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Review

Thylakoid membrane function in heterocysts☆

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ABSTRACT

Multicellular cyanobacteria form different cell types in response to environmental stimuli. Under nitrogen limiting conditions a fraction of the vegetative cells in the filament differentiate into heterocysts. Heterocysts are specialized in atmospheric nitrogen fixation and differentiation involves drastic morphological changes on the cellular level, such as reorganization of the thylakoid membranes and differential expression of thylakoid membrane proteins. Heterocysts uphold a microoxic environment to avoid inactivation of nitrogenase by developing an extra polysaccharide layer that limits air diffusion into the heterocyst and by upregulating heterocyst-specific respiratory enzymes. In this review article, we summarize what is known about the thylakoid membrane in heterocysts and compare its function with that of the vegetative cells. We emphasize the role of photosynthetic electron transport in providing the required amounts of ATP and reductants to the nitrogenase enzyme. In the light of recent high-throughput proteomic and transcriptomic data, as well as recently discovered electron transfer pathways in cyanobacteria, our aim is to broaden current views of the bioenergetics of heterocysts. This article is part of a Special Issue entitled Organization and dynamics of bioenergetic systems in bacteria, edited by Conrad Mullineaux.

1. Introduction

1.1. Heterocyst membrane heterogeneity

Since the nitrogenase enzyme is rapidly inactivated by molecular oxygen, diazotrophic cyanobacteria have developed ways of separating nitrogen fixation from photosynthetic oxygen evolution, either temporally or spatially [1,2]. Filamentous heterocystous cyanobacteria are multicellular species, where nitrogen starvation leads to differentiation of 5 to 10% of the cells into heterocysts, where nitrogen fixation takes place. Extensive modifications on a physiological level create a microoxic environment in the heterocyst, such as the creation of an extra thick cell wall that moderates diffusion of air into the cell.

Heterocyst differentiation involves changes on the morphological level, including structural changes of the thylakoid membranes [3–5]. The thylakoid membrane is an intracellular membrane system which fills a large part of the cyanobacterial cytoplasm, where the photochemical reactions that are the basis for photosynthesis take place (reviewed in [6]). During heterocyst differentiation, an additional membrane domain develops, that is most likely functionally separate from the thylakoid membranes. The additional membranes, dubbed "honeycombs" due to their appearance, are located close to the cell poles and harbour heterocyst specific terminal oxidases [7–10]. Besides the honeycomb membranes, mature heterocysts contain thylakoid membranes that are more "contorted" compared to the thylakoids in vegetative cells (Fig. 1) [11]. However, the protein content of the heterocyst thylakoids is highly similar to that in vegetative cells [12,13]. The function of the honeycomb membranes has been suggested to be consuming molecular oxygen that enters the heterocyst through the polar connections [9]. It is unclear where the line should be drawn between honeycombs and thylakoid membranes, and whether or not they constitute a continuous membrane. Nevertheless, the honeycombs are functionally distinct from the thylakoids and are outside the scope of this review.

1.2. The heterotrophic heterocyst

The nitrogenase reaction demands an astonishing amount of ATP and reductants—a minimum of 8 electrons and 16 ATP molecules are required per dinitrogen molecule that is reduced to ammonia [14]. When a vegetative cell differentiates into a heterocyst, most genes involved in carbon fixation are repressed, disabling its capacity for photoautotrophy [15–17]. Due to the considerable oxygen sensitivity of nitrogenase, PSII is thought to be inactivated in the heterocysts (but see section on PSII below) and oxygen consumption via respiration and Mehler-type reactions is increased [1,18–22]. To fuel nitrogen fixation under these circumstances, the heterocysts need to be supplied with an electron source other than water. The rate of nitrogen fixation is dependent on the availability of fixed carbon from photosynthesis in nearby vegetative cells, and movement of reduced carbon compounds from the vegetative cells into the heterocysts was observed already in the late 1960's [15,23,24]. Since then it has been established that carbohydrates are imported into the heterocysts via specific transporters [17,25–27]. The

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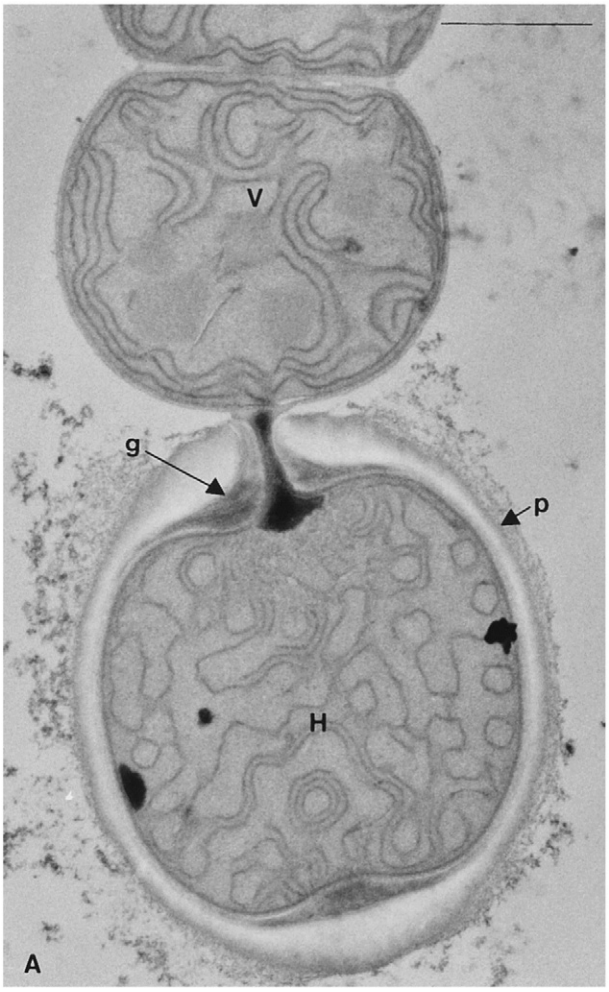

Fig. 1. A transmission electron micrograph of wild-type *Nostoc* PCC 7120. Both vegetative cells (V) and a heterocyst (H) are shown. The thylakoid membranes of the vegetative cell are smoothly layered, while in the heterocyst the membranes are less ordered and more contorted. The fibrous and homogeneous polysaccharide layers (p) and glycolipid layer (g) are visible around the heterocyst. Bar = 1 μm. Reprinted with permission from reference [176] © (1995) American Society for Microbiology.

carbohydrates are primarily catabolized by the oxidative pentose phosphate (OPP) pathway in the heterocysts [28–31]. In the OPP pathway, glucose-6-phosphate is oxidized to ribulose-5-phosphate yielding NADPH, which can serve as electron carrier to nitrogenase (section 3.2) [28,32–34].

1.3. Classification and studied strains

Only a handful of heterocystous cyanobacterial strains have been characterized on a molecular level and a few words on classification is in place before going into further details. Cyanobacteria are traditionally classified in five sections based on morphological traits and the mode of cell division [35]. Sections I to III include unicellular cyanobacteria and filamentous cyanobacteria incapable of cell differentiation. Sections IV and V, also classified as the order Nostocales and Stigonematales respectively, include all filamentous heterocystous cyanobacteria. More recent phylogenetic analyses have shown that cyanobacteria could be classified in seven to ten major clades, with sections I to III making polyphyletic clades and only the heterocystous cyanobacteria (sections IV and V) as true monophyletic [36,37].

The earliest genome sequencing of members of the Nostocales were made in *Nostoc* (or *Anabaena*) PCC 7120 and *Nostoc punctiforme* PCC 73102 (identical with strain ATCC 29133, and hereafter referred to as *N. punctiforme*) [38,39]. *Anabaena variabilis* has been a subject for mutagenesis and genetic studies long before its genome was sequenced. A large body of the research on heterocystous cyanobacteria that we are referring to has been made in these three strains, but we also include observations from less well-known strains. Throughout this review we will occasionally attribute observations to particular strains; otherwise we will refer to publications where information about the organism can be found.

2. Light harvesting in heterocysts

2.1. Occurrence of phycobiliproteins

One of the most noticeable characteristics of the heterocyst is the significantly lower autofluorescence from photosynthetic pigments compared to vegetative cells. In the absence of fixed nitrogen, the nitrogen rich phycobilisome antennae are degraded in all cells before heterocysts start to appear [40–43]. The phycobiliproteins continue to be degraded by a protease which is specific for this purpose, until heterocysts have formed and nitrogen fixation has been established [44–46]. After that, the vegetative cells regain their normal pigment composition, while in heterocysts both phycobilisome content and fluorescence intensity remain low [31,47–49]. Heterocysts contain large amounts of Photosystem I (PSI), so there is plenty of chlorophyll and most of the fluorescence that can be observed at room temperature from heterocysts comes from PSI (see Section 3 below). At 77 K however, the fluorescence spectrum reveals that some phycobilisomes remain in the heterocysts [50].

Heterocyst fluorescence spectra often display characteristics of altered phycobilisomes [12,51–56]). These are not merely remnants waiting to be degraded however. Fluorescence from the primary donor chlorophyll in PSI, P700, can be observed in heterocysts at 77 K after excitation in the phycobiliprotein absorbance bands. This indicates that phycobilisomes continue to function as antennae for PSI in heterocysts [12,50,51,57]. The *in vivo* action spectrum for nitrogenase was early on found to coincide with the absorption spectrum of the heterocyst phycobiliproteins, suggestive of the importance of PSI for nitrogen fixation [52,54].

2.2. Phycobilisome composition

Phycobilisomes (PBSs) are the main light harvesting antenna complexes in cyanobacteria, absorbing within a wide range between ca 500–675 nm (reviewed in [58,59]). The PBSs are extremely large protein complexes, several MDa in molecular weight. A PBS is generally composed of a central core, made of two to five cylindrical protein complexes, onto which six to eight cylindrical complexes ("rods") are attached (Fig. 2). Both core and rods are made of a protein class known as phycobiliproteins. The core consists of a small number of cylinders (typically 3–5) that are built up by trimers of allophycocyanin (APC) [60]. The rods consist of several disc-shaped protein complexes that are stacked onto each other. Each disc in a rod is a hexamer of phycobiliprotein, usually phycocyanin (PC) or phycoerythrin (PE), which bind phycobilins, the characteristic pigments of PBSs. The discs in each rod are held together by linker proteins that are essential for the structural integrity and energy transfer of the PBS.

The phycobiliproteins have different absorbance maxima: PE absorbs the shortest wavelengths, between 490 and 550 nm, and PC and APC have absorbance maxima at 620–640 nm and 650–670 nm respectively. PE discs are usually located at the peripheral end of the PBS rods, and PC discs closer to the core. This arrangement ensures that light energy is "funnelled" from the peripheral phycobiliproteins to the core. The APC core also has a heterogeneous composition. Several α (ApcA) and β (ApcB) subunits constitute the main body of the core together with the ApcE protein. The latter has a special function in the phycobilisome core. In older literature ApcE is often referred to as L_{CM} since it contains a linker (L) domain which connects the core (C) with