Changes in transfer ribonucleic acids of Bacillus subtilis during different growth phases.

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ABSTRACT

The transfer ribonucleic acids (tRNAs) of B. subtilis at different growth phases are examined for changes in the composition and the methylation of minor constituents. The composition of the tRNAs indicates about equal amounts of adenosine and uridine, and of guanosine and cytidine. About 3-4 residues are present as modified bases in the average tRNA molecule. The net composition of tRNAs appears to remain unaltered during different growth phases. In vitro methylation of tRNAs indicates lack of methyl groups in both exponentially growing cells and spores. In vivo methylation studies show tRNA methylation occurs during the stationary phase in the absence of net tRNA synthesis. Thus, both in vitro and in vivo methylation indicate that the tRNAs in exponentially growing cells do not contain their full complement of modified bases. More complete modification is noted in tRNAs from stationary cells or spores. Hence, tRNA modifications in general are preserved with fidelity even in the dormant spore but the possibility is left open that specific modifications of selected isoacceptors of tRNAs may occur.

INTRODUCTION

The developmental cycle of B. subtilis offers the extremes of growth phases from actively dividing cells to dormant spores. Spore tRNAs may represent a unique situation with respect to tRNA modification. The quantities of certain isoacceptor tRNAs have been reported to vary between the exponential growth phase and the spore phase1-7. Also, 30 per cent of spore tRNAs, compared to 2 per cent of exponential tRNAs, have been shown to lack adenosine at their 3' ends8,9. Although a partial or total cessation of net tRNA synthesis occurs10 at the end of the exponential growth phase, turnover and varied transcriptions during the stationary phase11 have been reported. A DNA methylase occurs principally in the early exponential phase, but declines rapidly thereafter12. Like DNA methylases, RNA methylases can be expected to differ with changes in the developmental cycle. However, no tRNA-modifying enzymes have been studied as a function of cell development.
Transfer RNAs from *B. subtilis* are examined in this report. Since very little is known about the chemistry of tRNAs from this spore-forming organism, chemical composition, nature of minor, modified constituents and *in vivo* methylation at different developmental stages are studied.

**METHODS**

**Growth of cells.** Cells were grown in either a fully-supplemented normal medium, called Tryptone-yeast extract medium or in the minimal salts medium. Cultures were started at an absorbance of 0.1 optical density unit measured at 660 nm and were grown with shaking at 37°.

**Isolation of tRNAs.** Cells were harvested by centrifugation; washed twice with 10 mM Tris pH 8.0, 0.15 M NaCl, 1 mM EDTA, 20% sucrose, and suspended in 50 mM Tris pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 20 mM 2-mercaptoethanol. Cultures not having a radioactive label were broken by sonication. After sonication, electrophoretically purified deoxyribonuclease was added (10 µg per ml) and the lysate centrifuged. The supernatant fluid was removed and made 0.2% with respect to sodium dodecyl sulfate and 0.5 M to NaCl. An equal volume of water saturated phenol was added and the phases were mixed and separated by centrifugation. The aqueous phase was collected. The phenol phase was washed once with a small volume of buffer. The aqueous phases were combined and extracted once with chloroform/isoamyl alcohol (24:1 v/v). After ethanol precipitation, the RNA was fractionated on a Sephadex G-100 column. The tRNA peak was pooled and precipitated with ethanol. The tRNA was freed of any contaminant DNA by treating once again with pure deoxyribonuclease, followed either by chromatography on a small DEAE-cellulose column or by dialysis and precipitation with ethanol.

Isotopically-labeled tRNAs were prepared in the same manner except that the cells were broken by lysis with lysozyme (1 mg per ml) in the presence of deoxyribonuclease (10 µg per ml). The final treatment with deoxyribonuclease and dialysis were omitted in this case.

**Methylation of tRNAs in vivo.** Transfer RNAs were methylated *in vivo* by growing cells in the minimal salts medium in the presence of L-[methyl-¹⁴C]methionine. Precultures were grown in Spizizen's minimal salts, 0.5% glucose, 0.1% casein hydrolysate, and 40 µg L-tryptophan per ml. Cells from the preculture were washed twice with sterile minimal medium and inoculated into a final culture containing Spizizen's minimal salts, 0.5% glucose, 1 mM adenosine, 1 mM guanosine, 1 mM thymidine, 1 mM cysteine, 40 µg L-tryptophan per ml, 2 µg L-tyrosine per ml, and 15 other L-amino acids at a concentration of 20 µg per ml. The amount of nonradioactive L-methionine
Methylation of tRNAs in vitro. The methyl donor for in vitro studies was S-adenosyl-L-[methyl-\(^{14}\)C]methionine. A typical reaction mixture of 200 µl contained 10% glycerol, 40 mM Tris pH 8.9, 8 mM 2-mercaptoethanol, 4 mM MgCl\(_2\), 6 µmol L-methionine, 1.4 µg (0.2 µCi) S-adenosyl-L-[methyl-\(^{14}\)C]-methionine, various concentrations of tRNAs (see Table III), and either 2 mg protein from a partially purified tRNA methylase preparation or 2.9 mg protein from a crude extract (see below). Reaction mixtures were incubated at 30° for 1 h. Samples of 40 µl were withdrawn in duplicate and spotted on paper discs, which were washed twice with cold 5% trichloroacetic acid and once with methanol-ether mixture (1:1 v/v), put into scintillation fluid, and quantitated for radioactivity in a scintillation spectrometer. The radioactivity thus assayed represents net incorporation of the radio-carbon in tRNAs. After removal of the first samples, 10 µg pancreatic ribonuclease was added to the reactions and the incubation continued for another 30 min at 37°. A second set of samples was withdrawn and treated as described. The radioactivity in these samples represents the activity of the nucleic acid preparation that cannot be removed by the ribonuclease. Hence, the radioactivity removed by the ribonuclease action is determined from the difference between the first and second set of samples. Results are expressed as radioactivity in methyl groups of tRNAs not removed by pancreatic ribonuclease action (see Table III).

Methylase Preparations. The partially purified methylase source was treated with streptomycin sulfate, precipitated with ammonium sulfate and chromatographed on Sephadex G-100 and DEAE-cellulose columns. The crude extract was prepared by breaking exponentially growing cells by sonication in 50 mM Tris pH 7.5, 10 mM MgCl\(_2\), 1 mM EDTA, 20 mM 2-mercaptoethanol. After centrifugation, the supernatant fluid was dialyzed overnight in the cold against the same solution, which also contained ten percent glycerol.

Hydrolysis of tRNAs. Transfer RNAs were hydrolyzed to the nucleoside level by two different methods. (1) A mixture of pancreatic ribonuclease, bacterial alkaline phosphatase (both from Worthington), and phosphodiesterase (Russell's viper's venom, from Calbiochem) was prepared and tRNAs hydrolyzed as described elsewhere\(^1\). Incubation was carried out in 0.2 M (NH\(_4\))\(_2\)CO\(_3\) containing 1 mM Mg(OAc)\(_2\), pH 8.7 at 37° for 15 h. (2) Transfer RNAs were first hydrolyzed with ribonuclease-T\(_2\) to nucleotides, and then dephosphorylated to nucleosides by treatment with alkaline phosphatase\(^2\).

Separation of Nucleosides. Nucleosides were separated by anion-ex-
elution chromatography on a cation-exchange column. Results were confirmed by cation-exclusion chromatography on an anion-exchange column.

For the anion-exclusion chromatography, a column of Aminex A-6 (Bio-Rad) (6.35 mm x 40 cm) (bed volume: 12.66 ml; void volume: 3.9 ml) was eluted with 20 mM (NH₄)₂CO₃ brought to pH 9.8 by adding concentrated NH₄OH. The column was maintained at 50°C. The effluent was monitored at 254 nm and 280 nm simultaneously with a dual wavelength and dual pen detector, using a 20 μl, 10 mm light path flow cell (Model 152, Altex Scientific Inc., Berkeley, California). Both major and minor nucleosides were quantitated in the same chromatography by setting the two channels at one wavelength (254 nm) but at two different sensitivity ranges. Peak areas were calculated from the peak height, H and the peak width at 50% of the maximum height, W. (Peak area = H x W x 1.06, where 1.06 is the correction factor; see reference 19). The peak width was expressed in volume units, obtained from the known chart speed and the flow rate. Each peak area, corresponding to the total absorbance at 254 nm and pH 9.8, was converted to nanomoles by dividing it by the appropriate extinction coefficient determined under identical conditions. Millimolar extinction coefficients (at 254 nm) for different compounds used in these experiments at pH 9.8 were as follows: adenosine, 13.9; 6-methyladenosine, 12.5; guanosine, 11.43; 1-methylguanosine, 10.5; cytidine, 6.88; uridine, 7.36; pseudouridine, 5.05; 5-methyluridine, 5.98.

Radioactivity in the effluent was assayed by collecting 0.3 ml fractions in scintillation vials and counting them in a scintillation spectrometer after the addition of 2 ml scintillation fluid (Aquasol, New England Nuclear).

Peaks were characterized by their elution positions in the anion-exclusion chromatography (refer to figure 6 in reference 17). Their identity was further confirmed by rechromatography in the cation-exclusion system after freeze drying, and by comparison of elution positions with those of known nucleosides.

RESULTS

Constituents of B. subtilis tRNAs. A typical separation of nucleosides from B. subtilis tRNAs by anion-exclusion chromatography is shown in figure 1(a). Besides the major nucleosides, commonly occurring minor nucleosides pseudouridine ($\text{(P}rd$) and ribothymidine ($\text{SM}Urd$) were present. Other modified constituents included 5-hydroxymethyluridine ($\text{SHOUrd}$), 4-thiouridine ($\text{4Srd}$), 5-methylaminomethyl-2-thiouridine ($\text{SM}N\text{HMe2Srd}$), an uncharac-
Figure 1: (a) Separation of tRNAs hydrolysate of *B. subtilis* by anion-exclusion chromatography. Aminex A-6 column was eluted with 20 mM (NH₄)₂CO₃, pH 9.80; 0.20 ml per minute, and 50°. (b) Elution positions of constituents containing [¹⁴C]methyl groups of tRNAs (*B. subtilis*) in anion-exclusion chromatography. Incorporation of label into deoxyribonucleosides and the major ribonucleosides was suppressed by adding adenosine, guanosine, and thymidine to the medium.

terized pyrimidine nucleoside (MePyd), 1-methylguanosine (1MeGuo), 2-methylguanosine (2MeGuo), 7-methylguanosine (7MeGuo), and 6-methyladenosine (6MeAdo). The uridine peak also included the isocytosine derivative derived from the partial degradation of 7-methylguanosine during alkaline and temperature conditions of the enzymatic digestion. The value of 6-methyladenosine includes 1-methyladenosine, which rearranges into 7-methyladenosine under alkaline conditions. Strongly adsorbed 2-methylthio-6(2-isopentenyl)adenosine was also present in these tRNAs. This constituent was desorbed from the anion-exclusion column by the normal eluant to which an equal volume of ethanol was added. It was not quantitated in the present studies. Though tRNA preparations were treated with deoxyribonuclease, some samples still indicated DNA contamination. Apparently the deoxyribonucleoside peaks in figure 1(a) were derived from the contaminant DNA.
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hydrolyzed with tRNAs.

As a further aid in identification, tRNAs were labeled in vivo using L-[methyl-1^14C]methionine as the methyl donor. The elution position of these methylated species in anion-exclusion chromatography is shown in figure 1(b) (peak positions shown as horizontal bars). Though a peak corresponding to 5-methyluridine was characterized in figure 1(a), no radioactivity was associated with this component in figure 1(b) (see Discussion).

Changes in tRNAs as a Function of Growth Medium. Table I shows the composition of *B. subtilis* tRNAs grown in two different media, rich tryptone-yeast medium and minimal-salt medium supplemented with glucose, casein hydrolysate and tryptophan (see Methods). Further, the composition of tRNAs in each medium at three different growth stages is shown. It is important to note that after 24 hours, cells in the rich tryptone-yeast medium were present as spores (about 90%), but cells in the minimal salt medium were still in the vegetative stage.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Normal Culture Medium</th>
<th>Minimal Culture Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Cell growth stages</td>
<td>Minimal Cell growth stages</td>
</tr>
<tr>
<td>Nucleosides</td>
<td>Exponential</td>
<td>Stationary</td>
</tr>
<tr>
<td>Ado</td>
<td>15.6</td>
<td>15.3</td>
</tr>
<tr>
<td>6MeAdo</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Urd</td>
<td>15.3</td>
<td>15.9</td>
</tr>
<tr>
<td>Gyr</td>
<td>1.67</td>
<td>1.84</td>
</tr>
<tr>
<td>5MeUrd</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td>Guo</td>
<td>25.2</td>
<td>25.6</td>
</tr>
<tr>
<td>1MeGuo</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Cyd</td>
<td>22.3</td>
<td>22.3</td>
</tr>
</tbody>
</table>

Expressed as moles per 80 nucleoside residues.

Tryptone-yeast 10cm extract medium.

Spizizen's Minimal Medium, sporulation efficiency in this medium after 72h is about 0.1%.

Exponential growth phase.

Stationary growth phase, 10h of growth, just before the appearance of heat resistant spores.

Spore growth phase, 24h of growth (the stage used to purify spores from the parental strain grown on sporulation medium).

6-Methyladenosine is mainly derived from 1-methyladenosine under the alkaline conditions of hydrolysis and chromatography.

Note: The results indicated above were derived from at least triplicate analyses. (For typical deviations in ion-exclusion chromatography, see Tables I to VI in reference 23.)
The results indicate that about the same modified nucleosides are present in both tRNAs whether isolated from rich culture medium or from the minimal culture medium. The data in Table I also indicate that the chemical composition of the tRNAs remains unchanged in exponential and stationary phases. Hence, limitations in cell nutrients have no effect in the "maturation" of tRNAs.

Changes in tRNAs as a Function of Cell Development. The nucleoside composition of tRNAs from cells harvested at three developmental stages are shown in Table I. Transfer RNAs appear to be modified as much in the early developmental stage (exponentially growing cells) as in the stationary growth phase. The chemical nature of tRNAs appears to remain unaltered in general, even in spore tRNAs. However, the loss in pseudouridine and the gain in guanosine of tRNAs from spores exceed simple experimental variations. A similar increase in guanosine, but no change in pseudouridine of tRNAs from the minimal salt medium after 24 hours of growth, is noticeable.

Changes in 4-thiouridine. The work of Lipsett and Doctor, for purified tRNAs from Escherichia coli, shows that an absorbance at 335 nm equal to 4.03 per cent of the total absorbance at 260 nm indicates two 4-thiouridines for each molecule of tRNA. From this relationship, the tRNAs from exponentially growing cells and tRNAs from spores were found to contain 0.12 and 0.11 residues per tRNA, respectively. Thus, every eighth or ninth tRNA contains one 4-thiouridine. The small difference between the two values suggest that the degree of thiolation in tRNAs do not vary substantially when cells transform from vegetative to spore phase.

Methylation of Preformed tRNAs in vitro. To determine if the tRNA is under-methylated (lacking the full complement of methylated constituents), methylation of tRNAs in vitro was attempted with the methyl donor, S-adenosyl-L-[methyl-14C]methionine and a homologous methylase. As a control for homologous methyl acceptance, tRNA was prepared from a relaxed, methionine requiring strain, B. subtilis 168 trpC2 metBS rel- (#27) (see reference 24). The tRNA thus prepared was 57-67% less methylated than that from the parent strain of B. subtilis. A partially purified methylase was used for experiments described in Table II. Transfer of the methyl group into tRNAs was confirmed by the ribonuclease sensitivity of the product (see Methods). To establish the absence of any methyl-transfer inhibitor in tRNA preparations, mixing experiments were performed. Transfer RNAs from exponentially growing cells or from spores were combined with tRNAs from the mutant requiring methionine (strain #27). This showed no loss in the methyl acceptance by tRNAs from the mutant strain.
Table II

<table>
<thead>
<tr>
<th>Origin of tRNA</th>
<th>Concentration of tRNA (A_260 units/reaction)</th>
<th>Incorporation of [^{14}C]methyl group (cpm/A_260 units)</th>
<th>Methylation capacity(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Mutant strain #27(^c)</td>
<td>3.0</td>
<td>1,880</td>
<td>91</td>
</tr>
<tr>
<td>(b) Exponential growth stage(^d)</td>
<td>1.4</td>
<td>54</td>
<td>2.6</td>
</tr>
<tr>
<td>(c) Spores(^d)</td>
<td>1.4</td>
<td>24</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(a\) Using S-adenosyl-L-[methyL-^{14}C]methionine as methyl donor.

\(b\) The incorporation of 2070 cpm of methyl-^{14}C per A_260 unit of tRNA is taken as the full methylation (100%).

\(c\) Undermethylated tRNA obtained from a relaxed, methionine-requiring mutant: *Bacillus subtilis* 168trpC2metBSrel\^-#27\).

\(d\) Transfer RNAs from *Bacillus subtilis* 168trpC2; tRNAs were isolated from exponentially growing cells and from the purified spores.

The results (Table II) indicate some lack of methylation in tRNAs both from exponentially growing cells (up to 2.6%) and from spores (up to 1.2%). Since only about 50% of tRNAs from the mutant strain were under-methylated, lack of the methyl group in tRNAs was in fact twice as much.

**Methylation of tRNAs in vivo.** To study the modification of tRNAs at different growth stages, three cultures were grown and labeled for short periods with L-[methyL-^{14}C]methionine. A growth curve showing the labeling periods is shown in figure 2. About 0.5 mg corresponding to 50 μCi of L-[methyL-^{14}C]methionine was added to each culture at the beginning of the labeling period and harvested at the end of the labeling period. Cultures of 200 ml were grown and tRNAs were extracted as described in Methods. The tRNA from each growth stage is shown in Table III. The cessation of net tRNA synthesis occurs during immediately after the exponential stage. This is shown by the constant recovery of tRNAs in growth phases 2 and 3. However, the methyl group from L-[methyL-^{14}C]methionine continues to be incorporated into tRNAs although at a reduced rate (see Discussion).

The lower portion of Table III shows the separation of methylated constituents of tRNAs, methylated in vivo and isolated from three different development phases (see figure 2). There is a constant turnover of methyl nu-
Figure 2: Growth curve for *B. subtilis* 168trpC2 in minimal salt medium showing the length of time in which different cultures were labeled with L-[methyl-14C]methionine (see Table III).

cleosides as a result of tRNA turnover (7-methylguanosine and 5-methylaminomethyl-2-thiouridine) in both actively-dividing cells and stationary cells. However, as the cell passes into the resting stage, the methyl group incorporation increases in some constituents (guanosine methylated at 1 or 2 position), and decreases in others (uncharacterized methylated pyrimidine). Differences in the 6-methyladenosine value are probably due to differences in methylation of adenosine at the 1 or 6 position.

**DISCUSSION**

Constituents of *B. subtilis* tRNAs. Very little is known about tRNAs from this spore forming bacterium. Although some data on the nucleoside composition of *Bacillus subtilis* and related *Bacillus* species exists, only one report has been concerned with differences of major nucleosides between vegetative cells and spores. In that report, the four major nucleosides were compared and the authors concluded that there were no differences in the major nucleosides of tRNAs from the two phases. The composition of the tRNAs (Table I) from *B. subtilis* indicates about equal amounts of adenosine and uridine, and of guanosine and cytidine. About 3-4 residues are present as modified bases in the average tRNA molecule. Modified adenosine is present as the 1-methyl- or N6-methyl adenosine and as methylthio(isopentenyl)adenosine. We were unable to detect 2-methyladenosine, which is commonly found in *E. coli* tRNAs. Among the modified guanosines, 7-methyl derivative is present in twice the amount of the 1-methyl derivative. A very small quantity of 2-methylguanosine (about 2% of all methylated species) is present. No modified
cytidines were detected. A modified pyrimidine containing a methyl group and an anionic charge at alkaline pH is yet to be characterized. Contrary to the case in *E. coli*, we find that the methyl group of 5-methyluridine in *B. subtilis* tRNAs is not derived from methionine. Other workers report that it is derived from a tetrahydrofolate derivative. The ratio of pseudouridine to 5-methyluridine in *B. subtilis* is similar to that found in *E. coli* tRNAs. Among sulfur-containing uridines (see figure 1), we find 5-methylaminomethyl-2-thiouridine and a very small quantity of 4-thiouridine in *B. subtilis* tRNAs, the latter in about 15% of the amount reported for *E. coli* tRNAs. Thus, there appear to be basic differences between the tRNAs from *E. coli* and from *B. subtilis*.

**Influence of nutrients on tRNA Population.** The net chemical composition of tRNAs remains unchanged when *B. subtilis* is grown either in the nutritionally rich medium or in the poor medium. Minor bases appear to be equally modified in the two different media, and limitations in the cell nutrients do not appear to cause any adverse effect in the "maturation" of tRNAs. However, differences in the number of serine isoacceptor tRNAs occur when this bacterium is grown in two different media.

**Changes in tRNAs during Development of *B. subtilis*.** The analysis of the tRNA patterns of sporulating *B. subtilis* shows that during the process of morphogenesis a shift occurs in the ratios of two isoacceptor species of valine tRNA. Our results indicate minor differences in the net composition of tRNAs among the three different phases (Table I). Studies on the methylation of tRNAs in vitro indicate little under-methylation of tRNAs (1.3% in exponentially growing cells and 0.6% in spores, see Table II). (In Table II, an incorporation of 2070 cpm per A260 unit of tRNA is taken as full methylation. Since the substrate is already about 50% methylated, a totally unmethylated substrate would be expected to accept twice as many methyl groups. On this basis, the methylation capacities listed in Table II are divided by two.) The results indicate an uptake of roughly less than 3 moles of [14C]methyl groups per mole of tRNA in the in vitro studies. Though tRNAs from different growth phases (figure 2) appear to be methylated (in vivo) to different degrees (64% from exponentially growing cells, 24% from stationary cells and 12% from spores, see Table III), they are synthesized at different rates in different growth phases.

If new isoacceptor species are required for the synthesis of new proteins during cell differentiation, our results indicate that they are perhaps derived from subtle modifications of certain residues in the
TABLE III

Methylation of B. subtilis tRNAs in vivo at three growth stages

<table>
<thead>
<tr>
<th>Growth stage, when cells harvested</th>
<th>(1) Time of incorporation of methyl-(^{14}C) donor</th>
<th>(2) Time of incorporation of methyl-(^{14}C) donor</th>
<th>(3) Time of incorporation of methyl-(^{14}C) donor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
<td>1.5 h</td>
<td>2 h</td>
</tr>
<tr>
<td>tRNA yield (A(_{260}) units)</td>
<td>5.8</td>
<td>26.7</td>
<td>25.1</td>
</tr>
<tr>
<td>Incorporation of (^{14}C)methyl per 1 A(_{260}) unit of tRNA (cpm)</td>
<td>48,340</td>
<td>18,360</td>
<td>9,330</td>
</tr>
</tbody>
</table>

Relative Proportions of Methylated Nucleosides

<table>
<thead>
<tr>
<th>Methylated nucleosides(^a)</th>
<th>Percentage of total (^{14}C)methyl incorporated to tRNAs (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7MeGua (M2)</td>
<td>31</td>
</tr>
<tr>
<td>6MeAdo (M6)</td>
<td>28</td>
</tr>
<tr>
<td>MePyd (M1)</td>
<td>22</td>
</tr>
<tr>
<td>1MeCuo (M5)</td>
<td>9</td>
</tr>
<tr>
<td>SMeNHMe2Srd (M3)</td>
<td>8</td>
</tr>
<tr>
<td>2MeGua (M4)</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\)Cells were grown in the Minimal Medium\(^b\) following labelling procedure as described in the Methods section. A growth curve with labelling times from (1) to (3) is shown in figure 2.

\(^b\)Methylated nucleosides correspond to their elution positions on the anion-exclusion column, shown as bars in figure 1(b), and numbered from M1 to M6 as eluted. The labelled material 2-methylthio-6-(2-isopentenyl)adenosine which was strongly adsorbed to the column and was eluted with 10% ethanol was not considered in these calculations. The 6-methyladenosine peak also contains this compound derived from 1-methyladenosine during alkali conditions. The first peak, M1 appears to be a methylated pyrimidine (MePyd).

In conclusion, tRNAs of B. subtilis differ from tRNAs of E. coli in their overall composition and in their modified constituents. Changes in the nutrients of the growth medium do not appear to cause compositional changes in the tRNAs. In different growth stages, minor compositional differences of tRNAs exist. However, both in vivo and in vitro methylation studies indicate that the tRNAs in exponentially growing cells do not contain their full complement of modified bases. More complete modification is noted in tRNAs from stationary cells or spores. The results also indicate methylation of tRNAs in the stationary growth phase after the cessation of net tRNA synthesis.

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Note added in the revision. Keith et al. report trNA^1^ and trNA^2^ from B. subtilis differ due to post-transcriptional modification (FEBS Letters, 61, 1976, p. 120). They find trNA^1^ typical of the exponentially growing cells contains only isopentenyladenosine, but trNA^2^, typical of the stationary cells contains its full modification, 2-methylthiopentenyladenosine. The results confirm our findings that after cessation of trNA transcription, trNA structure changes by minor modifications and not by overall composition.

REFERENCES
