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Absence of 3'-Terminal Residues from Transfer Ribonucleic Acid of Dormant Spores of *Bacillus megaterium*

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Essentially all (>97%) of the transfer ribonucleic acid (tRNA) in log-phase and sporulating cells of *Bacillus megaterium* contains a complete 3'-cytidyl-cytidyl-adenosine terminus. However, about one-third of the tRNA in the dormant spore lacks the 3'-terminal adenosine 5'-monophosphate (AMP) residue, and some of the adjacent cytosine monophosphate residues are also missing. Examination of specific tRNAs indicated that those specific for isoleucine, leucine, and methionine are missing 30 to 40% of their terminal residue, whereas tRNAs specific for tyrosine lack 88% of the 3'-terminal AMP. Defective spore tRNA is not degraded during germination, but the missing residues are added back in the first minutes of the process. The enzyme catalyzing the addition reaction, tRNA nucleotidyltransferase, is present in the dormant spore at a level similar to that found in the vegetative cell.

All transfer ribonucleic acids (tRNAs) contain the common sequence cytidyl-cytidyl-adenosine (C-C-A) at their 3'-terminus (26). Since the 3'-terminal adenosine monophosphate (AMP) residue is the site of attachment of the activated amino acid, tRNA molecules lacking this moiety do not accept amino acids and are inactive in protein synthesis (7, 16). Removal of the 3'-terminal AMP residue from one, or several, tRNA species might be a possible mechanism for turning off or slowing down protein synthesis in vivo. Indeed, there is evidence that the 3'-terminal residue is absent from some of the tRNA of stationary-phase yeast (17), nonlactating mammary gland (8), and unfertilized sea urchin eggs (6), and in the latter case protein synthesis is known to be very slow (23).

Dormant *Bacillus* spores carry out no detectable protein synthesis despite the presence of much of the protein-synthesizing apparatus (5, 12). The lack of protein synthesis in dormant spores is probably due to a number of factors including defective ribosomes (10), low or absent messenger RNA (5, 12), lack of amino acids (15), and lack of adenosine triphosphate (ATP) and guanosine triphosphate (21). It has been thought that the tRNA of dormant spores is fully active in protein synthesis, since tRNAs for all amino acids are present and only a few differences in isoaccepting tRNA species between dormant spores and log-phase cells are known to exist (1, 11, 13, 24). However, it has been reported that up to 30% of the tRNA in dormant spores lacked a 3'-terminal AMP residue (5). Because of the implications of this finding as a possible mechanism for controlling protein synthesis in sporulating cells and dormant or germinating spores, we have reexamined this question in detail. We found that 35% of the tRNA in the dormant spore lacked a 3'-terminal AMP and that 7% of the adjacent cytidine moieties were also absent. These missing residues were returned to the defective tRNA during the first minutes of spore germination. On the other hand, essentially all (>97%) of the tRNA in log-phase and sporulating cells contained an intact C-C-A terminus.

MATERIALS AND METHODS

Chemicals, tRNAs, and enzymes. [^{14}C]ATP and [^{14}C]cytidine triphosphate (CTP) were obtained from Schwarz-Mann; [^{32}P]orthophosphate (carrier free), [^{3}H]leucine, [^{3}H]isoleucine, [^{3}H]tyrosine, and [^{14}C]methionine were from New England Nuclear Corp. ATP, CTP, lysozyme, and chloramphenicol were from Sigma Chemical Co., and actinomycin D was a generous gift from Merck, Sharpe, and Dohme. Yeast tRNA (about 50% tRNA-C-C) was purchased from Schwarz-Mann, and *Escherichia coli* tRNA was from General Biochemicals Corp. Rabbit liver tRNA nucleotidyltransferase (EC 2.7.7.25) was purified as described previously (3), and enzyme from step 6 was used for analysis of the AMP and cytidine monophosphate (CMP) acceptor activities of the various tRNAs. Purified *E. coli* aminoacyl-tRNA synthetases were a gift from Paul Berg (Stanford University, Stanford, Calif.).

Growth and harvesting of cells and spores. All work was carried out with *Bacillus megaterium* QM B1551 originally obtained from Hillel Levinson (U.S. Army Laboratories, Natick, Mass.). Dormant spores and ^{32}P-labeled spores were prepared in supplemented nutrient broth, harvested, washed, lyophilized, and stored as described previously (20, 21). All spore preparations used were >95% refractile and were free from cell debris as seen in the phase-contrast microscope. Cells were also grown on supplemented nutrient broth, chilled by addition of ice, and harvested by centrifugation (10 min, 10,000 × *g*). The pellets were immediately frozen in dry ice-ethanol and then lyophilized.

Germination of spores. Unless otherwise noted, all spore germinations were carried out as follows. Dormant spores (25 mg [dry weight]/ml) were heat-shocked (10 min, 60 C) in water and then cooled in ice; germination was at 30 C in 50 mM KPO_4 (pH 7.4) and 100 mM glucose at a spore concentration of 2.5 mg (dry weight)/ml. In all experiments reported here, germination was >95% complete within 10 min as judged by the loss of spore refractility in the phase-contrast microscope. NaF, KCN, chloramphenicol, and actinomycin D did not inhibit this initial step in the germination process. Germinated spores were chilled and harvested by centrifugation for 3 min at 17,000 × *g*.

Extraction procedures. tRNA was extracted from both dry cells and spores (200 mg) after dry rupture in a Wig-L-Bug with glass beads (250 mg) as the abrasive (18). Ten 1-min periods of shaking with intermittent cooling in ice sufficed to rupture >90% of all cells or spores. The dry powder from the Wig-L-Bug was suspended in 5 ml of phenol, containing 1 mM 8-quinolinol, and mixed well, and then 5 ml of 0.15 M NaCl was added. After shaking for 60 min at room temperature, the mixture was centrifuged (30 min, 20,000 × *g*), the aqueous layer was removed, and the phenol was re-extracted with an additional 5 ml of 0.15 M NaCl. The two aqueous layers were pooled, made 2% in potassium acetate, and precipitated overnight at −20 C with two volumes of ethanol. tRNA was isolated from this nucleic acid mixture by the isopropanol fractionation method of Zubay (27). Cell tRNA was purified further by gradient elution from diethylaminoethyl-cellulose to remove a viscous material which inhibited tRNA nucleotidyltransferase. Purity of tRNA was determined by chromatography on Sephadex G-100. Yields of tRNA through the isopropanol step were similar (within 30%) to values reported by other workers (5, 25), and the tRNA was active in accepting amino acids (see Table 4; P. Setlow, unpublished results [1973]). In a few cases dried cells were extracted with phenol without prior rupture in the Wig-L-Bug. However, this method gave poor yields of tRNA with dormant spores, germinating spores, and stationary-phase cells, and therefore was not routinely used.

Assay for tRNA missing terminal residues. Reaction mixtures contained in 0.2 ml: 50 mM glycine-NaOH, pH 9.4; 5 mM $MgCl_2$; 0.5 mM [^{14}C]ATP (about 10^3 counts per min per nmol) or [^{14}C]CTP (about 10^3 counts per min per nmol); about 100 μg of the tRNA to be tested; and 0.05 U of rabbit liver tRNA nucleotidyltransferase (12 U/mg). For determination of CMP incorporation, 0.38 M KCl was also present to prevent anomalous incorporation (4). Mixtures were incubated for 30 min at 37 C, and the reaction was terminated with 3 ml of 10% trichloroacetic acid-0.02 M sodium pyrophosphate. After 10 min in ice, the precipitate was collected on Whatman GF/C filters and washed six times with 3-ml portions of 2.5% trichloroacetic acid-0.02 M pyrophosphate and once with ethanol-ether (1:1). Filters were dried under an infrared lamp and counted in 5 ml of toluene-based scintillation fluid.

Assays were performed at several enzyme concentrations to ensure that the maximal amount of AMP or CMP was incorporated. In addition, all tRNA samples were tested for the presence of inhibitors by performing mixing experiments with known tRNA acceptors to ensure that the level of incorporation was not affected by possible contaminants.

Assay for amino acid acceptor activity of tRNA. The acceptor activity of spore tRNA was determined by a modification of the procedure of Muench and Berg (14). The reaction mixture (0.1 ml) contained 50 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4), 4 mM ATP, 10 mM $MgCl_2$, 5 mM KCl, 2 mM 2-mercaptoethanol, 20 μM ^{14}C-labeled amino acids (20 counts per min per pmol) or ^3H-labeled amino acids (300 to 1000 counts per min per pmol), tRNA (5 to 25 μg), and an excess of the required purified *E. coli* aminoacyl-tRNA synthetase. CTP when added was present at 0.1 mM. The reaction mixture was incubated for 30 min at 37 C, and the reaction was terminated by addition of 1 ml of cold 5% trichloroacetic acid. The precipitate was collected on a Whatman GF/C filter and washed six times with 5 ml of cold 5% trichloroacetic acid and three times with ethanol. The filters were dried and counted in a scintillation counter.

AMP and CMP residues were added back to tRNA by incubation in the reaction mixture described above (minus aminoacyl-tRNA synthetase and amino acid) with 0.02 U of tRNA nucleotidyltransferase and CTP; for AMP addition alone CTP was omitted. After 15 min at 37 C the amino acid and aminoacyl-tRNA synthetase were added, and acceptor activity was determined as described above.

The percentage of tRNA missing AMP was calculated as the acceptor activity of tRNA after addition of AMP and CMP residues minus the acceptor activity of untreated tRNA, divided by the acceptor activity of tRNA after addition of AMP and CMP residues. Values for tRNA missing CMP residues were calculated similarly, but using the difference between the acceptor activities of tRNA after addition of AMP and CMP residues, and AMP residues alone in the numerator.

Other procedures. tRNA nucleotidyltransferase was assayed with yeast tRNA and [^{14}C]ATP as described previously (3). ATP was extracted with boiling 80% *n*-propanol and assayed on a scintillation counter by using the luciferase reaction (21). Enzymes were extracted from cells and spores by using lysozyme as described previously (19). Protein was deter-

mined by the Lowry procedure, and tRNA concentrations were determined using an absorbancy at 260 nm (A_{260}) of 22.0/mg.

RESULTS

Levels of tRNA with defective C-C-A termini at various stages of growth. tRNAs were isolated from *Bacillus megaterium* cells at various stages of growth, sporulation and germination (Fig. 1), and the state of their C-C-A termini was examined by their ability to accept AMP and CMP. Greater than 97% of the tRNA in log-phase or sporulating cells (to stage 8 in sporulation) contained an intact C-C-A terminus. In contrast, one-third of the tRNA isolated from dormant spores lacked the 3'-terminal AMP residue (Table 1). These values were similar to those observed previously (5). The values for the amount of defective cell and spore tRNA have been corrected for the presence of a 25% contamination of 5*S* and high-molecular-weight RNA as determined by analysis on Sephadex G-100. In addition, one sample of spore tRNA was purified further than usual by chromatography on Sephadex G-100, and incorporation of AMP gave the same level of tRNA molecules with defective termini. Therefore, the finding that only 33% of the tRNA molecules were not intact was not due to contamination of the spore tRNA with other nucleic acids. It was also possible that this value was low because of addition of AMP residues during the extraction and purification of the spore tRNA. However, this was probably not the case since: (i) rupture of dormant spores was in the dry state, precluding enzyme action; (ii) no ATP was formed during the dry rutpure and subsequent mixing of the dry powder with phenol (Table 2); and (iii) the ATP level in the dormant spore was only 5% that of defective tRNA (Table 2). The latter value was calculated by assuming 10 mg of tRNA per g of dry spores, a molecular weight for tRNA of 2.5×10^4, and the value for defective spore tRNA in Table 1. Thus, it is extremely unlikely that sufficient ATP could have been produced to affect the state of the C-C-A terminus of tRNA.

In addition to lacking a 3'-terminal AMP residue, dormant spore tRNA also lacked a small amount of the CMP residues in the C-C-A sequence (Table 3). However, we do not know whether these values represent tRNA chains lacking the whole C-C-A terminus or only one CMP and the AMP residue. As was observed with the terminal AMP residue, log-phase and sporulating cell (to stage 8 of sporulation) tRNA lacked no CMP residues (Table 3).

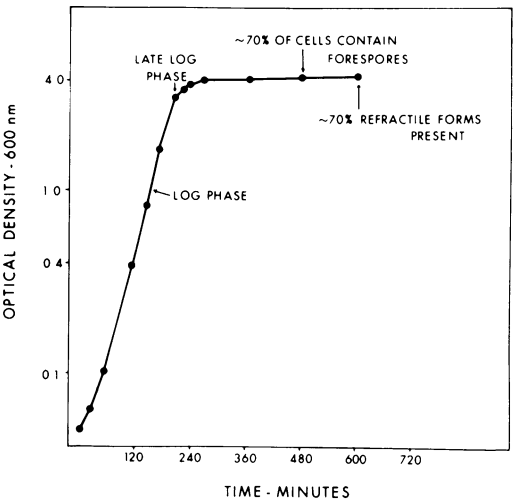

FIG. 1. *Growth and sporulation of B. megaterium. Cells were grown at 30 C in supplemented nutrient broth, and the optical density was measured at 600 nm in a Gilford spectrophotometer. The percentages of forespores and refractile spores in sporangia were determined by counting about 100 cells in the phase-contrast microscope.*

TABLE 1. *Amount of tRNA lacking a 3'-terminal AMP residue at various stages of growth of B. megaterium*[a]

Stage of growth[b]	AMP added to tRNA (nmol/mg)	Termini missing (%)
Mid-log-phase cell	1.1	3
Late-log-phase cell	0.8	2
Cells containing forespores (~70%)	0.3	1
Cells containing refractile spores (~70%)	0.7	2
Dormant spore	11.7[c]	33

[a] tRNA was isolated from 0.7 to 1.1 g (dry wt) of cells or spores and assayed as described in Materials and Methods. All values have been corrected for the non-tRNA material in the samples.

[b] Refer to Fig. 1 for exact time of harvesting cultures.

[c] This value corresponds to 130 nmol of defective tRNA per g (dry weight) of spores.

Levels of defective C-C-A termini in dormant spore tRNAs specific for individual amino acids. Since the high level of defective tRNA in dormant spores could be due to defective 3'-termini in either one-third of the molecules of all tRNA species or all of the molecules of some tRNA species, it was of interest to investigate possible defects in dormant spore tRNAs specific for individual amino acids. tRNAs accepting three different amino acids

TABLE 2. *ATP levels in spores*[a]

Treatment of dormant spore	ATP level (nmol/g dry wt)
None	7
Dry ruptured and mixed with phenol	3
Germinated 7 min	425

[a] ATP was extracted from 1 to 2 mg (dry wt) of spores and assayed as described in Materials and Methods.

TABLE 3. *Amount of tRNA lacking CMP residues at various stages of growth of B. megaterium*[a]

Stage of Growth[b]	tRNA missing CMP (nmol/mg)
Mid-log-phase cell	0.8
Late-log-phase cell	0.3
Cells containing forespores (~70%)	0.3
Cells containing refractile spores (~70%)	0.3
Dormant spore	2.2

[a] tRNA samples were identical to those of Table 1.
[b] Refer to Fig. 1 for exact time of harvesting cultures.

lacked 30 to 43% of the 3'-terminal AMP residue, and some CMP residues were also missing (Table 4). The similarity in the levels of these defective tRNAs with the amount of total tRNA which was defective suggested initially that one-third of the molecules of all tRNA species might lack 3'-terminal residues. However, the absence of nearly 90% of the 3'-terminal AMP from $tRNA^{Tyr}$ appears to negate this suggestion. The reason for the high level of AMP residues absent from $tRNA^{Tyr}$ is not clear at present.

Disappearance of defective tRNA during spore germination. Since dormant spores tRNA lacked significant amounts of AMP and CMP residues from its 3'-terminal sequence, it was of interest to determine the fate of these tRNA molecules upon germination. All of these defects disappeared in the first minutes of spore germination (Tables 4 and 5). After 7 min of germination, >95% of the spores had germinated and >98% of the tRNA molecules contained an intact C-C-A sequence. The most likely explanation for the loss of the defective tRNA early in germination is the rapid addition of the missing AMP and CMP residues, utilizing the ATP and CTP produced during this period (21). In support of this explanation is the finding that tRNA nucleotidyltransferase was present in the dormant spores at a level similar to that in the log-phase cells (Table 6). In addition, it was calculated that the amount of enzyme in 1 g of dry spores is capable in vitro of adding an AMP residue to 100 nmol of tRNA per min, whereas only 130 nmol of defective tRNA is present per g of dry spores. This value was calculated as described above. Thus, sufficient enzyme was present in the dormant spores to repair the defective tRNA rapidly as soon as sources of nucleoside triphosphates became available.

The disappearance of defective tRNA from spores germinating in the presence of NaF and KCN was surprising (Table 5), since little high-energy phosphate was produced under these conditions (Fig. 2, [21]). However, some ATP (and presumably CTP) actually did accu-

TABLE 4. *Amount of 3'-terminal residues missing from spore tRNAs specific for individual amino acids*[a]

Amino acid	tRNA missing terminal AMP (%)		Dormant spore tRNA missing at least one CMP (%)
	Dormant spore	Germinated spore	
Isoleucine	31	2	11
Leucine	43	0	13
Methionine	31	1	5
Tyrosine	88	2	6

[a] tRNA was isolated from 0.7 g (dry wt) of dormant or germinated (20 min) spores and assayed for amino acid acceptor activity with and without addition of AMP and CMP residues or AMP alone as described in Materials and Methods. Calculations were also performed as described in Materials and Methods. Maximal specific acceptor activities (pmol/A_{260}) for individual amino acids were: isoleucine, 40; leucine, 52; methionine, 66; and tyrosine, 33. These values have been corrected for the presence of non-tRNA material in the samples.

TABLE 5. *Absence of defective tRNA early in spore germination*[a]

Type of spores extracted for tRNA	tRNA missing terminal AMP (nmol/mg)	tRNA missing CMP (nmol/mg)
Dormant spore	11.7	2.2
Germinated 7 min	0.5	0.3[b]
Germinated 7 min with NaF (10 mM) and KCN (10 mM)	0.8	0.3

[a] tRNA was isolated from 0.5 to 1.0 g (dry wt) of spores and assayed for AMP and CMP acceptance as described in Materials and Methods. Values have been corrected for the 25% of non-tRNA material in the samples. By 7 min, germination was >95% complete.
[b] This sample was germinated for 20 min.

TABLE 6. *Levels of tRNA nucleotidyltransferase in cells and spores of B. megaterium*[a]

Stage of growth	tRNA nucleotidyltransferase (nmol of AMP incorporated per h per mg of protein)
Log-phase cell	13.8
Dormant spore	19.0

[a] Cell or spore protein (25 to 100 μg) was assayed for tRNA nucleotidyltransferase as described in Materials and Methods.

mulate in NaF and KCN (Fig. 2), and in fact the ATP level after 5 min of germination under these conditions was one-half the level of defective tRNA in the dormant spore. Furthermore, it should be emphasized that this value for ATP in the germinating spores represents only the steady-state level and that the ATP pool probably turns over rapidly (see below). Therefore, it appears likely that more than enough high-energy phosphate is generated in spores germinating in NaF and KCN to add AMP and CMP residues to the defective tRNA of the dormant spores.

Determination of the extent of tRNA degradation during germination. Although the addition of AMP and CMP residues was the most likely explanation for the disappearance of defective tRNA during spore germination, it was also possible that the defective tRNA was merely degraded rapidly. That this is more than a formal possibility was suggested by the fact that 5 to 10% of the RNA of dormant spores is known to be degraded early in germination (22) and that defective tRNA accounts for approximately 7% of total spore RNA. This value was calculated by using a value of 21% for the percentage of total spore RNA as tRNA (2) and the value for defective spore tRNA in Table 1. Indeed, there was a small amount of tRNA degradation during the first 20 min of germination, but this amounted to an average of only 6% of the tRNA under three separate germination conditions (Table 7). Since the defective tRNA was one-third that of the tRNA fraction, this value was too low by a factor of 6 to account for the disappearance of defective tRNA, and it may simply reflect the random degradation of 5 to 10% of the total dormant spore RNA during germination.

Although the larger value for tRNA degradation in spores germinating with actinomycin D may suggest that the other values are too low due to net tRNA synthesis during germination, we feel this is extremely unlikely since (i) there was no net increase in total RNA content during the first 20 min of spore germination due to the absence of de novo nucleotide biosynthesis (22) and (ii) there is little, if any, tRNA synthesis early in spore germination (P. Setlow, unpublished results, 1973). The higher amount of degradation in actinomycin D is probably due to a small experimental variation.

DISCUSSION

Although as much as 60% of the tRNA chains of stationary-phase yeast may lack a 3'-terminal AMP residue (17), the absence of about 35% of the 3'-termini from the tRNA of dormant spores of *B. megaterium* is another dramatic example of this type of defect. It is clear that this situation is rectified in the first minutes of spore germination, but at present we have no information on the mechanism whereby a large amount of tRNA loses its 3'-terminal residues. However, this must be an extremely late event in sporulation since it occurs after the appearance of refractile spores. Enzymes are known which can remove 3'-terminal residues from tRNA (M. P. Deutscher, Fed. Proc. **29**:871, 1970), but we have not yet looked for such activities during sporulation.

The fact that the missing residues are added to spore tRNA early in germination suggests that this may be a favored event during the process. Such a conclusion is further suggested by the rapid occurrence of this reaction despite the fact that little ATP was present (plus NaF

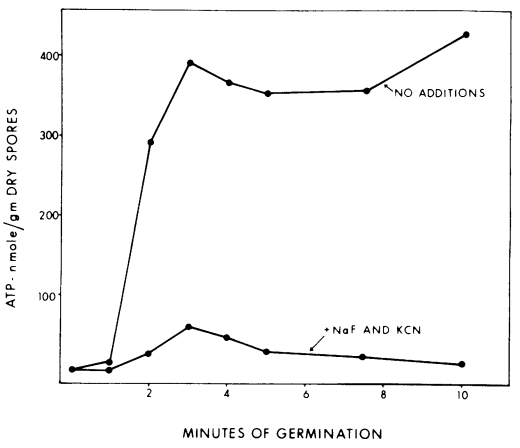

FIG. 2. *ATP levels in spores germinating in the presence or absence of NaF and KCN. Dormant spores were heat-shocked and germinated with or without NaF (10 mM) and KCN (10 mM). At the indicated times, samples (0.5 ml) were injected into 2 ml of boiling n-propanol, boiled for 7 min, cooled, and flash-evaporated, and the residue was suspended in 1 ml of water prior to ATP analyses.*

TABLE 7. *Absence of significant tRNA degradation during the early minutes of spore germination*[a]

Treatment of spores	Specific radioactivity of purified tRNA (counts per min per A_{260})
Dormant heat shocked	100[b]
Germinated for 20 min	97
Germinated for 20 min with NaF (10 mM) and KCN (10 mM) .	93
Germinated for 20 min with actinomycin D (100 μg/ml)	91

[a] ^{32}P-labeled dormant spores (4×10^6 counts per min per mg) were heat shocked and then cooled. Samples of 5.5 mg were suspended in 8.5 ml of water and either centrifuged immediately (dormant heat-shocked) or germinated for 20 min under various conditions and then centrifuged. *E. coli* tRNA (0.5 ml of 15 mg/ml in 5 mM ethylenediaminetetraacetic acid) was added to all spore pellets as a carrier, and this mixture was frozen and lyophilized. The dry powder was ruptured in the Wig-L-Bug, and tRNA was extracted and purified as described in Materials and Methods. A sample of each of the final solutions (0.4 ml, about 2 mg/ml) was chromatographed on a Sephadex G-100 column (1.5 by 140 cm) in 1 M NaCl to separate tRNA from 5S RNA and other high- or low-molecular-weight contaminants. The region of constant specific radioactivity across the tRNA peak was pooled, precipitated with two volumes of ethanol (−20 C), and dissolved in a small volume of water. Determinations of specific radioactivity were performed on this final fraction which was completely (>98%) sensitive to ribonuclease.

[b] Value for dormant spores was set at 100. This was the average value for two different samples of spores and varried only 1% from the average.

and KCN, Table 5 and Fig. 2). That a low steady-state level of ATP is indeed sufficient for addition of terminal residues to defective spore tRNA is suggested strongly by the absence of significant tRNA degradation under these conditions (Table 7). Furthermore, although the steady-state ATP level is low during germination in NaF and KCN, under these conditions there is mobilization of >75% of the large depot (16 μmol/g of dry spores [21]) of 3-phosphoglyceric acid (3-PGA) in the dormant spore (P. Setlow, unpublished results, 1973). This amount of 3-PGA is sufficient to form enough high-energy phosphate to phosphorylate the adenine nucleotide pool 15 times over (21), or to repair the defects in spore tRNA about 50 times over. This value was calculated by assuming that two molecules of 3-PGA are needed to generate one molecule of ATP via the glycolytic pathway and that the dormant spore contains 130 nmol of defective tRNA per g of dry spores, necessitating 160 nmol of ATP plus CTP for its repair. Thus, it appears likely that during spore germination the ATP pool turns over very rapidly as has been observed in other systems (9).

The defect in dormant spore tRNA, and the obvious possibilities for shutting off or slowing down protein synthesis by removing the 3′-terminal AMP from some key tRNA species suggest that this defect may serve a control function either late in sporulation or in the dormant spore. Although it has been reported that log-phase and dormant spore tRNA have about the same specific acceptor activities for all amino acids tested with only a few differences in isoaccepting species (24), these experiments may have utilized a preparation of aminoacyl-tRNA synthetases which probably contained significant amounts of tRNA nucleotidyltransferase (P. Setlow, unpublished results, 1973). Therefore, it is still possible that a specific tRNA species in dormant spores might lack its terminal AMP residue. On the other hand, it is possible that the absence of the terminal residue from one-third of the dormant spore tRNA serves no control function at all, but is only a consequence of the dephosphorylation of the ribonucleotide pool which must take place in the developing spore late in sporulation (21). This absence of ATP would prevent the repair of defective tRNA species, and under such conditions, one might expect that one-third of all tRNA species would be lacking their terminal residue. The finding that tRNAs specific for isoleucine, leucine, and methionine lack, on the average, 35% of their 3′-terminal residues certainly supports the latter possibility. However, the absence of about 90% of the 3′-terminal AMP from $tRNA^{Tyr}$ and its rapid addition in germination also suggest that all chains of one or more isoaccepting tRNAs may lack complete 3′-termini, and might possibly be important in regulating protein synthesis late in sporulation. Clearly the significance of these findings requires further study.

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