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

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Evidence has been obtained recently which suggests that tRNA[1] 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 medium[5] 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 mμ per hr), respectively. Cells were harvested in log phase at a density of 1–2 × $10^8$ 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 $A^{260}$ 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-$C^{14}$, 120 mc/mmole; L-serine-$H^3$, 870 mc/mmole; L-valine-$C^{14}$, 200 mc/mmole; L-valine-$H^3$, 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.

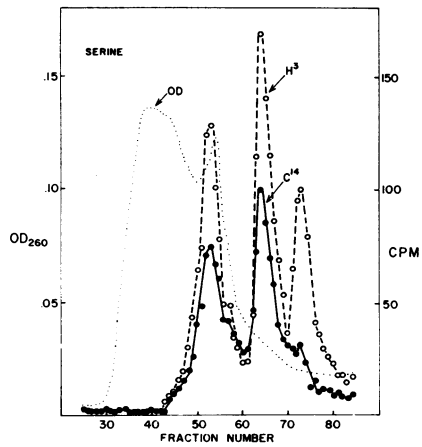


FIG. 1.—MAK-column cochromatography of seryl-tRNA-$H^3$ from cells grown in Penassay medium and seryl-tRNA-$C^{14}$ from cells grown in SCM medium. The closed circles and open circles represent seryl-tRNA from cells grown in SCM medium and in Penassay medium, respectively.

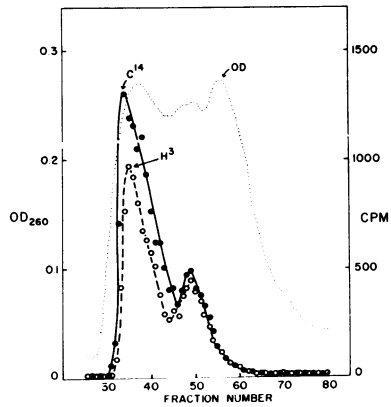


FIG. 2.—MAK-column cochromatography of valyl-tRNA-$C^{14}$ from cells grown in Penassay medium and valyl-tRNA-$H^3$ from cells grown in SCM medium. The closed circles and open circles represent valyl-$C^{14}$-tRNA and valyl-$H^3$-tRNA, respectively.

(*b*) Lindahl *et al.*[9] 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* $MgCl_2$, and 0.01 *M* Tris-HCl, pH 8.0.[9] 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 4*a* 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 4*a* and *b*. The mild iodine oxidation of tRNA prevents some tRNA species from accepting amino acids if they contain thiobases.[8] Oxidation of tRNA from Penassay-grown cells prevented peaks 2 and 3 from accepting serine (Fig. 4*a*). 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 4*b*. Both peak 2 and peak 3 are again capable of accepting serine after reduction.

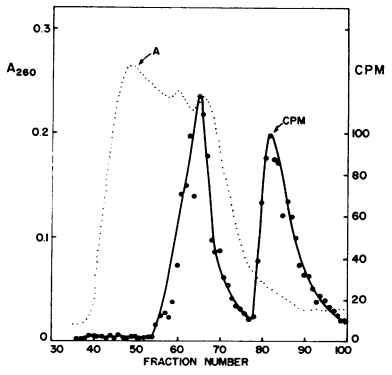


FIG. 3.—MAK column chromatography of seryl-$C^{14}$-tRNA from cells grown in SCM medium. The tRNA was heated in the presence of $Mg^{++}$ before aminoacylation (see *Methods*) and chromatography.

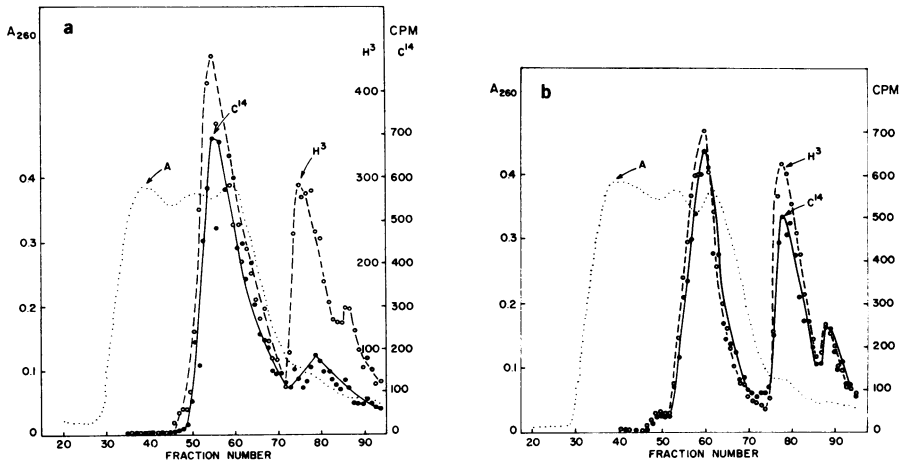


FIG. 4.—MAK-column chromatography of seryl-tRNA. (*a*) The tRNA was oxidized with mild iodine treatment before aminoacylation with $C^{14}$-serine. The unoxidized control was aminoacylated with $H^3$-serine. Both tRNA preparations were heated in the presence of $Mg^{++}$ before the oxidation and aminoacylation steps.

(*b*) The oxidized tRNA was reduced with thiosulfate before aminoacylation with $C^{14}$-serine. The unoxidized control was labeled with $H^3$-serine. Both tRNA preparations were heated in the presence of $Mg^{++}$ before oxidation-reduction treatment or MAK-column 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*[10] 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[11] and ribosomal RNA[12] 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.

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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.

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