JOURNAL OF BACTERIOLOGY, May 1975, p. 526-531
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Vol. 122, No. 2
Printed in U.S.A.

Alteration of Tyrosine Isoaccepting Transfer Ribonucleic Acid Species in Wild-Type and Asporogenous Strains of *Bacillus subtilis*

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Received for publication 20 February 1975

The relative amounts of two isoaccepting species of tyrosine transfer ribonucleic acid, tRNA$_{\mathrm{I}}^{\mathrm{Tyr}}$ and tRNA$_{\mathrm{II}}^{\mathrm{Tyr}}$, determined from reversed phase 5 profiles of tyrosyl-tRNA, prepared from *Bacillus subtilis* strain W168, were growth phase and medium dependent. The growth phase-dependent alterations in the relative amounts of tRNA$^{\mathrm{Tyr}}$ species were also demonstrated in 11 asporogenous strains of *B. subtilis*. The proportion of tRNA$^{\mathrm{Tyr}}$ species and the extent of the alteration in their relative amounts during the transition from the exponential to the stationary phase of growth of these strains was not directly correlated with the formation of spores by strain W168 grown in various media or the stage at which the asporogenous strains are blocked in the process of sporulation.

Specific alterations in the relative amounts of certain isoaccepting species of transfer ribonucleic acid (tRNA) have been demonstrated during differentiation, speciation, tumor formation (31, 34), viral infection (9, 16), and growth of cells under varying conditions (5, 18-21, 33, 35). Since tRNA serves as an adaptor molecule for the transfer of amino acids into polypeptides during the process of protein synthesis, alterations in the relative amounts of isoaccepting species of tRNA may exert control on translation of messenger RNA. In addition, tRNA may be involved in regulation of transcription and other cellular processes (3, 14, 24, 28). Alterations in the relative proportions of isoaccepting species of tRNA may result from selective transcription of two or more tRNA genes or from selective control of post-transcriptional modification of one tRNA gene product.

An alteration in the ratio of the isoaccepting species of tyrosine tRNA (tRNA$^{\mathrm{Tyr}}$), tRNA$_{\mathrm{I}}^{\mathrm{Tyr}}$ and tRNA$_{\mathrm{II}}^{\mathrm{Tyr}}$, has been observed during the shifts from the lag to the exponential and from the exponential to the stationary phases of growth of *Bacillus subtilis* strain W168 in Penassay broth (2, 29, 30). A similar alteration was also reported to occur during the transition from the exponential to the stationary phase of growth of *B. subtilis* strain W23 in Penassay broth (30). In this study, the alteration in the ratio of tRNA$_{\mathrm{I}}^{\mathrm{Tyr}}$ to tRNA$_{\mathrm{II}}^{\mathrm{Tyr}}$ in *B. subtilis* strain W168 grown in various media and in several asporogenous strains of *B. subtilis* was determined by reversed phase 5 (RPC-5) chromatography of tyrosyl-tRNA prepared from these strains.

MATERIALS AND METHODS

Organisms and conditions for culture. The strains of *B. subtilis* used in this study are listed in Table 1. Cultures were incubated on a gyratory shaker (New Brunswick model G-25, 300 rpm) at 37 C, except in the case of strain ts-14 which was incubated at 47.5 C. Growth of the cultures was measured with a Klett-Summerson spectrophotometer (no. 66 filter).

Strain W168 was cultivated in 1.75% Penassay broth (Difco), Penassay broth supplemented with tyrosine (100 μg/ml), medium C (1), medium GM-11 ([17]; medium C supplemented with the following amino acids in μg/ml: alanine, 50; arginine, 20; asparagine, 50; glutamic, 100; histidine, 50; isoleucine, 10; leucine, 5; serine, 5; threonine, 20; valine, 20; and methionine, 5), or medium SCM (15). The approximate generation times observed with strain W168 were as follows: Penassay broth or Penassay broth supplemented with tyrosine, 25 min; medium C, 75 min; medium GM-11, 48 min; and medium SCM, 40 min. The asporogenous strains were cultivated in Penassay broth; the broth was supplemented with amino acids (50 μg/ml) required by these strains as indicated in Tables 1 and 3. The generation time for all asporogenous strains grown in Penassay broth or supplemented Penassay broth at 37 C was approximately 25 min; the generation time for strain ts-14 at 47.5 C was approximately 15 min.

Sporulation. Sporulation in strain W168 was determined from smears by microscopic counts of spores stained with malachite green and vegetative cells counter-stained with safranin. Asporogenous strains were also examined by this procedure; no spores were observed in these strains. Strain ts-14 did not sporulate at 47.5 C.

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Preparation of aminoacyl-tRNA synthetase, bulk tRNA, and tyrosyl-tRNA. Aminoacyl-tRNA synthetase was prepared from exponential phase cells of *B. subtilis* strain W168 harvested from Penassay broth at a density of 55 Klett units (approximately 2.4 × 10^8 colony-forming units/ml). For the preparation of bulk tRNA, exponential phase cells (40 Klett units; approximately 1.5 × 10^8 colony-forming units/ml) and early stationary phase cells (4 to 5 h after the onset of the transition from the exponential to the stationary phase of growth; approximately 6 × 10^8 colony-forming units/ml) were chilled in an ice-water bath and harvested by continuous flow centrifugation (Sorvall RC-2 Centrifuge, Sorvall Type KSB apparatus) at 0 C. In the case of strain W168 grown in medium C, stationary phase samples were taken up to 14 h after the onset of the decelerated growth phase. The procedures used to prepare aminoacyl-tRNA synthetase, bulk tRNA, and tyrosyl-tRNA have been described previously (2). The specific activities of L-[3,5-^{3}H]tyrosine and L-[^{14}C]tyrosine were 43.8 Ci/mmol and 374 mCi/mmol, respectively (New England Nuclear Corp.).

Chromatography of tyrosyl-tRNA on reversed phase 5. A column (0.9 by 12 cm) of reversed phase 5 (RPC-5) (trialkylmethylammonium chloride on polychlorotrifluoroethylene; Miles Laboratories, Inc.) (23) was equilibrated with 0.01 M sodium acetate buffer, pH 4.5, containing 0.01 M MgCl$_2$, 0.35 M NaCl, and 0.001 M 2-mercaptoethanol (equilibration buffer). The aminoacyl-tRNA mixture in 40 ml of equilibration buffer was loaded on the column and washed with 50 ml of equilibration buffer. A linear gradient (220 ml) of 0.35 M to 0.95 M NaCl in equilibration buffer used to elute tyrosyl-tRNA was delivered to the column by a Buchler Polystaltic Pump at a flow rate of 1 ml per 4 min, and 2-ml fractions were collected.

Scintillation counting. The radioactivity of samples was measured in a Packard Tri-Carb 3003 liquid scintillation spectrometer (Omnifluor-toluene scintillation fluid; New England Nuclear Corp.). The efficiency of ^{14}C and ^{3}H counting was 56 and 22%, respectively; the data were corrected for background and spill over.

RESULTS

Comparison of tyrosyl-tRNA from exponential and stationary phase cells by RPC-5 chromatography. Bulk tRNA preparations from exponential and early stationary phase cells of the various strains of *B. subtilis* (Table 1) were acylated with [^{3}H]tyrosine and [^{14}C]tyrosine, respectively, in the presence of 19 other nonradioactive amino acids; the radioactive labels were reversed in control experiments. The kinetics of acylation were similar to that reported earlier (2). The samples of aminoacyl-tRNA were extracted, mixed, and analyzed by RPC-5 chromatography.

Tyrosyl-tRNA from strain W168 grown in Penassay broth was resolved into two major species, tyrosyl-tRNA$_{\rm I}$ and tyrosyl-tRNA$_{\rm II}$; tyrosyl-tRNA$_{\rm I}$ was eluted from RPC-5 before tyrosyl-tRNA$_{\rm II}$ (Fig. 1). Both species of tyrosyl-tRNA are present in exponential and stationary phase cells. The ratio of tyrosyl-tRNA$_{\rm I}$ to tyrosyl-tRNA$_{\rm II}$ observed in exponential phase cells was 1:0.15; during the shift from the exponential to the stationary phase of growth, the relative amounts of tRNA^{Tyr} species underwent a rapid progressive change to 1:11.5. The identity of the two species (2) of tyrosyl-tRNA on

TABLE 1. *Strains of B. subtilis used in this investigation*

Strain	Origin	Characteristics[a]	Source
W168	168	Wild type 168	N. Sueoka
3NA[b]	Marburg W	OA, Ab$^-$, Prot$^-$, Cpt$^-$	P. Schaeffer
9V	168	OC	P. Schaeffer
4Z	GSY 254	IIB, Ab$^-$, *lys-1 trpC2*	P. Schaeffer
94U	168	III, *trp*	P. Schaeffer
11T	168	IV, *trp*	P. Schaeffer
ts-14[c]	168	Sporulates at 35 C, but not at 47.5 C	R. H. Doi
SCR100[d]	168	*spoA12 leu*	J. Ito
SCR119	168	*spoB107 trpC2 lys-1*	J. Ito
SCR158	168	*spoE80 trpC2 lys-1*	J. Ito
SCR162	168	*spoC81 trpC2 lys-1*	J. Ito
SCR166	168	*spoC90 lys-1*	J. Ito

[a] Symbols: Ab, antibiotic production; Cpt, competence for transformation; *lys*, lysine; Prot, protease; spo, sporogenous; *trp*, tryptophan; and OA, OC, IIB, III, and IV, stages of sporulation.

[b] Strains 3NA, 9V, 4Z, 94U, and 11T: References 6 and 11.

[c] Reference 25: Strain ts-14 is a temperature-sensitive RNA polymerase mutant conditionally defective in an early aspect of sporulation.

[d] Strains SCR100, SCR119, SCR158, SCR162, and SCR166 are blocked in early aspects of sporulation (J. Ito, personal communication; references 12 and 13).

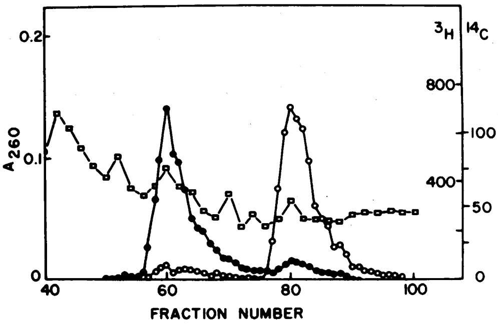

FIG. 1. *Comparison of the relative amounts of tyrosyl-tRNA$_{I}$ and tyrosyl-tRNA$_{II}$ in exponential and stationary phase cells of B. subtilis strain W168 by RPC-5 chromatography. [^{3}H]tyrosyl-tRNA (16,800 counts/min) and [^{14}C]tyrosyl-tRNA (3,300 counts/min) were mixed and chromatographed on RPC-5. Tyrosyl-tRNA$_{I}$ is eluted before tyrosyl-tRNA$_{II}$ on RPC-5. Symbols: (●) exponential phase [^{3}H]tyrosyl-tRNA; (○) stationary phase [^{14}C]tyrosyl-tRNA; (□) absorbency at 260 nm.*

RPC-5 was confirmed in this study by chromatography of samples on methylated albumin kieselguhr (MAK). It should be noted that the total amount of tyrosine acceptor activity in exponential phase cells, stationary phase cells, and spores of strain 168 are similar (32); furthermore, the relative amounts of tyrosyl-tRNA_{I} and tyrosyl-tRNA_{II} in early stationary phase cells (Fig. 1) and late stationary phase cells (2) are similar to that reported from spores (32).

The ratios of $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ to $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ in exponential and stationary phase cells of strain W168 grown in Penassay broth supplemented with tyrosine, medium C, medium GM-11, and medium SCM were determined from RPC-5 profiles of tyrosyl-tRNA. The ratios of tRNA^{Tyr} species have been normalized (Table 2) in order that the quantity of $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ in each sample equals 1. In exponential phase cells, $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ is the predominant species of tRNA^{Tyr}; the amount of $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ relative to $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ in strain W168 ranges from 0.09 in medium SCM to 0.48 in medium GM-11.

The extent of the alteration in the relative amounts of the tRNA species during the transition from the exponential to the stationary phase of growth of strain W168 is medium dependent (Table 2). In early stationary phase cells of strain W168 grown in Penassay broth, the amount of $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ is 11.5 times greater than $\text{tRNA}_{\text{I}}^{\text{Tyr}}$. The extent of the alteration in strain W168 during the transition to the stationary phase was reduced considerably in Penassay broth supplemented with tyrosine. Early stationary phase cells of strain W168 grown in medium C, a chemically defined medium that does not contain tyrosine, have 3.7 times more $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ than $\text{tRNA}_{\text{I}}^{\text{Tyr}}$; a similar observation was made 14 h after the onset of the transition from the exponential to the stationary phase of growth of strain W168 in medium C. The extent of the growth phase dependent alteration in the relative amounts of tRNA^{Tyr} species in strain W168 was reduced considerably in medium GM-11 compared to that observed in medium C; in fact, the ratio of the isoaccepting species was altered only slightly during growth of strain W168 in medium GM-11. In medium SCM, which contains casein hydrolysate, the amount of $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ relative to $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ was 0.33 in early stationary phase cells of strain W168.

TABLE 2. *Growth phase and medium dependent alterations in the ratio of tRNA^{Tyr} species in B. subtilis strain W168*

Strain	Medium	$\text{tRNA}_{\text{I}}^{\text{Tyr}}:\text{tRNA}_{\text{II}}^{\text{Tyr}}$	
		Exponential phase	Early stationary phase
W168	Penassay broth[a]	1:0.15	1:11.5
W168	Penassay broth + 100 µg of tyrosine/ml	1:0.11	1:1.59
W168	C	1:0.25	1:3.70
W168	GM-11	1:0.48	1:0.46
W168	SCM	1:0.09	1:0.33

[a] Figure 1.

The alteration during the transition to the stationary phase of growth of the asporogenous strains 3NA, 9V, 4Z, 94U, and 11T (Table 3) was less extensive than that observed with strain W168 grown in Penassay broth. In stationary phase cells of strains 3NA and 4Z, $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ was present in larger amounts than $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ The relative amounts of tRNA^{Tyr} species in exponential and early stationary phase cells of strains 3NA and 9V, both grown in Penassay medium, and the extent of the growth phase alteration in the relative amounts of tRNA^{Tyr} species in these strains were different; both strains are blocked at stage 0 in the process of sporulation. On the other hand, the results obtained with strain 94U (blocked at stage III) and strain 11T (blocked at stage IV) were essentially identical; both of these strains were grown in Penassay broth supplemented with tryptophan. A 7.4-fold increase in the amount of $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ compared to $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ was observed during the transition from the exponential to the stationary phase of growth of strain ts-14 at 47.5 C (Table 3).

The relative amounts of the isoaccepting species of tRNA^{Tyr} in exponential and early stationary phase cells of the asporogenous strains SCR 100, SCR 119, SCR 158, SCR 162, and

TABLE 3. *Growth phase dependent alterations in the ratio of tRNA^{Tyr} species in Asporogenous strains of B. subtilis*

Strain	Medium	$\text{tRNA}_{\text{I}}^{\text{Tyr}}:\text{tRNA}_{\text{II}}^{\text{Tyr}}$	
		Exponential phase	Early stationary phase
3NA	Penassay broth	1:0.09	1:0.59
9V	Penassay broth	1:0.16	1:3.76
4Z	Penassay broth + 50 µg of lysine and 50 µg of tryptophan/ml	1:0.11	1:0.49
94U	Penassay broth + 50 µg of tryptophan/ml	1:0.22	1:1.30
11T	Penassay broth + 50 µg of tryptophan/ml	1:0.23	1:1.30
ts-14[a]	Penassay broth	1:0.24	1:1.78

[a] Grown at 47.5 C.

SCR 166 were estimated from MAK chromatograms are described previously (2). The resolution of the isoaccepting species on MAK does not permit an accurate determination of the ratios of the isoaccepting species of $tRNA^{Tyr}$; nevertheless, the profiles demonstrated that $tRNA_{I}^{Tyr}$ is the predominant species present in exponential phase cells of these strains, whereas $tRNA_{II}^{Tyr}$ is the predominant species present in stationary phase cells. Compared to strain W168 grown in unsupplemented Penassay broth, strains SCR 158 and SCR 162 contain proportionally greater amounts of $tRNA_{II}^{Tyr}$ in the exponential phase, and strains SCR 158 and SCR 166 contain proportionally greater amounts of $tRNA_{I}^{Tyr}$ in the early stationary phase.

Sporulation in B. subtilis strain W168. Smears prepared from cultures of strain W168 at time intervals up to 20 h after the onset of the decelerated growth phase in various media were examined microscopically. Neither free spores nor sporangia were observed in the exponential phase samples harvested for preparation of bulk tRNA, in smears prepared during the decelerated growth phase, or in early stationary phase samples harvested for preparation of bulk tRNA. Smears of strain W168 grown in Penassay broth or Penassay broth supplemented with tyrosine demonstrated less than 1% sporulation of cells from 8 to 20 h after the onset of the decelerated growth phase. No free spores or sporangia were observed during this period in smears prepared from cultures grown in medium C or medium GM-11. As early as 6 h after the onset of the decelerated growth phase of strain W168 in medium SCM, the cells exhibited approximately 50% sporulation; this value increased to 85% during the 20 h period. It is apparent that the ability of strain W168 to produce sporangia and free spores in these media is not directly correlated with ratios of $tRNA^{Tyr}$ reported in Table 2.

DISCUSSION

Alterations in the relative or absolute amounts and the functional competence of isoaccepting tRNAs may result from selective control of transcription of multiple tRNA genes or from post-transcriptional modification of one tRNA gene product (4, 7-10, 16, 26, 27, 30). Since alterations in isoaccepting tRNA have been reported to be associated with changes in growth conditions, viral infection, and differentiation of tissues, it has been suggested that they may exert cellular control at the level of translation.

An alteration in the relative amounts of tyrosine isoaccepting species of tRNA, $tRNA_{I}^{Tyr}$ and $tRNA_{II}^{Tyr}$, during the shift from the exponential to the early stationary phase of growth in *B. subtilis* was demonstrated under conditions in which acylation had proceeded to completion. The two species of $tRNA^{Tyr}$ may represent metastable conformational forms with identical primary sequences; however, it has not been possible to interconvert the two species (2). The rates of enzymatic acylation, enzymatic deacylation, and nonenzymatic deacylation of the two species in vitro are similar. Enzymatic exchange of radioactive tyrosine between species of $tRNA^{Tyr}$ demonstrated that both species are recognized by the same amino-acyl-tRNA synthetase molecule. Preferential binding of either species of tyrosyl-tRNA to ribosomes was not observed from MAK profiles of polysomal bound tyrosyl-tRNA. In addition, no significant difference was observed in the capacity of exponential and stationary phase preparations of tyrosyl-tRNA to transfer tyrosine into polypeptides in response to poly (U_2, A) or poly (U, A, C) in an in vitro system derived from *Escherichia coli* (2). It should be noted, however, that specific tRNA molecules have been shown to play a role in cellular processes other than translation. For example, glycyl-tRNA plays a role in peptidoglycan synthesis (24, 28); histidyl-tRNA, in repression of the histidine operon (3); and tyrosyl-tRNA, in the activity of tryptophan pyrrolase in *Drosophila* (14). The alteration in isoaccepting species of $tRNA^{Tyr}$ in *B. subtilis* may be involved in aspects of control related to induction and repression of genetic information or the activity of a specific metabolic pathway.

Isoaccepting species for 16 aminoacyl-tRNAs in *B. subtilis* strain 168 have been reported. An alteration in the ratios of isoaccepting species of glycyl-, tyrosyl-, leucyl-, seryl-, threonyl-, asparaginyl-, and arginyl-tRNAs was observed when aminoacyl-tRNAs from exponential phase cells and spores were compared; the appearance of disappearance of uniques species of lysyl-, glutamyl-, and tryptophanyl-tRNAs was also reported (32).

The data presented here demonstrate the medium and growth phase dependent alterations in the relative amounts of isoaccepting species of $tRNA^{Tyr}$ in strain W168 and the growth phase dependent alterations in several asporogenous strains of *B. subtilis*. The effect of supplemental tyrosine in reducing the extent of the alteration in strain W168 grown in Penassay broth may suggest a role for this amino acid in the regulation of the level of $tRNA^{Tyr}$ species; its role may be elucidated further with tyrosine-requiring strains. A comparison of the results obtained from strain W168 grown in medium C and medium GM-11 suggests that other amino acids may also play a role in this regard. Various strains of *B. subtilis* resistant to tyrosine or histidine analogues exhibit elevated levels of enzymes in both the tyrosine and histidine biosynthetic pathways; however, the RPC-5 profile of tyrosyl-tRNA strain 2802, a representative strain resistant to the histidine analog 1,2,4-triazole-3-alanine was similar to that of a wild-type strain designated WB746. In these strains, tyrosyl- or histidyl-tRNA species or aminoacyl-tRNA synthetases do not appear to serve as the common element in the control of these biosynthetic pathways (22). Further studies are necessary to define the nature of the difference(s) between $\text{tRNA}_{\text{I}}^{\text{Tyr}}$ and $\text{tRNA}_{\text{II}}^{\text{Tyr}}$ and delineate potential control functions that may be attributed to the tRNA^{Tyr} species in *B. subtilis*.

ACKNOWLEDGMENTS

The research was supported by Public Health Service Training grant AI-69, on which R.A.M. was a predoctoral Trainee, and the Public Health Service General Research Support grant RR05386.

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