Septal Junctions in Filamentous Heterocyst-forming Cyanobacteria

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Abstract

In the filaments of heterocyst-forming cyanobacteria, septal junctions that traverse the septal peptidoglycan join adjacent cells allowing intercellular communication. Perforations in the septal peptidoglycan have been observed, and proteins involved in the formation of such perforations and putative protein components of the septal junctions have been identified, but their relationships are debated.

The N_2-fixing Cyanobacterial Filament

Some cyanobacteria grow as chains of cells (filaments or trichomes) that can be hundreds of cells long. The cyanobacteria bear a Gram-negative type of cell envelope, and the cyanobacterial filament consists of individual cells surrounded by their peptidoglycan layers but enclosed in a continuous outer membrane that defines a continuous periplasm [1]. Under nitrogen-limiting conditions, the filaments of heterocyst-forming cyanobacteria contain two cell types with specialized functions: the vegetative cells that carry out oxygenic photosynthesis and the heterocysts that perform nitrogen fixation (Figure 1A). Growth of the filament as the organismic unit depends on the coordinated activity of these cell types in a simple example of multicellularity. An intercellular exchange of regulators and nutrients takes place in the filament, and the structures and mechanisms involved are now actively being investigated. Here we wish to provide a clarifying view of the current knowledge on septal communication structures in the cyanobacterial filament, highlighting issues that need further research.

Intercellular Molecular Exchange

Heterocysts differentiate from vegetative cells, and heterocyst differentiation is subjected to regulation by intercellularly transferred inhibitors produced by developing and mature heterocysts [2]. Additionally, in the diazotrophic filament, the vegetative cells provide the heterocysts with reduced carbon, including sugars, and the heterocysts feed the vegetative cells with fixed nitrogen in the form of amino acids [1]. Two possible pathways have been discussed for intercellular molecular exchange in heterocyst-forming cyanobacteria: the continuous periplasm and direct cell-cell connecting structures [1]. Intercellular communication involving the continuous periplasm would require transport across the cytoplasmic membranes of vegetative cells

and heterocysts, and cytoplasmic membrane transporters required for optimal diazotrophic growth have indeed been identified [1]. On the other hand, the use of fluorescent tracers, including calcein, to probe intercellular molecular exchange has shown that a rapid intercellular exchange of the tracer takes place in the filament, and that this exchange has properties of diffusion [3]. This observation suggests the presence of structures directly connecting adjacent cells in the filaments.

Septal Junctions and Septal Peptidoglycan Nanopores

Structures observed by electron microscopy apparently joining adjacent cells in the filament (Figure 1B,C) have been known for years as "microplasmodesmata" [1, 2], then termed "septosomes" [4] and now "septal junctions" to better reflect their possible analogy to metazoan gap junctions [5, 6]. To mediate intercellular molecular transfer, these structures must traverse the septal peptidoglycan. Consistently, perforations termed "nanopores" have been observed in isolated septal peptidoglycan [7]. The septal "channels" recently observed by electron tomography [8] would correspond to such nanopores. A peptidoglycan amidase of the AmiC type has been identified to be responsible for nanopore formation [7]. Furthermore, a novel type of peptidoglycan-binding protein, SjcF1, influences their size [6]. Both proteins have a preferential location in the septal regions of the filaments and corresponding mutants exhibit impaired intercellular exchange of calcein [6, 9].

Putative Septal Junction Proteins

The septal junctions likely contain protein [4]. In the model heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120 (Figure 1), SepJ (also known as FraG), FraC, and FraD are integral membrane proteins that, as shown with GFP fusions, are

located at the cell poles in the intercellular septal regions of the filament, with SepJ being located in more focused regions than FraC and FraD (see [10, 11] and references therein). Mutants lacking these proteins have a decreased number of nanopores and are impaired in the intercellular exchange of fluorescent tracers, relating these proteins to the septal junctions that traverse the nanopores [3, 5]. SepJ has a long extra-membrane section containing large coiled-coil motifs [10], which are known to participate in protein-protein interactions. This led to the hypothesis that SepJ proteins from adjacent cells interact and contribute to the formation of septal junctions, implying that the extra-membrane section of SepJ is periplasmic [10]. FraD has an extra-membrane section that, as shown by immunogold-labeling, is located in the septum between adjacent vegetative cells [11], making FraD a possible component of septal junctions. FraC and FraD are encoded in an operon (*fraCDE*) that is strongly conserved in filamentous cyanobacteria and, based on the similar phenotype of their mutants, could work together [11].

The extra-membrane section of SepJ has now been proposed to be cytoplasmic, which would imply that SepJ is not a component of septal junctions [8]. Instead, it was proposed that SepJ is a docking protein for septal channels [8]. Because the C-terminus of SepJ is most likely cytoplasmic [10, 12], a periplasmic or cytoplasmic location of its N-terminal extra-membrane section will depend on the number (odd or even, respectively) of transmembrane segments in its integral membrane section. Different protein topology prediction programs render diverse numbers (from 9 to 11) of transmembrane segments for *Anabaena* SepJ [6]. Furthermore, a particular program, TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), predicts 9, 10 or 11 transmembrane segments for the SepJ protein from different heterocyst-forming cyanobacteria whose genomic sequence is available. Thus, in the absence of structural

information, the number of transmembrane segments in SepJ and, hence, its topology are uncertain. Nonetheless, a SepJ topology with a periplasmic N-terminal extra-membrane section is supported by available experimental evidence: the SepJ extra-membrane section interacts with the peptidoglycan-binding protein SjcF1 [6] and with a periplasmic domain of the divisome protein FtsQ [12]. Additionally, immunogold-labeling of the SepJ coiled-coil domain (detected in a strain overexpressing SepJ) clearly indicates a preferential localization in the septa between vegetative cells [8]. Localization in a ring, whose position is similar to that of a Z ring, of GFP fused to the extra-membrane section of SepJ has been interpreted to suggest a cytoplasmic location [8]. Nevertheless, the interaction of SepJ with FtsQ [12] suggests rather localization in the periplasm of the SepJ extra-membrane section fused to the GFP. The possible mechanism of translocation into the periplasm is however unknown and represents an important research objective. More generally, investigating the topology of SepJ is imperative.

Summarizing, SepJ and FraD are candidate components of the septal junctions, and FraC is a likely companion of FraD. Interestingly, FraC also appears to interact with the nanopore-related, peptidoglycan-binding protein SjcF1 [6], further indicating a relationship between putative septal junction proteins and nanopores. Whereas a similar decrease (by about 90%) in the number of nanopores in *sepJ* and *fraCD* mutants [5] suggests that SepJ and FraCD together are needed to make a normal number of nanopores, differential impairment in the intercellular transfer of different fluorescent tracers (calcein, 5-carboxyfluorescein and the sucrose analog esculin) in those mutants suggests that septal junctions with somewhat different specificities are present in the cyanobacterial filament [5, 11].

Heterocyst-vegetative Cell Junctions

In the heterocyst-vegetative cell septum, in which the heterocyst "neck" contacts the polar region of a vegetative cell (Figure 1C), SepJ has a distinct location: whereas SepJ-GFP is seen as a single fluorescent spot in septa between vegetative cells, two spots are seen in the heterocyst-vegetative cell septa [1, 8, 10; see Figure 1D]. This observation suggests a re-localization of SepJ that may accompany the differentiation of the heterocyst neck. In the heterocysts, immunogold-labeling has been reported to place the SepJ coiled-coil domain surrounding the polar region where the cyanophycin granule (a multi-L-arginyl-poly [L-aspartic acid] reserve polymer), lost during sample preparation, is normally located [8]. We consider that this connection of SepJ with the cyanophycin granule needs corroboration. If, as discussed earlier, the SepJ extra-membrane section has a periplasmic location, localization of the coiled-coil domain towards the cyanophycin granule in the heterocyst neck could imply that the heterocyst-polar cyanophycin granule is located inside a compartment topologically equivalent to the periplasm. Such compartment ought to be surrounded by a membrane, reminiscent of the thylakoid lumen surrounded by the thylakoid's photosynthetic membranes. Although the presence of a membrane specifically surrounding the heterocyst-polar cyanophycin granule has not been described, this granule is frequently seen in electron microscopy surrounded by an electro-dense layer of unknown composition [9-11]. Investigating the fine structure of the heterocyst neck would thus be of interest.

Concluding Remarks

Multicellularity requires cell-cell attachment and communication, and the septal junctions discussed here appear to represent a unique development in evolution that contributes to multicellularity in cyanobacteria. Three putative protein components of

the septal junctions, SepJ, FraC, and FraD, have been identified, but the precise composition of the septal junctions and their possible interactions with peptidoglycan and peptidoglycan-related proteins remain to be fully explored. Finally, the special construction of the heterocyst-polar regions, including the heterocyst-vegetative cell septa, remains intriguing and deserves further research.

Acknowledgments

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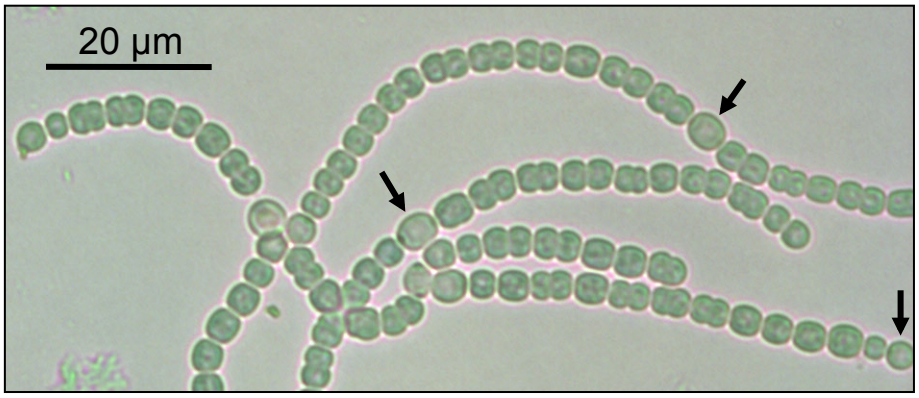
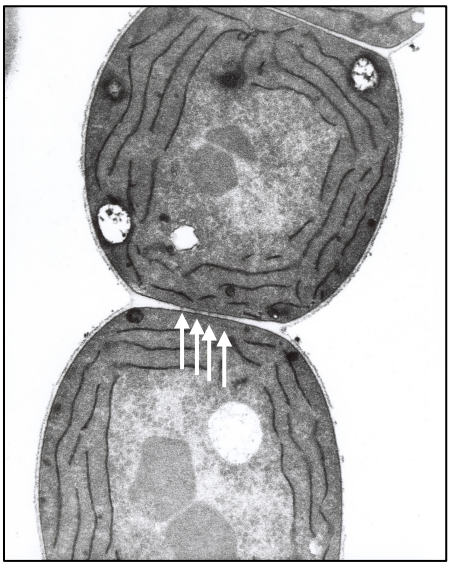
Figure 1. The Filament of an N_2-fixing Heterocyst-forming Cyanobacterium, *Anabaena* sp. Strain PCC 7120. (A) Optical micrograph showing fragments of *Anabaena* filaments consisting of vegetative cells and heterocysts (some indicated by arrows). (B) Electron micrograph of a portion of a filament of *Anabaena* showing the septum between two vegetative cells in which thin structures perpendicular to the cytoplasmic membranes of the adjacent cells are visible (white arrows). These structures are known as septal junctions and thought to join the adjacent cells. (C) Electron micrograph of the junction between a heterocyst (top) and a vegetative cell (bottom) where septal junctions are visible (white arrows). The polar region of the heterocyst known as the 'heterocyst neck' is indicated (Het neck). The place of the

cyanophycin granule (a cell inclusion that serves as a nitrogen reservoir), lost during sample preparation, is seen as a split white space in the heterocyst neck and close to it. (Samples prepared and electron micrographs taken as described [7, 9].) (D) Fragment of a filament of *Anabaena* sp. strain CSAM137 containing vegetative cells and a heterocyst (Het). Strain CSAM137 is *Anabaena* sp. strain PCC 7120 bearing a *sepJ-gfp* gene fusion [10]. Bright field (top) and GFP green fluorescence (bottom) are shown. The GFP fluorescence is observed as single spots in the septa between vegetative cells (single arrow), a localization that identifies SepJ as a possible component of septal junctions, and as two spots in the heterocyst-vegetative cell septa (double arrow). (Micrographs taken as described [10].) All micrographs are from the authors' laboratories.

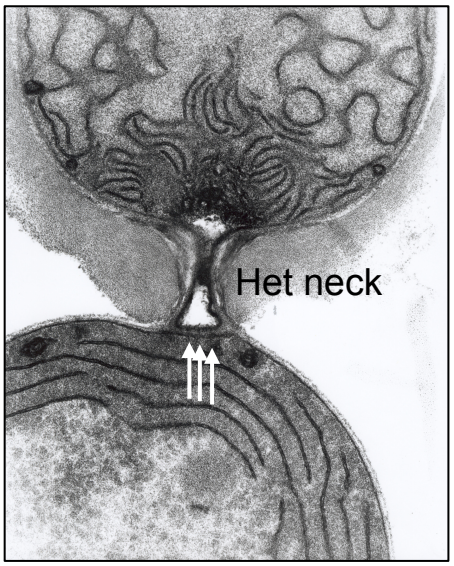
(A)



(B)



(C)



(D)