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Macromolecular Composition of Spores from the Filamentous Cyanobacterium *Anabaena cylindrica*

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Spores were isolated from the filamentous cyanobacterium *Anabaena cylindrica*, and their deoxyribonucleic acid, ribonucleic acid, and protein compositions were determined.

No direct measurements of the macromolecular composition of spores from the filamentous cyanobacteria have been reported, even though such measurements are an important preliminary step in the analysis of sporulation. Ueda has used the fluorochrome coriphosphin to cytochemically measure the relative amount of deoxyribonucleic acid (DNA) in vegetative cells and spores of several filamentous cyanobacteria (18, 19) and has shown that spores appear to contain 5 to 30 times the amount of vegetative cell DNA. This note reports the result of direct measurements of the macromolecular composition of *Anabaena cylindrica* spores.

Table 1 shows the macromolecular composition of intact filaments and isolated spores from *A. cylindrica*. Heterocysts constitute 4 to 5% of the cells in cultures (8), so that intact filaments approximate a population of vegetative cells. Measurements on intact filaments were made during both exponential growth (average growth rate constant of 0.040 h^{-1}) and late logarithmic growth just prior to sporulation. The DNA content of exponentially growing vegetative filaments is twice that found during the late logarithmic culture phase. However, the ratio of ribonucleic acid (RNA) to DNA between the two culture stages is not significantly different. The amount of DNA per spore is approximately equal to that from an exponentially growing vegetative cell, whereas the ratios of protein/spore and RNA/spore are, respectively, three and four times that of exponentially growing cells.

The amount of DNA present in vegetative cells agrees with that previously reported. Since Craig et al. (4) found that *A. cylindrica* contained 0.62% of its dry weight as DNA, it can be calculated that there is between 10.2×10^{-4} and 26.8×10^{-14} g of DNA per vegetative cell (6, 8). Herdman and Carr estimated the genome size of several cyanobacteria from the renaturation kinetics of isolated DNA (10). The genome molecular weight of *A. cylindrica* is 2.47×10^9 g/mol. By this figure, a vegetative cell of *A. cylindrica* has 25 genome copies during late logarithmic growth, whereas a spore contains an average of 57 genome copies. It is difficult to understand why so much DNA is present per cell, and Mann and Carr (14) have commented that in *Anacystis nidulans* they are unable to explain the number of genome equivalents per cell in terms of the relationship between the cell division cycle time and the growth rate.

The spore DNA content reported here does not agree with cytochemical measurements in other species, which show that spores contain 5 to 30 times the amount of vegetative cell DNA (18, 19). Cytochemical measurements were carried out with two species of filamentous cyanobacteria which are not available for analysis (K. Ueda, personal communication), and attempts to repeat these observations in *A. cylindrica* were unsuccessful.

Mature spores of *A. cylindrica* have approximately 10 times the volume of a vegetative cell (7, 8). An examination of *Bergy's Manual* (2) shows that members of the cyanobacteria are unusual in that the resting stages produced in some species are significantly larger than the vegetative cells. Donachie (5) has proposed that in procaryotic organisms there is a critical cell size at which a new round of replication is initiated. During sporulation in *A. cylindrica*, DNA replication must be uncoupled from this control, because there is a significant increase in cell size but no concomitant replication. An analysis of this system offers an opportunity for investigating the factors involved in coordinating replication with cell size.

The constancy of the ratios of RNA/DNA and transfer RNA/ribosomal RNA (11, 13, 14) for at least two species of cyanobacteria growing at several growth rates has been used in support of the argument by Carr that for the most part the cyanobacteria do not regulate macromolecular synthesis at the transcriptional level (3). In the sporulation of *A. cylindrica*, the RNA/DNA ratio changes as a result of the termina-

TABLE 1. *Macromolecular composition of intact filaments and isolated spores of A. cylindrica*

Material assayed[b]	Growth phase	Macromolecular composition and ratios of components[a]				
		DNA	RNA	Protein	RNA/DNA	Protein/DNA
Spores[c]		23.3 ± 6.70	1,260 ± 170	4,916 ± 403	54.1	211
Intact filaments	Exponential	22.4 ± 0.76	295 ± 44	1,659 ± 68	13.2	74
	Late log	10.2 ± 0.51	126 ± 8.0	1,063 ± 41	12.4	104

[a] The mean macromolecular composition was determined from four spore and filament preparations and is given in grams per cell × 10^{+14} ± the standard deviation from the mean. Ratios of components are on a weight basis. The RNA and DNA content of intact filaments and spores was determined using a modification of the Schneider procedure (15), which is described by Mann and Carr (14). Protein was determined by solubilizing hot perchloric acid-insoluble material in 0.5 N NaOH and measuring the protein present with the Lowry procedure (12) using bovine serum albumin as a standard. Cell number was determined using a Levy ultraplane counting chamber (C. A. Hauser and Son, Philadelphia, Pa).

[b] *A. cylindrica* Lemm. was grown axenically in 3-liter batches of the medium of Allen and Arnon (1) that had been diluted 16-fold. Cultures, which were grown in 5-liter diptheria bottles (16), were started with a 0.25% (vol/vol) inoculum and illuminated from one side with 40 W cool white fluorescent lamps. Growth rate was measured by following the change in the optical density of the culture at 730 nm.

[c] Spores were isolated from stationary-phase cultures by a method that uses lysozyme for the selective disruption of vegetative cells and is similar in form to one previously published (8, 9). After isolation, diluted spore samples were counted in a Levy ultraplane counting chamber to determine yield and purity. In addition, 2-μl spots of spore suspension (10^6 spores/ml) were placed on 1% purified agar (Difco) containing the BG-11 growth medium of Stanier (17). The plates were incubated at 25°C under dim cool white fluorescent light and were observed at daily intervals to determine the percentage of spore germination. Chemical analysis was limited to those preparations that were >90% spores on a cell basis and that showed >10% germination 48 to 72 h after plating.

tion of DNA replication. It is not known if the ratio of transfer RNA/ribosomal RNA is affected by the sporulation process. If a minimal number of steps in the sporulation process are controlled at the transcriptional level, the system could provide an extremely simple model for studying the regulation of macromolecular synthesis during development.

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