REGULATION OF A SERINE TRANSFER RNA OF BACILLUS SUBTILIS
UNDER TWO GROWTH CONDITIONS*

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Communicated by H. A. Barker, September 6, 1966

Evidence has been obtained recently which suggests that tRNA1 modifications
may be involved during shifts in metabolism.2-4 Analyses of tRNA obtained from
Bacillus subtilis cells grown in a rich broth medium and a semisynthetic sporulation
medium were performed to determine whether the valyl-tRNA pattern change
during sporulation could be attributed to a change in growth rate or medium condition.
Although the valyl-tRNA pattern was unchanged under these two growth conditions,
the seryl-tRNA pattern was modified. Evidence will be presented which indicates
that the relative amount of one of the three seryl-tRNA’s varies depending
on the growth rate and condition.

Materials and Methods.—B. subtilis W23 cells were used as the source of transfer
RNA and aminoacyl-transfer RNA synthetase. The cells were grown in SCM
medium5 and in Penassay broth (Difco) at 37°C. The growth rates of the cells in
Penassay and SCM media were 1.5 and 1.2 (doublings of optical density at 660
μμ per hr), respectively. Cells were harvested in log phase at a density of 1-2 × 108
cells per ml. The tRNA was extracted from cells as described by von Ehrenstein
and Lipmann.6 The synthetase and methylated albumin kieselguhr (MAK)
column were prepared as described by Sueoka and Yamane.7 Details of these
methods and the preparation of aminoacyl-transfer RNA with B. subtilis cell
extracts were described previously.4 The serine-accepting capacities of the tRNA
preparations from Penassay- and SCM-grown cells were 64.4 and 85.9 μμmoles
per A260 unit, respectively.

The iodine oxidation and thiosulfate reduction of tRNA were performed according
to the method of Carbon et al.8 The Mg++ treatment was done according to the procedures of Lindahl et al.9

Materials: Reagents were obtained from the following sources: Schwarz
BioResearch, Inc., L-serine-C14, 120 mc/mmole; L-serine-H3, 870 mc/mmole;
L-valine-C14, 200 mc/mmole; L-valine-H3, 1.2 c/mmole.

Results.—Change in seryl-tRNA pattern during growth in different media: A
comparison of aminoacyl-tRNA from cells grown in rich and poor media by MAK-
column chromatography revealed very slight differences in elution patterns, except
for seryl-tRNA (Fig. 1). For seryl-tRNA from cells grown in a rich medium, three
distinct elution peaks were obtained. The cells grown in the semisynthetic sporulation
medium contained two major peaks and a very minor third peak. The valyl-
tRNA pattern was identical under the two growth conditions (Fig. 2). Although
none of the other 14 amino acids tested showed any differences, the lack of sensitivity
of the MAK-column procedure may have precluded any observation of minor
changes with other aminoacyl-tRNA.

Validity of seryl-tRNA pattern change: (a) The tRNA preparations were stripped
of amino acids by incubating for 3 hr at 35°C in Tris-HCl buffer, pH 8.8, before
aminoacylation. No differences in the patterns were observed.
(b) Lindahl et al. demonstrated that tRNA's lacking Mg++ were incapable of accepting amino acids. Therefore, the tRNA preparations were heated for 5 min at 60°C in the presence of 0.001 M EDTA, 0.02 M MgCl₂, and 0.01 M Tris-HCl, pH 8.0. After this treatment they were aminoacylated and eluted from a MAK column. Figure 3 shows the results with tRNA from cells grown in SCM medium. The control in Figure 4a illustrates the results with tRNA from cells grown in Penassay medium. The Mg++ treatment did not alter either pattern.

(c) Evidence for the presence of more than one seryl-tRNA is presented in Figure 4a and b. The mild iodine oxidation of tRNA prevents some tRNA species from accepting amino acids if they contain thiobases. Oxidation of tRNA from Penassay-grown cells prevented peaks 2 and 3 from accepting serine (Fig. 4a). Oxidation of tRNA from SCM-grown cells also prevented peak 2 (and perhaps the very minor peak 3) from accepting serine. Therefore, at least peaks 1 and 2 are structurally different. The constant presence of peak 3 in Penassay-grown cells suggests that it is also an independent peak. The oxidation of the serine-specific tRNA preparations is reversible by thiosulfate reduction as indicated in Figure 4b. Both peak 2 and peak 3 are again capable of accepting serine after reduction.

Fig. 1.—MAK-column cochromatography of seryl-tRNA-H³ from cells grown in Penassay medium and seryl-tRNA-C¹⁴ from cells grown in SCM medium. The closed circles and open circles represent seryl-tRNA from cells grown in SCM medium and in Penassay medium, respectively.

Fig. 2.—MAK-column cochromatography of valyl-tRNA-C¹⁴ from cells grown in Penassay medium and valyl-tRNA-H³ from cells grown in SCM medium. The closed circles and open circles represent valyl-C¹⁴-tRNA and valyl-H³-tRNA, respectively.

Fig. 3.—MAK column chromatography of seryl-C¹⁴-tRNA from cells grown in SCM medium. The tRNA was heated in the presence of Mg++ before aminoacylation (see Methods) and chromatography.
Discussion.—Two explanations are possible for the results which have been presented: (1) the third serine-specific tRNA in SCM-grown cells has been modified in vivo so that it is incapable of being aminoacylated in vitro; or (2) during growth in different media, there is differential transcription of serine-specific tRNA cistrons resulting in an altered ratio of the three serine-specific tRNA's.

The presence of serine as one of the N-terminal amino acids in B. subtilis suggests that the latter explanation may be more probable. Since cells growing more rapidly would require a higher rate of initiation of translation, the possibility exists that the third serine-specific tRNA is involved in the initiation process and is therefore required in higher concentrations under faster rates of cell growth.

If this is indeed so, it would be the third case in which a regulatory mechanism has been demonstrated for RNA synthesis. Evidence for such a mechanism in messenger RNA and ribosomal RNA synthesis was presented previously.

Summary.—Evidence has been presented for differential transcription of serine-specific tRNA during growth in rich and poor media. The results suggest that tRNA synthesis may be under regulatory control.

The authors thank Richard T. Igarashi for his superb technical assistance.

* The research was supported by National Science Foundation grant no. GB-3694 and U.S. Atomic Energy Commission grant no. AT(11-1)-34.
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1 The following abbreviations are used: tRNA, transfer ribonucleic acid; MAK, methylated albumin kieselguhr; Tris buffer, tris(hydroxymethyl)aminomethane buffer; EDTA, ethylenediamine tetraacetic acid.

2 Peterkofsky, A., these PROCEEDINGS, 52, 1233 (1964).
3 Sueoka, N., and T. Kano-Sueoka, these PROCEEDINGS, 52, 1535 (1964).
7 Sueoka, N., and T. Yamane, these Proceedings, 48, 1454 (1962).