論文内容の要旨

論文題目 **Studies on the heterocyst pattern formation in filamentous cyanobacterium *Anabaena* sp. PCC 7120**

(糸状性シアノバクテリアにおけるヘテロシストパターンの形成に関する研究)

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The filamentous cyanobacterium *Anabaena* sp. PCC 7120 fixes dinitrogen facultatively. Upon depletion of combined nitrogen, about 10% of vegetative cells within the filaments (also called trichomes) differentiate terminally into nitrogen-fixing cells. The heterocyst has been studied as a model system of prokaryotic cell differentiation, with major focus on signal transduction and pattern formation. The fate of heterocyst differentiation is determined at about the eighth hour of induction (point of no return), well before conspicuous morphological or metabolic changes occur. However, little is known about how the initial heterocysts are selected after the induction by nitrogen deprivation.

In chapter I, I studied whether cell division is a prerequisite for heterocyst differentiation. For this purpose, I developed a microscopy-based analytical system that allows following the complete cell lineage after nitrogen depletion in single trichomes of *Anabaena* sp. PCC 7120. The rate of heterocyst differentiation was higher at higher light intensity, whereas the division rate was not affected until early phase of heterocyst formation. I found that 10% of initial heterocysts were formed without prior cell division since the start of nitrogen starvation under continuous illumination at 60 μE $m^{-2}s^{-1}$. Inhibition of cell division by aztreonam resulted in elongated, but clearly identifiable, heterocysts. Single cells prepared by sonication were able to form heterocyst-like cells without cell division though at a low rate. These results suggest that cell division is not essential for heterocyst differentiation.

In chapter II, I visualized the expression of the *ntcA*, *nrrA* and *hetR* genes using green fluorescent protein (GFP) as a reporter of gene expression related to heterocyst differentiation. I obtained results showing that the onset of increase in the expression of *hetR* was earlier than the start of expression of *ntcA* and *nrrA* in the future heterocysts. This is the first evidence that early *hetR* expression is localized in the future heterocysts. The start of expression of *ntcA* in the future heterocysts was not dependent on the time of visually detectable differentiation of heterocysts. These results suggest that the early differentiating heterocysts began to express the *hetR* gene higher than the neighboring cells at a very early time during the induction of differentiation, clearly before the time of commitment (about 8 h). I expected to find a sign of future heterocysts, if the differentiation process as shown by the expression of *hetR* gene starts soon after

nitrogen deprivation. I analyzed the profile of phycobilin fluorescence along a trichome. In a typical trichome that showed a sharp peak of four cells in periodicity analysis of fluorescence, the cells within a trichome were clustered as four-cell units (quartets). 75.6% of heterocysts that initiated differentiation at 16 h originated from the outer cells of quartets. The percentage was 72.3% and 85.0% for the heterocysts that initiated differentiation at 20 h and 24 h, respectively. This suggests that the outer cells of quartets have a high chance of becoming a heterocyst at the beginning of nitrogen deprivation.

Based on the results obtained in the experiments, I propose the early candidacy hypothesis, in which future heterocysts are selected, without a need for unequal cell division, soon after nitrogen step-down and before heterocyst differentiation is irreversibly determined.