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Degree of Completion of 3'-Terminus of Transfer Ribonucleic Acids of *Bacillus subtilis* 168 at Various Developmental Stages and Asporogenous Mutants

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Transfer ribonucleic acids from sporulating cells, spores, sporangia, or stationary-phase asporogenous mutants of *Bacillus subtilis* all showed a deficiency in the 3'-terminal adenosine moiety.

As part of a larger investigation of the structure and function of transfer ribonucleic acids (tRNAs) of *Bacillus subtilis*, the status of the 3'-terminus was studied as a function of developmental stage. This determination is important because tRNA molecules with an incomplete -pCpCpA terminus cannot be aminoacylated, and, therefore, cannot be active in protein synthesis.

Cells were broken by treatment with lysozyme, and spores were broken with a Braun homogenizer (B. S. Vold, Methods in enzymology, in press). tRNA preparations were made as previously described. The procedure included deoxyribonuclease treatment, deproteinization with phenol and $CHCl_3$-isoamyl alcohol, and chromatography over Sephadex G-100 (B. S. Vold, in press). In addition, tRNAs from *B. subtilis* strain 168 were purified by batchwise elution from reversed-phase column RPC-5 (5). The incorporation of [^{14}C]adenosine 5'-monophosphate (AMP) was assayed in a 250-μliter reaction containing 50 μg of bovine serum albumin, 100 mM tris(hydroxymethyl)aminomethane, pH 8.5, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.16 mM [^{14}C]adenosine 5'-triphosphate (ATP), 180 μg of protein (from aminoacyl-tRNA synthetase fraction, prepared as described [B. S. Vold, in press], which contained tRNA nucleotidyl-transferase activity), and 2 nmol of tRNA. The reactions were incubated at 30 C for 30 min, and samples were withdrawn, washed on paper disks, and quantitated for radioactivity as previously described (10). Background values were obtained by adding 10 μg of ribonuclease to each reaction after the initial 30 min-incubation and continuing the incubation for 30 min at 37 C. Samples were then withdrawn and treated as before. This background was used to verify that all of the radioactivity was incorporated into an RNA moiety. No incorporation above background was taken as 0%. Incorporation of 100% was determined from mixed *Escherichia coli* tRNApCpC- prepared at the Oak Ridge National Laboratory and a gift from G. David Novelli.

Results are shown in Table 1. In general, our data are in agreement with others (1, 2, 8). However, the time at which the loss of the terminal adenosine can be detected in *B. subtilis* 168 differs from the report of *Bacillus megaterium*, in which the loss was determined to occur after the appearance of refractile spores (8). The earliest culture to show a loss was at 550 Klett units (Table 1). This loss could not be due to the presence of a percentage of cells in later stages arising from an asynchrony in the culture, because no refractile objects could be detected by phase-contrast microscopy, and colony counts of heated or unheated samples gave a value of less than 0.5% heat-resistant cells (9).

Table 1 also shows that sporangial tRNA, obtained by mild sonic treatment of a sporulating culture, possessed tRNAs lacking a percentage of the terminal adenosines, indicating that this phenomenon is not compartmentalized to the spore. Further studies with the two asporogenous mutants demonstrate that tRNAs from cells not capable of septation, which occurs in sporulating cells at stage II, tended to lose the terminal adenosines in stationary phase even though the tRNAs of normally sporulating cells at stage II were complete. The latter observation indicates that the loss is probably related to the aging of the cells rather than the differentiation of the spore. Because nucleotidyl-transferase activity is present even in the dormant spores of *B. subtilis* 168 (unpublished data), the failure to repair the termini is not due to a

TABLE 1. *In vitro assay for incorporation of [^{14}C]AMP*

Klett units[a]	Time after inoculation (h)[b]	Growth stage[b]	Organism[c]	tRNAs lacking 3'-terminal adenosine (%)
20	3.5	Early exponential	168	2.3
266	5.5	0, I	168	2.3
351	8.5	I, II	168	3.0
550	14.0	III	168	10.6
800	24.0	Spore	168	19.3
1,000	72.0	Spore	168	23.9
800	24.0	Sporangia	168	30.5
75	4.5	Exp.	spoA	0
525	24.0	Stationary	spoA	11.3
75	6.5	Exp.	spoF	4.2
414	24.0	Stationary	spoF	12.4

[a] Klett-Summerson colorimeter, 660-nm filter; values above 100 were corrected for light scattering.

[b] Cells were grown in tryptone-yeast extract medium (6) at 37 C with constant shaking, by using 1 liter of medium per 2.8-liter Fernbach flask. Cultures were inoculated with a loop of cells from a plate. Because of the large amount of medium per flask, the time course of sporulation was longer than that presented by Mandelstam (7). Therefore, the stage of sporulation under our conditions was approximated by following the turbidity of the culture and the appearance of refractility and heat-resistant forms. The end of exponential growth was taken as stage 0. Heat resistance began to appear at 640 Klett units. At 550 Klett units, the last vegetative stage shown in the table, the cells showed no refractility by phase-contrast microscopy and formed no heat-resistant colonies (less than 0.5%).

[c] 168, *B. subtilis* 168 *trpC2;* spoA, *B. subtilis* 168 *spoA12 trpC2*, which is blocked at stage 0 (3); spoF, *B. subtilis* 168 *spoF138 trpC2 phe*-1, which is blocked before septation (J. Hoch, personal communication).

lack of this enzyme. It is likely that the decrease of ATP concentration, which in *B. subtilis* 168 Marburg commences at the end of growth (4), causes a limitation of substrate necessary for the repair.

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