterocyst, external to the outer membrane, specific glycolipid and polysaccharide layers are present. The different distributions of thylakoids in the two types of cells (peripheral in vegetative cells and polar in the heterocyst) and the presence of carboxysomes in vegetative cells but not in heterocysts are indicated. HEP, heterocyst polysaccharide layer; HGL, heterocyst glycolipid layer. Part **a** courtesy of Iris Maldener, Universität Tübingen, Germany.

of cyanobacteria with complex developmental processes by around 2.1 billion years ago[63]. Whereas the akinetes are resistance cell forms, the function of the heterocyst is nutritional, and N_2 fixation in an oxic environment could have set the selection pressure for the evolution of heterocysts shortly after the oxidation of Earth's atmosphere[63]. The two differentiated cell types share some components of their special cell envelopes, suggesting that they are evolutionarily related[39]. Whereas akinetes and hormogonia are transient cell forms that will produce growing filaments, heterocysts are terminally differentiated, non-growing cells that serve an altruistic function. Finally, comparison of the genomes of some algae and plants with those of cyanobacteria suggests that the endosymbiotic ancestor of the chloroplast was a heterocyst-forming cyanobacterium[64]. As infection units, hormogonia could have had a key role in the original endosymbiosis, which took place around 1.5 billion years ago[65].

Heterocyst differentiation

Developmental processes in bacteria rely on two types of circuits for information sensing and processing: regulation of gene expression and generation of positional information. Regulation of gene expression takes place in response to cell cycle progression or, in the case of adaptive cell differentiation processes, to external cues or metabolic conditions, and it frequently follows a temporal sequence. The generation of positional information is often linked to cell cycle progression and is responsible for the establishment of the spatial pattern characteristic of the developmental process. Moreover, these two circuits are usually interconnected at specific points.

Activation of gene expression. Heterocyst differentiation can be considered to be one extreme of the range of the organism's responses to nitrogen stress, which are orchestrated by NtcA, a global transcriptional regulator found in all cyanobacteria[66,67]. NtcA belongs to the cyclic AMP receptor protein family of regulators and binds to specific DNA sites that bear the signature sequence $GTAN_8TAC$, which are found in the promoter regions of multiple genes involved in nitrogen assimilation and related functions[68]. The standard NtcA-dependent promoters bear an NtcA-binding site centred at around –41.5 nucleotides from the transcription start site and a –10 sequence in the form TAN_3T (REF. 66). NtcA perceives nitrogen stress by responding to 2-OG, which has been shown to increase NtcA binding to its targets in several regulated promoters (for examples, see REFS 69–71) and to be required for open-complex formation, and hence transcription, at the promoters of several *Anabaena* sp. PCC 7120 genes that are involved in heterocyst differentiation[72].

Microarray analysis of transcript abundance at 3 hours, 8 hours and 24 hours after combined-nitrogen deprivation of nitrate-grown cells has shown significant changes for 495 upregulated and 196 downregulated genes in *Anabaena* sp. PCC 7120 (REFS 34,73). The earliest induced genes increase the scavenging of traces of ammonium, nitrate, nitrite or urea, which are sources of combined nitrogen[66,74]. If nitrogen stress persists, the cyanobacterium will enter the heterocyst differentiation process (FIG. 5). The differentiation of a single cell into a heterocyst is governed by a sequence of gene activation events triggered by the concerted action of two regulators: NtcA[75,76] and heterocyst differentiation control protein (HetR)[77]. At initiation, the two regulatory genes are induced in a mutually dependent manner, with induction of *hetR* preceding that of *ntcA*[78]. Moreover, positive autoregulation seems to be crucial for increasing the expression of *hetR* and *ntcA* up to the levels that are required for the establishment of heterocyst differentiation[71,79]. Increased expression of both *ntcA*[80] and *hetR*[79] is mainly localized to certain spatially distributed cells that eventually become heterocysts.

When heterocyst differentiation has been completed, the expression of many of the genes that have increased expression on combined-nitrogen deprivation return to initial levels[34,73]. Around 30 of the transiently induced genes have putative or demonstrated regulatory functions affecting the expression of other genes (TABLE 1). However, inactivation of these genes has a far smaller effect on heterocyst differentiation than inactivation of *ntcA* or *hetR*, which prevents differentiation[75–77].

Many of the genes that act at different stages of the differentiation process or in the mature heterocyst, including *ntcA* and *hetR*, bear complex promoter regions that allow the start of transcription at several sites, each directed by a different promoter (FIG. 6). These include constitutive promoters with recognizable –10 sequence and –35 sequence hexamers (ensuring a low level of gene expression that is independent of the nitrogen regime), promoters that are activated by direct binding of NtcA, either to sites located at the standard –41.5 nucleotide position or further upstream, and promoters that are dependent on NtcA or on NtcA and HetR but that have no apparent NtcA-binding site or determinant other than a –10 sequence[67]. Some of the promoters that are directly activated by NtcA can function in the absence of the accompanying promoters, as has been shown *in vivo* for the *ntcA* and glutamine synthetase (*glnA*) genes[80,81]. Indeed, standard NtcA-dependent promoters such as those of the heterocyst differentiation gene *hetC* (TABLE 1), *devBCA* (discussed above) and *nrrA* (discussed below) can be activated *in vitro* in the presence of only RNA polymerase, NtcA and 2-OG[72]. We speculate that this kind of promoter could be activated on combined-nitrogen deprivation in all cells of the filament, although the increased levels of NtcA in the differentiating cells, which result from the localized increase of expression of *ntcA* at a certain time during differentiation[80], would potentiate direct activation by NtcA in those particular cells[67].

The requirement of NtcA for activation of the promoters that do not bear canonical NtcA-binding sites could be an indirect effect, which would call for the participation of additional regulators. NrrA, a nitrogen- and NtcA-regulated response regulator[82], has been proposed to serve such a role in a *hetR* promoter[73,83]. However, the fact that NrrA-deficient mutant strains still undergo heterocyst differentiation and the fact that induction of *nrrA* on combined-nitrogen deprivation occurs in all cells of the filament[82], independent of HetR, suggest that the role of NrrA is not specifically related to heterocyst differentiation.

Finally, for the HetR-dependent promoters that do not show a recognizable promoter structure, the mechanism for activation of cell-specific transcription is unclear. HetR has been reported to bind DNA upstream of *hetR* and two other genes that are also activated during heterocyst differentiation, although the protein has no recognizable DNA-binding domain[84,85]. However, conflicting results have been reported concerning the necessity of the protein dimerization and autoproteolytic activity of HetR for DNA binding and heterocyst differentiation[85,86]. Indeed, unravelling the mechanism of action of HetR will be essential for understanding heterocyst differentiation at the molecular level.

Establishment of the spatial pattern of heterocysts. In the cyanobacterial filaments, heterocysts occupy strain-characteristic positions, a trait that arguably represents the simplest example of patterning in multicellular organisms. In diazotrophically grown *Anabaena* sp. PCC 7120, heterocysts are found at semiregular intervals along the filament, with 10–15 vegetative cells

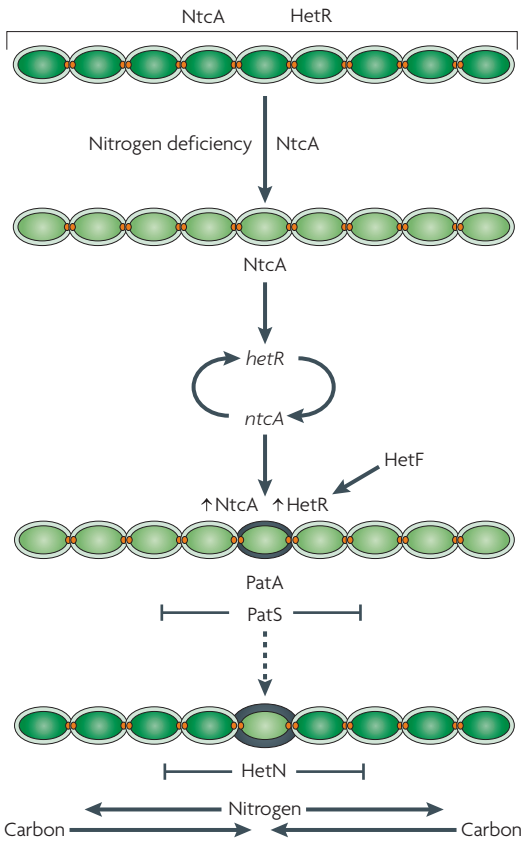


Figure 5 | **Progress of heterocyst differentiation.** The scheme represents a filament of a heterocyst-forming cyanobacterium grown with a source of fixed nitrogen, in which NtcA and heterocyst differentiation control protein (HetR) are present. In response to nitrogen deficiency, NtcA is activated, probably by interaction with its effector, 2-oxoglutarate. In some cells or cell clusters (as discussed in the main text), NtcA activates expression of *hetR*, and the NtcA–HetR amplification loop, which includes autoregulation of *ntcA* and *hetR*, results in increased levels of the two proteins. NtcA stays active possibly because of high levels of 2-oxoglutarate present in the heterocysts (including differentiating heterocysts), and HetR activity is positively influenced by heterocyst differentiation protein F (HetF). The differentiating heterocyst produces heterocyst inhibition-signalling peptide (PatS), which inhibits neighbouring cells from differentiating, and PatA, which counteracts the action of differentiation inhibitors in the differentiating cell. Finally, by 18 to 24 hours after the onset of nitrogen deprivation, a thick-walled, mature heterocyst has been formed, which fixes N_2 and produces other inhibitors such as ketoacyl reductase HetN-dependent inhibitor. In the N_2-fixing filament, fixed carbon moves from vegetative cells to heterocysts, and fixed nitrogen moves from heterocysts to vegetative cells. Intercellular molecular exchange of metabolites and regulatory molecules could take place through the continuous periplasm or cell-to-cell connecting structures. Note the changing pigmentation of the vegetative cells (during the differentiation process, these cells lose phycobiliproteins, which are regained when nitrogen compounds produced by the heterocysts reach the vegetative cells) and the different pigmentation of the heterocyst (the phycobiliprotein content of which is lower than that of vegetative cells).

Table 1 | **Further genes regulating heterocyst differentiation**

Gene*	Protein function or motif	Role in heterocyst differentiation	References
Heterocyst differentiation and patterning			
hetC	ABC-type exporter	Commitment to differentiation	108–110
ccbP	Ca^{2+}-binding protein	Ca^{2+} balance	111
asr1734	Unknown	Response to nutrient stress	112
hetZ	Helix–turn–helix motif	Coordinating differentiation and pattern formation	113
Heterocyst maturation			
devR	Response regulator	Polysaccharide layer synthesis	114
hepK	DevR histidine kinase	Polysaccharide layer synthesis	115
hepN	Histidine kinase	Polysaccharide layer synthesis	116
hepS	Serine/threonine kinase	Polysaccharide layer synthesis	117
devH	CRP-type transcriptional regulator	Glycolipid synthesis	118
prpJ	Protein phosphatase	Glycolipid synthesis	119
pkn44	HstK family protein kinase	Glycolipid synthesis	120
pkn30	HstK family protein kinase	Glycolipid synthesis	120
xisA	Site-specific recombinase	*nifHDK* operon recreation	121
patB	Bacterial ferredoxin and helix–turn–helix motifs	Unknown	122,123
Transcription			
sigC	Group 2 σ-factor	Induced in differentiating heterocysts	124
sigE	Group 2 σ-factor	Induced in differentiating heterocysts	124
sigG	Group 2 σ-factor	Induced in differentiating heterocysts	124

*Many genes have been identified in *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120) and *Nostoc punctiforme* PCC 73102 mutants as being responsible for the inability of these mutants to fix N_2 under oxic conditions. Some genes were cloned by genetic complementation of mutants, and others were cloned by transposon mutagenesis that marked the inactivated gene. CRP, cAMP receptor protein; *hetC*, heterocyst differentiation protein C gene; *xisA*, excisase A.

separating 2 heterocysts. However, when filaments of vegetatively growing cells are subjected to combined-nitrogen deprivation, differentiation often begins in clusters of cells and proceeds up to a certain point, when only one of the cells in the cluster is committed to continue differentiation[87]. Several genes have been identified that alter the pattern of heterocyst distribution in *Anabaena* sp. PCC 7120 filaments when they are mutated. Inactivation of *patS* (the heterocyst inhibition-signalling peptide gene), which is induced in the cells undergoing differentiation, leads to the formation of multiple contiguous heterocysts (the Mch phenotype) and short vegetative-cell intervals, whereas its overexpression suppresses differentiation[88]. *patS* encodes a 17- or 13-amino-acid product, the carboxy-terminal pentapeptide (RGSGR; also known as PatS-5) of which suppresses differentiation when added to the external medium[88]. It has been proposed that PatS could be exported from the producing cell to inhibit differentiation in its neighbours, leading to resolution of the initial clusters of differentiating cells[88]. This model requires that the inhibitory activity is different in the producing and receptor cells. In contrast to the full PatS, PatS-5 expression in differentiating heterocysts failed to restore a wild-type heterocyst pattern in a PatS-deficient mutant[89], which is consistent with PatS being processed during intercellular transfer.

In cells lacking a functional *patS* gene, the Mch phenotype is evident after 24 hours of combined-nitrogen deprivation, when the first round of differentiation is complete, but after 48 hours the heterocyst pattern in the mutants resembles that of the wild type. Consistently, expression of *patS* returns to basal levels following 24 hours of combined-nitrogen deprivation. By contrast, inactivation of the gene encoding ketoacyl reductase HetN produces no discernible effect during the first round of heterocyst differentiation but results in a Mch phenotype after 48 hours of combined-nitrogen deprivation[90,91]. Expression of *hetN* is activated later than that of *patS* during the differentiation process and, as is the case for *patS*, overexpression of *hetN* suppresses heterocyst formation. Therefore, whereas PatS is involved in the *de novo* pattern formation, HetN participates in the maintenance of the pattern of heterocyst distribution once it has been established. It has been suggested that both PatS and HetN influence the activity of HetR[92]. Whether this influence is restricted to an effect on the reported DNA-binding activity of HetR, which is inhibited by PatS-5 (REFS 84,85), remains to be discerned. Interestingly, HetN also carries an RGSGR pentapeptide in its sequence.

The *patA* gene encodes a protein with a chemotaxis protein Y-like phospho-acceptor domain and a PATAN (PatA N-terminus) domain that is possibly

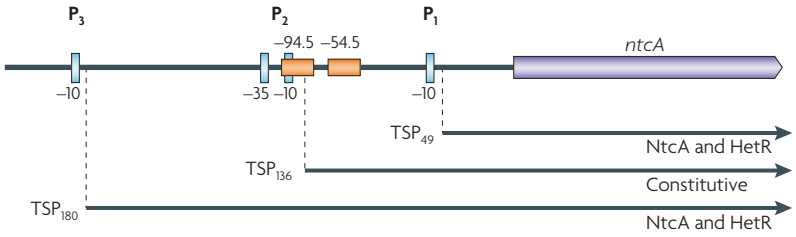


Figure 6 | **A complex gene promoter region.** Some *Anabaena* sp. PCC 7120 (also known as *Nostoc* sp. PCC 7120) genes that are expressed during heterocyst differentiation and in mature heterocysts bear complex promoter regions containing several promoters that generate different transcription start points (TSPs). The promoter of the regulatory *ntcA* gene is depicted as an example (the gene and promoter elements are not presented to scale). This gene carries three promoters that generate TSPs at 49, 136 and 180 nucleotides upstream from the gene translation start. P_1 is used at a low level in filaments grown in the presence of combined nitrogen and is transiently induced, depending on NtcA and heterocyst differentiation control protein (HetR), during heterocyst differentiation; it has a recognizable –10 promoter sequence and two NtcA-binding sites (orange boxes) that are centred at nucleotides –54.5 and –94.5 with regard to the TSP[71]. P_2 is constitutively active and has recognizable –10 and –35 promoter sequences. P_3 is transiently induced, depending on NtcA and HetR, during heterocyst differentiation but has no recognizable promoter elements other than a putative –10 promoter sequence.

involved in protein–protein interactions[93], and its inactivation leads to heterocyst differentiation mostly at the ends of the filament[94]. Epistasis analysis with mutants that are altered in *patA*, *patS* and *hetN* shows that PatA is required only in the presence of wild-type *patS* or *hetN* alleles, suggesting a role for PatA in attenuation of the negative effects of PatS and, later, of HetN[95]. The heterocyst differentiation gene *hetF* encodes a putative cysteine protease that is required for differentiation[96] and for activation of several HetR-dependent promoters[97]. Although no direct action has been demonstrated yet, both PatA and HetF could influence the levels of the HetR protein[97].

Therefore, the correct spatial pattern of heterocyst distribution along the filament, so that mature heterocysts do not occupy adjacent positions, seems to rely on interference with the stream of gene transcription activation that sustains the differentiation of a given cell. However, molecular details of the equilibrium between positive and negative factors that determine which cell in a neighbourhood will differentiate are still largely unresolved. Two other important aspects of the pattern that are yet to be resolved at the molecular level are why, on perception of nitrogen stress, differentiation begins in clustered cells[87], and how the sites of the differentiation of new heterocysts are selected during sustained diazotrophic growth[98]. Perhaps initiation by grouped cells responds to a requirement for a given stage in the cell cycle, which could be shared by neighbouring cells. The reported requirement of DNA replication[60] and cell division[99] for heterocyst differentiation supports this notion. In addition, differentiation of new heterocysts approximately midway between pre-existing heterocysts might involve regulation by nitrogenous compounds that are exported by the pre-existing, N_2-fixing heterocysts.

Epistasis analysis
Investigation of the interaction between genes, such that a gene with a mutant phenotype that prevails is said to be epistatic over another related gene.

Diazotrophic physiology
The physiology related to the growth of an organism that uses N_2 as a source of nitrogen.

Molecular exchange in the filament

As mentioned above, during both heterocyst differentiation and diazotrophic growth, exchange of metabolites and regulatory molecules takes place between the cells of the filament. A technique for studying cell-to-cell communication in filamentous cyanobacteria has been recently developed and consists of loading a fluorescent tracer (calcein) into the cytoplasm of the cells, where it is stably retained[100]. Fluorescence recovery after photobleaching (FRAP) experiments show rapid transfer of the calcein between the cytoplasm of the cells in filaments of *Anabaena* spp. As no specific permeases are expected to be present for an artificial substrate such as calcein, these results suggest the presence of direct cell-to-cell communication conduits. Calcein transfer is impaired in a SepJ-deficient mutant, suggesting that the proteinaceous cell-to-cell joining structures discussed above might be involved in molecular transfer directly between the cytoplasms of two adjacent cells[100]. Whereas calcein (which is 623 Da in size) can be transferred, there is no evidence for intercytoplasmic exchange of GFP[25,88]. Exchange of calcein is faster between vegetative cells than between vegetative cells and heterocysts, suggesting a different structure to the communication conduits between the different cell types[100]. This is consistent with the different fluorescence patterns of SepJ–GFP at the junctions between heterocysts and vegetative cells (which have a double fluorescence spot) and between vegetative cells (which have a single spot), as discussed above. Unlike simple cytoplasmic bridges, proteinaceous structures could be regulated and therefore behave as gates rather than simple pores. Additionally, the possible processing of PatS to direct its action to neighbours of the producing cell, as discussed above, would not be compatible with cytoplasmic bridges that were freely open to diffusion.

Alternatively, intercellular molecular exchange could take place through the periplasm, with cytoplasmic membrane permeases mediating the export and import of different molecules in the different cell types. For example, if the periplasm were important for exchange of nutrients, sugar exporters would be expected to be present in the vegetative cells and sugar importers would be expected to be present in the heterocysts; similarly, amino acid exporters would be expected to be present in the heterocysts and amino acid importers would be expected to be present in the vegetative cells[15]. In *Anabaena* sp. PCC 7120, inactivation of two ABC-type transporters that mediate amino acid uptake, one of which is expressed only in vegetative cells, specifically impairs N_2-dependent growth, indicating a role for these transporters in diazotrophic physiology[101,102]. A role for the periplasm as a metabolic communication conduit would require the outer membrane to have a low permeability for the exchanged metabolites, which has been recently shown for sucrose and glutamate[103].

We suggest that a specific use of each of the two possible pathways for moving different types of compounds between the two cell types might be a key feature in cyanobacterial multicellularity. An important property of the paths for intercellular molecular exchange in the filament is that they should permit the formation of

gradients of nitrogenous compounds or regulatory molecules. Consumption by the vegetative cells could contribute to the establishment of gradients of molecules (such as nutrients) that originate in the heterocysts, but regulated gates could also influence the formation of such gradients.

Conclusions

Consistent with the description that reproduction in heterocyst-forming cyanobacteria takes place 'by random trichome breakage' (REF. 8), the filament (or trichome), which is now viewed as a string of cells encapsulated by the outer membrane, can be considered as an organismic unit in these bacteria. Heterocyst-forming cyanobacteria are unique in that they can form two different types of metabolically active cells, photosynthetic vegetative cells and N_2-fixing heterocysts, the activities of which are necessary and complementary for the growth of the organism. Specific mechanisms seem to keep cells together in the cyanobacterial filament and to allow the intercellular exchange of nutrients that is necessary for growth. Of particular importance will be an increased understanding of the role of a shared periplasm and of SepJ-containing cell-to-cell connecting structures. Heterocyst differentiation from a vegetative cell also depends on intercellular communication, with exchange of regulatory molecules, such as a PatS-related compound, that influence the execution of a genetic differentiation programme. This programme is started as a nitrogen deprivation response that is mediated by NtcA, the global nitrogen-control transcription factor of cyanobacteria, and requires specific regulators, of which HetR is essential to differentiation. Knowledge of the mechanism of functional interactions between NtcA and HetR and of how other regulators inhibit differentiation of too many cells into heterocysts will be key to gaining a final understanding of this process that leads to a truly multicellular bacterium.

1. Cavalier-Smith, T. Cell evolution and Earth history: stasis and revolution. *Phil. Trans. R. Soc. Lond. B* **361**, 969–1006 (2006).
2. Falkowski, P. G., Fenchel, T. & Delong, E. F. The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**, 1034–1039 (2008).
3. Carroll, S. B. Chance and necessity: the evolution of morphological complexity and diversity. *Nature* **409**, 1102–1109 (2001).
4. Blankenship, R. E., Sadekar, S. & Raymond, J. in *Evolution of Primary Producers in the Sea* (eds Falkowski, P. G. & Knoll, A. H.) 21–35 (Academic Press–Elsevier, Amsterdam, 2006).
5. Knoll, A. H. in *The Cyanobacteria: Molecular Biology, Genomics and Evolution* (eds Herrero, A. & Flores, E.) 1–19 (Caister Academic, Norfolk, 2008).
6. Giovannoni, S. J. *et al.* Evolutionary relationships among cyanobacteria and green chloroplasts. *J. Bacteriol.* **170**, 3584–3592 (1988).
A classic phylogenetic study using 16S ribosomal RNA sequences that corroborates the hypothesis that chloroplasts belong to the cyanobacterial radiation and shows that heterocyst-forming cyanobacteria constitute a monophyletic group.
7. Shi, T. & Falkowski, P. G. Genome evolution in cyanobacteria: the stable core and the variable shell. *Proc. Natl Acad. Sci. USA* **105**, 2510–2515 (2008).
8. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. Generic assignments, strain stories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**, 1–61 (1979).
A classic study of the taxonomy of cyanobacteria, presented from a microbiological perspective.
9. Angert, E. R. Alternatives to binary fission in bacteria. *Nature Rev. Microbiol.* **3**, 214–242 (2005).
10. Haselkorn, R. Heterocysts. *Annu. Rev. Plant Physiol.* **29**, 319–344 (1978).
11. Drews, G. & Weckesser, J. in *The Biology of Cyanobacteria* (eds Carr, N. G. & Whitton, B. A.) 333–357 (Blackwell, Oxford, 1982).
12. Hoiczyk, E. & Hansel, A. Cyanobacterial cell walls: news from an unusual prokaryotic envelope. *J. Bacteriol.* **182**, 1191–1199 (2000).
13. Ris, H. & Singh, R. N. Electron microscopy studies on blue-green algae. *J. Biophys. Biochem. Cytol.* **9**, 63–80 (1961).
14. Wildon, D. C. & Mercer, F. V. The ultrastructure of the heterocyst and akinete of the blue-green algae. *Arch. Microbiol.* **47**, 19–31 (1963).
15. Flores, E., Herrero, A., Wolk, C. P. & Maldener, I. Is the periplasm continuous in filamentous multicellular cyanobacteria? *Trends Microbiol.* **14**, 439–443 (2006).
16. Nierzwicki-Bauer, S. A., Balkwill, D. L. & Stevens, S. E. Jr. Heterocyst differentiation in the cyanobacterium *Mastigocladus laminosus*. *J. Bacteriol.* **157**, 514–525 (1984).
17. Schneider, D., Fuhrmann, E., Scholz, I., Hess, W. R. & Graumann, P. L. Fluorescence staining of live cyanobacterial cells suggest non-stringent chromosome segregation and absence of a connection between cytoplasmic and thylakoid membranes. *BMC Cell Biol.* **8**, 39 (2007).
18. Bos, M. P., Robert, V. & Tommassen, J. Biogenesis of the Gram-negative outer membrane. *Annu. Rev. Microbiol.* **61**, 191–214 (2007).
19. Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **67**, 593–656 (2003).
20. Moslavac, S. *et al.* Proteomic analysis of the outer membrane of *Anabaena* sp. strain PCC 7120. *J. Proteome Res.* **4**, 1330–1338 (2005).
21. Kaneko, T. *et al.* Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. *DNA Res.* **8**, 205–213 (2001).
22. Weckesser, J., Katz, A., Drews, G., Mayer, H. & Fromme, I. Lipopolysaccharide containing L-acofriose in the filamentous blue-green alga *Anabaena variabilis*. *J. Bacteriol.* **120**, 672–678 (1974).
23. Xu, X., Khudyakov, I. & Wolk, C. P. Lipopolysaccharide dependence of cyanophage sensitivity and aerobic nitrogen fixation in *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **179**, 2884–2891 (1997).
24. Wilcox, M., Mitchison, G. J. & Smith, R. J. Pattern formation in the blue-green alga *Anabaena*. *J. Cell Sci.* **13**, 637–649 (1973).
25. Mariscal, V., Herrero, A. & Flores, E. Continuous periplasm in a filamentous, heterocyst-forming cyanobacterium. *Mol. Microbiol.* **65**, 1139–1145 (2007).
26. Zhang, L.-C., Chen, Y.-F., Chen, W.-L. & Zhang, C.-C. Existence of periplasmic barriers preventing green fluorescent protein diffusion from cell to cell in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* **70**, 814–823 (2008).
27. Buikema, W. J. & Haselkorn, R. Isolation and complementation of nitrogen fixation mutants of the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **173**, 1879–1885 (1991).
28. Ernst, A. *et al.* Synthesis of nitrogenase in mutants of the cyanobacterium *Anabaena* sp. strain PCC 7120 affected in heterocyst development or metabolism. *J. Bacteriol.* **174**, 6025–6032 (1992).
29. Bauer, C. C., Buikema, W. J., Black, K. & Haselkorn, R. A short-filament mutant of *Anabaena* sp. strain PCC 7120 that fragments in nitrogen-deficient medium. *J. Bacteriol.* **177**, 1520–1526 (1995).
30. Nayar, A., Yamaura, H., Rajagopalan, R., Risser, D. D. & Callahan, S. M. FraG is necessary for filament integrity and heterocyst maturation in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Microbiology* **153**, 601–607 (2007).
31. Flores, E. *et al.* Septum-localized protein required for filament integrity and diazotrophy in the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **189**, 3884–3890 (2007).
This article describes the subcellular location of SepJ at the cell poles in the intercellular septa, paving the way for the molecular study of intercellular connections in the cyanobacterial filament.
32. Lang, N. J. & Fay, P. The heterocysts of blue-green algae. II. Details of ultrastructure. *Proc. R. Soc. Lond. B* **178**, 193–203 (1971).
33. Giddings, T. H. & Staehelin, L. A. Plasma membrane architecture of *Anabaena cylindrica*: occurrence of microplasmodesmata and changes associated with heterocyst development and the cell cycle. *Cytobiologie* **16**, 235–249 (1978).
34. Xu, X., Elhai, J. & Wolk, C. P. in *The Cyanobacteria: Molecular Biology, Genomics and Evolution* (eds Herrero, A. & Flores, E.) 383–422 (Caister Academic, Norfolk, 2008).
35. Moslavac, S. *et al.* A TolC-like protein is required for heterocyst development in *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **189**, 7887–7895 (2007).
36. Fiedler, G., Arnold, M., Hannus, S. & Maldener, I. The DevBCA exporter is essential for envelope formation in heterocysts of the cyanobacterium *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* **27**, 1193–1202 (1998).
37. Fan, Q. *et al.* Clustered genes required for synthesis and deposition of envelope glycolipids in *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* **58**, 227–243 (2005).
38. Huang, G. *et al.* Clustered genes required for the synthesis of heterocyst envelope polysaccharide in *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **187**, 1114–1123 (2005).
39. Wolk, C. P., Ernst, A. & Elhai, J. in *The Molecular Biology of Cyanobacteria* (ed. Bryant, D. A.) 769–823 (Kluwer Academic, Dordrecht, 1994).
40. Walsby, A. E. Cyanobacterial heterocysts: terminal pores proposed as sites of gas exchange. *Trends Microbiol.* **15**, 340–349 (2007).
41. Valladares, A., Herrero, A., Pils, D., Schmetterer, G. & Flores, E. Cytochrome *c* oxidase genes required for nitrogenase activity and diazotrophic growth in *Anabaena* sp. PCC 7120. *Mol. Microbiol.* **47**, 1239–1249 (2003).
42. Wolk, C. P. Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica*. *J. Bacteriol.* **96**, 2138–2143 (1968).
43. Curatti, L., Flores, E. & Salerno, G. Sucrose is involved in the diazotrophic metabolism of the heterocyst-forming cyanobacterium *Anabaena* sp. *FEBS Lett.* **513**, 175–178 (2002).
44. Summers, M. L., Wallis, J. G., Campbell, E. L. & Meeks, J. C. Genetic evidence of a major role for glucose-6-phosphate dehydrogenase in nitrogen fixation and dark growth of the cyanobacterium *Nostoc* sp. strain ATCC 29133. *J. Bacteriol.* **177**, 6184–6194 (1995).
45. Wolk, C. P., Thomas, J., Shaffer, P. W., Austin, S. M. & Galonsky, A. Pathway of nitrogen metabolism after fixation of ^{13}N-labeled nitrogen gas by the cyanobacterium, *Anabaena cylindrica*. *J. Biol. Chem.* **251**, 5027–5034 (1976).

46. Martín-Figueroa, E., Navarro, F. & Florencio, F. J. The GS-GOGAT pathway is not operative in the heterocysts. Cloning and expression of *glsF* gene from the cyanobacterium *Anabaena* sp. PCC 7120. *FEBS Lett.* **76**, 282–286 (2000).
47. Thomas, J. *et al.* Formation of glutamine from [^{13}N] ammonia, [^{13}N]dinitrogen, and [^{14}C]glutamate by heterocysts isolated from *Anabaena cylindrica*. *J. Bacteriol.* **129**, 1545–1555 (1977).
48. Sherman, D., Tucker, D. & Sherman, L. A. Heterocyst development and localization of cyanophycin in N_2-fixing cultures of *Anabaena* sp. PCC 7120 (Cyanobacteria). *J. Phycol.* **36**, 932–941 (2000).
49. Carr, N. G. in *Photosynthetic Prokaryotes. Cell Differentiation and Function* (eds Papageorgiou, G. C. & Packer, L.) 265–280 (Elsevier, New York, 1983).
50. Ziegler, K., Stephan, D. P., Pistorius, E. K., Ruppel, H. G. & Lockau, W. A mutant of the cyanobacterium *Anabaena variabilis* ATCC 29413 lacking cyanophycin synthetase: growth properties and ultrastructural aspects. *FEMS Microbiol. Lett.* **196**, 13–18 (2001).
51. Picossi, S., Valladares, A., Flores, E. & Herrero, A. Nitrogen-regulated genes for the metabolism of cyanophycin, a bacterial nitrogen reserve polymer. *J. Biol. Chem.* **279**, 11582–11592 (2004).
52. Adams, D. G. & Duggan, P. S. Heterocyst and akinete differentiation in cyanobacteria. *New Phytol.* **144**, 3–33 (1999).
53. Argueta, C., Yuksek, K., Patel, R. & Summers, M. L. Identification of *Nostoc punctiforme* akinete-expressed genes using differential display. *Mol. Microbiol.* **61**, 748–757 (2006).
54. Cardemil, L. & Wolk, C. P. The polysaccharides from heterocyst and spore envelopes of a blue-green alga. Structure of the basic repeating unit. *J. Biol. Chem.* **254**, 736–741 (1979).
55. Soriente, A. *et al.* Heterocyst glycolipids of the cyanobacterium *Cyanospira rippkae*. *Phytochemistry* **33**, 393–396 (1993).
56. Leganés, F. Genetic evidence that *hepA* gene is involved in the normal deposition of the envelope of both heterocysts and akinetes in *Anabaena variabilis*. *FEMS Microbiol. Lett.* **123**, 63–67 (1994).
57. Zhou, R. & Wolk, C. P. Identification of an akinete marker gene in *Anabaena variabilis*. *J. Bacteriol.* **184**, 2529–2532 (2002).
58. Campbell, E. L. *et al.* Global gene expression patterns of *Nostoc punctiforme* in steady-state dinitrogen-grown heterocyst-containing cultures and at single time points during differentiation of akinetes and hormogonia. *J. Bacteriol.* **189**, 5247–5256 (2007).
This is the first study to present an estimation of the number and identity of genes needed for the differentiation of various specialized cell types in a filamentous cyanobacterium with different developmental possibilities.
59. Tandeau de Marsac, N. in *The Molecular Biology of Cyanobacteria* (ed. Bryant, D. A.) 825–842 (Kluwer Academic, Dordrecht, 1994).
60. Meeks, J. C. & Elhai, J. Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. *Microbiol. Mol. Biol. Rev.* **66**, 94–121 (2002).
61. Campbell, E. L., Christman, H. & Meeks, J. C. DNA microarray comparisons of plant factor- and nitrogen deprivation-induced hormogonia reveal decision-making transcriptional regulation patterns in *Nostoc punctiforme*. *J. Bacteriol.* **190**, 7382–7391 (2008).
62. Duggan, P. S., Gottardello, P. & Adams, D. G. Molecular analysis of genes in *Nostoc punctiforme* involved in pilus biogenesis and plant infection. *J. Bacteriol.* **189**, 4547–4551 (2007).
63. Tomitani, A., Knoll, A. H., Cavanaugh, C. M. & Ohno, T. The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. *Proc. Natl Acad. Sci. USA* **103**, 5442–5447 (2006).
64. Deusch, O. *et al.* Genes of cyanobacterial origin in plant nuclear genomes point to a heterocyst-forming plastid ancestor. *Mol. Biol. Evol.* **25**, 748–761 (2008).
65. Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G. & Bhattacharya, D. A molecular timeline for the origin of photosynthetic eukaryotes. *Mol. Biol. Evol.* **21**, 809–818 (2004).
66. Herrero, A., Muro-Pastor, A. M. & Flores, E. Nitrogen control in cyanobacteria. *J. Bacteriol.* **183**, 411–425 (2001).
67. Herrero, A., Muro-Pastor, A. M. Valladares, A. & Flores, E. Cellular differentiation and the NtcA transcription factor in filamentous cyanobacteria. *FEMS Microbiol. Rev.* **28**, 469–487 (2004).
68. Luque, I., Flores, E. & Herrero, A. Molecular mechanism for the operation of nitrogen control in cyanobacteria. *EMBO J.* **13**, 2862–2869 (1994).
This article describes the structure of the standard gene promoters that are regulated by the cyanobacterial nitrogen control transcription factor NtcA, opening the way for the definition of the NtcA regulon.
69. Vázquez-Bermúdez, M. F., Herrero, A. & Flores, E. 2-Oxoglutarate increases the binding affinity of the NtcA (nitrogen control) transcription factor for the *Synechococcus glnA* promoter. *FEBS Lett.* **512**, 71–74 (2002).
70. Tanigawa, R., M. *et al.* Transcriptional activation of NtcA-dependent promoters of *Synechococcus* sp. PCC 7942 by 2-oxoglutarate *in vitro*. *Proc. Natl Acad. Sci. USA* **99**, 4251–4255 (2002).
71. Olmedo-Verd, E., Valladares, A., Flores, E., Herrero, A. & Muro-Pastor, A. M. Role of two NtcA-binding sites in the complex *ntcA* gene promoter of the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **190**, 7584–7590 (2008).
72. Valladares, A., Flores, E. & Herrero, A. Transcription activation by NtcA and 2-oxoglutarate of three genes involved in heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **190**, 6126–6133 (2008).
73. Ehira, S. & Ohmori, M. NrrA, a nitrogen-responsive response regulator facilitates heterocyst development in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* **59**, 1692–1703 (2006).
74. Luque, I. & Forchhammer, K. in *The Cyanobacteria: Molecular Biology, Genomics and Evolution* (eds Herrero, A. & Flores, E.) 335–382 (Caister Academic, Norfolk, 2008).
75. Frías, J. E., Flores, E. & Herrero, A. Requirement of the regulatory protein NtcA for the expression of nitrogen assimilation and heterocyst development genes in the cyanobacterium *Anabaena* sp. PCC 7120. *Mol. Microbiol.* **14**, 823–832 (1994).
76. Wei, T. F., Ramasubramanian, T. S. & Golden, J. W. *Anabaena* sp. strain PCC 7120 *ntcA* gene required for growth on nitrate and heterocyst development. *J. Bacteriol.* **176**, 4473–4482 (1994).
77. Buikema, W. J. & Haselkorn, R. Characterization of a gene controlling heterocyst differentiation in the cyanobacterium *Anabaena* 7120. *Genes Dev.* **5**, 321–330 (1991).
This work identifies HetR as a key positive regulator of heterocyst differentiation.
78. Muro-Pastor, A. M., Valladares, A., Flores, E. & Herrero, A. Mutual dependence of the expression of the cell differentiation regulatory protein HetR and the global nitrogen regulator NtcA during heterocyst development. *Mol. Microbiol.* **44**, 1377–1385 (2002).
79. Black, T. A., Cai, Y. & Wolk, C. P. Spatial expression and autoregulation of *hetR*, a gene involved in the control of heterocyst development in *Anabaena*. *Mol. Microbiol.* **9**, 77–84 (1993).
80. Olmedo-Verd, E., Muro-Pastor, A. M., Flores, E. & Herrero A. Localized induction of the *ntcA* regulatory gene in developing heterocysts of *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **188**, 6694–6699 (2006).
81. Valladares, A., Muro-Pastor, A. M., Herrero, A. & Flores, E. The NtcA-dependent P_1 promoter is utilized for *glnA* expression in N_2-fixing heterocysts of *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **186**, 7337–7343 (2004).
82. Muro-Pastor, A. M., Olmedo-Verd, E. & Flores, E. All4312, an NtcA-regulated two-component response regulator in *Anabaena* sp. strain PCC 7120. *FEMS Microbiol. Lett.* **256**, 171–177 (2006).
83. Ehira, S. & Ohmori, M. NrrA directly regulates expression of *hetR* during heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **188**, 8520–8525 (2006).
84. Huang, X., Dong, Y. & Zhao, J. HetR homodimer is a DNA-binding protein required for heterocyst differentiation, and the DNA-binding activity is inhibited by PatS. *Proc. Natl Acad. Sci. USA* **101**, 4848–4853 (2004).
85. Risser, D. D. & Callahan, S. M. Mutagenesis of *hetR* reveals amino acids necessary for HetR function in the heterocystous cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **189**, 2460–2467 (2007).
86. Dong, Y., Huang, X., Wu, X.-Y & Zhao, J. Identification of the active site of HetR protease and its requirement for heterocyst differentiation in the cyanobacterium *Anabaena* sp. PCC 7120. *J. Bacteriol.* **182**, 1575–1579 (2000).
87. Yoon, H.-S. & Golden, J. W. PatS and products of nitrogen fixation control heterocyst pattern. *J. Bacteriol.* **183**, 2605–2613 (2001).
88. Yoon, H.-S. & Golden, J. W. Heterocyst pattern formation controlled by a diffusible peptide. *Science* **282**, 935–938 (1998).
This investigation identifies *patS* and basic aspects of the PatS-related inhibition of heterocyst differentiation.
89. Wu, X., Liu, D., Lee, M. H. & Golden, J. W. *patS* minigenes inhibit heterocyst development of *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **186**, 6422–6429 (2004).
90. Black, T. A. & Wolk, C. P. Analysis of a Het$^-$ mutation of *Anabaena* sp. strain PCC 7120 implicates a secondary metabolite in the regulation of heterocyst spacing. *J. Bacteriol.* **176**, 2282–2292 (1994).
91. Callahan S. M. & Buikema, W. J. The role of HetN in maintenance of the heterocyst pattern in *Anabaena* sp. PCC 7120. *Mol. Microbiol.* **40**, 941–950 (2001).
92. Khudyakov, I. Y. & Golden, J. W. Different functions of HetR, a master regulator of heterocyst differentiation in *Anabaena* sp. PCC 7120, can be separated by mutation. *Proc. Natl Acad. Sci. USA* **101**, 16040–16045 (2004).
93. Makarova, K. S., Koonin, E. V., Haselkorn, R. & Galperin, M. Y. Cyanobacterial response regulator PatA contains a conserved N-terminal domain (PATAN) with an alpha-helical insertion. *Bioinformatics* **22**, 1297–1301 (2006).
94. Liang. J., Scappino, L. & Haselkorn, R. The *patA* gene product, which contains a region similar to CheY of *Escherichia coli*, controls heterocyst pattern formation in the cyanobacterium *Anabaena* 7120. *Proc. Natl Acad. Sci. USA* **89**, 5655–5659 (1992).
95. Orozco, C. C., Risser, D. D. & Callahan, S. M. Epistasis analysis of four genes from *Anabaena* sp. strain PCC 7120 suggests a connection between PatA and PatS in heterocyst pattern formation. *J. Bacteriol.* **188**, 1808–1816 (2006).
96. Wong, F. C. Y. & Meeks, J. C. The *hetF* gene product is essential to heterocyst differentiation and affects HetR function in the cyanobacterium *Nostoc punctiforme*. *J. Bacteriol.* **183**, 2654–2661 (2001).
97. Risser, D. D. & Callahan, S. M. HetF and PatA control levels of HetR in *Anabaena* sp. PCC 7120. *J. Bacteriol.* **190**, 7645–7654 (2008).
98. Wolk, C. P. & Quine, M. P. Formation of one-dimensional patterns by stochastic processes and by filamentous blue-green algae. *Dev. Biol.* **46**, 370–382 (1975).
99. Sakr, S., Jeanjean, R., Zhang, C.-C. & Arcondeguy, T. Inhibition of cell division suppresses heterocyst development in *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **188**, 1396–1404 (2006).
100. Mullineaux, C. W. *et al.* Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. *EMBO J.* **27**, 1299–1308 (2008).
This study develops a method to probe molecular exchange between the cells in the cyanobacterial filament by using a fluorescent tracer, calcein, which is a valuable tool to study intercellular communication.
101. Picossi, S. *et al.* ABC-type neutral amino acid permease N-I is required for optimal diazotrophic growth and is repressed in the heterocysts of *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* **57**, 1582–1592 (2005).
102. Pernil, R., Picossi, S., Mariscal, V., Herrero, A. & Flores, E. ABC-type amino acid uptake transporters Bgt and N-II of *Anabaena* sp. strain PCC 7120 share an ATPase subunit and are expressed in vegetative cells and heterocysts. *Mol. Microbiol.* **67**, 1067–1080 (2008).
103. Nicolaisen, K. *et al.* The outer membrane of a heterocyst-forming cyanobacterium is a permeability barrier for uptake of metabolites that are exchanged between cells. *Mol. Microbiol.* **74**, 58–70 (2009).
104. Herdman, M., Janvier, M., Rippka, R. & Stanier, R. Y. Genome size of cyanobacteria. *J. Gen. Microbiol.* **111**, 73–85 (1979).
105. Wolk, C. P., Vonshak, A., Kehoe, P. & Elhai, J. Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria. *Proc. Natl Acad. Sci. USA* **81**, 1561–1565 (1984).
This work develops a method for DNA transfer to heterocyst-forming cyanobacteria, leading to the modern study and genetic manipulation of these organisms.

106. Elhai, J. Vepritskiy, A., Muro-Pastor, A. M., Flores, E. & Wolk, C. P. Reduction of conjugal transfer efficiency by three restriction activities of *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **179**, 1998–2005 (1997).
107. Koksharova, O. A. & Wolk, C. P. Genetic tools for cyanobacteria. *Appl. Microbiol. Biotechnol.* **58**, 123–137 (2002).
108. Khudyakov, I. & Wolk, C. P. *hetC*, a gene coding for a protein similar to bacterial ABC protein exporters, is involved in early regulation of heterocyst differentiation in *Anabaena* sp. PCC 7120. *J. Bacteriol.* **179**, 6971–6978 (1997).
109. Muro-Pastor, A. M., Valladares, A., Flores, E. & Herrero, A. The *hetC* gene is a direct target of the NtcA transcriptional regulator in cyanobacterial heterocyst development. *J. Bacteriol.* **181**, 6664–6669 (1999).
110. Xu, X. & Wolk, C. P. Role for *hetC* in the transition to a nondividing state during heterocyst differentiation in *Anabaena* sp. *J. Bacteriol.* **183**, 393–396 (2001).
111. Zhao, Y. *et al.* CcbP, a calcium-binding protein from *Anabaena* sp. PCC 7120, provides evidence that calcium ions regulate heterocyst differentiation. *Proc. Natl Acad. Sci. USA* **102**, 5744–5748 (2005).
112. Wu, X., Lee, D. W., Mella, R. A. & Golden, J. W. The *Anabaena* sp. PCC 7120 *asr1734* gene encodes a negative regulator of heterocyst development. *Mol. Microbiol.* **64**, 782–794 (2007).
113. Zhang, W. *et al.* A gene cluster that regulates both heterocyst differentiation and pattern formation in *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* **66**, 1429–1443 (2007).
114. Campbell, E. L., Hagen, K. D., Cohen, M. F., Summers, M. L. & Meeks J. C. The *devR* gene product is characteristic of receivers of two-component regulatory systems and is essential for heterocyst development in the filamentous cyanobacterium *Nostoc* sp. strain ATCC 29133. *J. Bacteriol.***178**, 2037–2043 (1996).
115. Zhou, R. & Wolk, C. P. A two-component system mediates developmental regulation of biosynthesis of a heterocyst polysaccharide. *J. Biol. Chem.* **278**, 19939–19946 (2003).
116. Ning, D. & Xu, X. *alr0117*, a two-component histidine kinase gene, is involved in heterocyst development in *Anabaena* sp. PCC 7120. *Microbiology* **150**, 447–453 (2004).
117. Fan, Q. *et al.* Signal transduction genes required for heterocyst maturation in *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **188**, 6688–6693 (2006).
118. Ramírez, M. E., Hebbar, P. B., Zhou, R., Wolk, C. P. & Curtis, S. E. *Anabaena* sp. strain PCC 7120 gene *devH* is required for synthesis of the heterocyst glycolipid layer. *J. Bacteriol.* **187**, 2326–2331 (2005).
119. Jang, J., Wang, L., Jeanjean, R. & Zhang, C.-C. PrpJ, a PP2C-type protein phosphatase located on the plasma membrane, is involved in heterocyst maturation in the cyanobacterium *Anabaena* sp. PCC 7120. *Mol. Microbiol.* **64**, 347–358 (2007).
120. Shi, L. *et al.* Two genes encoding protein kinases of the HstK family are involved in synthesis of the minor heterocyst-specific glycolipid in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **189**, 5075–5081 (2007).
121. Golden, J. W., Robinson, S. J. & Haselkorn, R. Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium *Anabaena*. *Nature* **314**, 419–423 (1985).
122. Liang., J., Scappino., L. & Haselkorn, R. The *patB* gene product, required for growth of the cyanobacterium *Anabaena* sp. strain PCC 7120 under nitrogen-limiting conditions, contains ferredoxin and helix-turn-helix domains. *J. Bacteriol.* **175**, 1697–1704 (1993).
123. Jones, K. M., Buikema, W. J. & Haselkorn, R. Heterocyst-specific expression of *patB*, a gene required for nitrogen fixation in *Anabaena* sp. PCC 7120. *J. Bacteriol.* **185**, 2306–2314 (2003).
124. Aldea, M. R., Mella-Herrera, R. A. & Golden, J. W. Sigma factor genes *sigC, sigE,* and *sigG* are upregulated in heterocysts of the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **189**, 8392–8396 (2007).

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
fraC | *glnA* | *hetC* | *hetF* | *nrrA* | *patA* | *sepJ*
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Anabaena sp. PCC 7120 | *Anabaena variabilis* ATCC 29413 | *Escherichia coli* | *Nostoc punctiforme* PCC 73102 | *Synechocystis* sp. PCC 6803 | *Trichodesmium erythraeum* IMS101
UniProtKB: http://www.uniprot.org
HetN | HetR | NtcA | PatS

FURTHER INFORMATION
Enrique Flores and Antonia Herrero's homepage: http://www.ibvf.cartuja.csic.es/Cianobac_Diferenciacion/Cyanobac_Differentiation.htm
CyanoBase: http://genome.kazusa.or.jp/cyanobase
Gordon and Betty Moore Foundation Microbial Genome Sequencing Project: http://www.moore.org/microgenome
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