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Transcriptional Mapping: Functional Organization of the Ribosomal and Transfer Ribonucleic Acid Cistrons in the *Bacillus subtilis* Genome

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The transcriptional mapping method developed previously has been used to investigate the functional organization of the ribosomal ribonucleic acid (rRNA) and transfer ribonucleic acid (tRNA) cistrons in the *Bacillus subtilis* genome. The sensitivities of the syntheses of these RNA species to actinomycin D indicated that (i) large polycistronic groupings of the tRNA cistrons exist, but (ii) the 16*S* and 23*S* rRNA cistrons are probably transcribed as individual units, and (iii) each 5*S* rRNA cistron is part of an operon containing a 23*S* rRNA cistron.

In recent years it has been possible, through the use of nonclassical genetic mapping techniques (i.e., methods that are not based primarily upon recombination), to map the genes specifying the primary structures of the ribonucleic acid (RNA) components of the translation apparatus in several procaryotic organisms (3, 4, 9, 10). Although these cistrons manifest a strong tendency to be clustered, the studies in question have not—and probably cannot—reveal what functional relationships obtain among the cistrons. For example, do all the cistrons specifying ribosomal RNA (rRNA)—or, transfer RNA (tRNA)—constitute a single large operon? And, if so, what is the ordering of cistrons within such operons? Knowledge of these functional relationships is bound to be of greater general significance than a mere knowing of chromosomal locations of the cistrons in question.

A mapping technique utilizing properties of the transcription process offers considerable promise with regard to elucidating the functional organization of these and other genes (1). It is easy to see that the transcription polymerase molecule is in one sense a measuring device. In attaching to an operator locus and traversing the operon at a (presumably) constant speed, the polymerase measures (i) the length of an operon and (ii) the order of cistrons in the operon (1). By perturbing this process in the proper way(s), these two quantities can be determined.

We already have at our disposal at least one method for perturbing the transcription process in a way that reveals functional organization of genes. The method (1) is based upon the creation of (more or less) random blocks to polymerase travel within the operon by the antibiotic actinomycin D (AMD). Blocking transcription in this way means, first, that the larger a cistron or operon, the more sensitive is its (complete) expression to AMD, and, second, the further away from the operator locus a cistron, the more sensitive to AMD its expression in general becomes (1). Thus, "transcriptional mapping" should provide useful information regarding the functional organization of the (clustered) ribosomal and transfer RNA cistrons in *Bacillus subtilis*.

In the present communication we provide evidence indicating (i) that tRNA cistrons in *B. subtilis* are for the most part clustered into one (or a few) extremely large polycistronic (operon) groups; (ii) that the 16*S* and 23*S* rRNA cistrons appear to exist as "unit operons" (i.e., each cistron is transcribed as an independent unit); and (iii) that the 5*S* rRNA cistron is part of an operon that includes the 23*S* rRNA cistron.

MATERIALS AND METHODS

All of the general materials and methods used have been described in the preceding paper in this series or references cited therein (1). In the present study, however, RNA is analyzed by electrophoresis on higher resolution polyacrylamide gels than used previously. For 16*S* and 23*S* rRNA analysis, a 2.8% acrylamide (0.14% bisacrylamide) gel is used, length 7 cm, diameter 1.0 cm. It is run for 4.5 hr at 10 ma per gel, which necessitates a voltage drop of about 50 v under our conditions. For simultaneous analysis of

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all rRNA and tRNA species, a "split gel" is used. Such a gel has a bottom section of 6% acrylamide (0.3% bisacrylamide) and a top section of 2.8% acrylamide, the gel being 10 to 12 cm in length. The bottom section of the gel is polymerized from a solution containing 20% sucrose in order to assure a flat, uniform interface between the two halves of the gel. Running time, etc., is as just described. All further processing of RNA preparations and gels has been described previously (1).

Methylated albumin (MAK) chromatography of tRNA was performed by the procedure of Yamane and Sueoka (14) modified to a slight extent (M. Kondo, Ph.D. Thesis, University of Illinois, Urbana, 1967).

AMD was the generous gift of the Merck, Sharpe and Dohme Co. It should be noted that the effectiveness of AMD in our experience was a function of both the batch and its history. Therefore, as far as possible, precautions were taken to use the same batch of AMD in any set of experiments (and to do the experiments in a set as close together in time as possible).

RESULTS

By electrophoresis through polyacrylamide gels of the proper composition, it is possible to obtain pulse-labeled rRNA and tRNA profiles in which these RNA species are practically free from messenger RNA (mRNA) of similar (but nonidentical) electrophoretic mobilities (5, 6, Bleyman; *unpublished data*). This then permits an observation of the effect of AMD on the synthesis of rRNA and tRNA with negligible interference from other pulse-labeled species of RNA, RNA fragments, etc.

If the 5*S*, 16*S*, and 23*S*, rRNA and the tRNA were transcribed from single cistrons (i.e., cistrons not parts of larger operon groupings), then one would predict that the AMD sensitivities of their syntheses would be in the same ratios as the corresponding RNA sizes (1). However, various operonal arrangements of these cistrons would lead to different relative sensitivities of their syntheses, depending upon the exact nature of the arrangement. It is easy to see, for example, that if an operon comprised a 23*S* cistron followed by a 16*S* cistron, 16*S* rRNA synthesis could be more sensitive to AMD than would 23*S* rRNA synthesis.

Figures 1–3 show the gel electrophoretic profiles obtained for 5*S*, 16*S*, and 23*S* rRNA and tRNA synthesized in the presence and absence of AMD, at various concentrations of the antibiotic. Figures 1 and 2 show the 16*S*-23*S* and the 5*S*-tRNA regions, respectively, of gels on which a 6-min pulse-labeled RNA preparation was run. Figure 3 shows the results of a similar experiment in which the labeling time was 2 min. These profiles are typical of pulse-labeled RNA from *B. subtilis;* i.e., only the precursor forms of 16*S* and

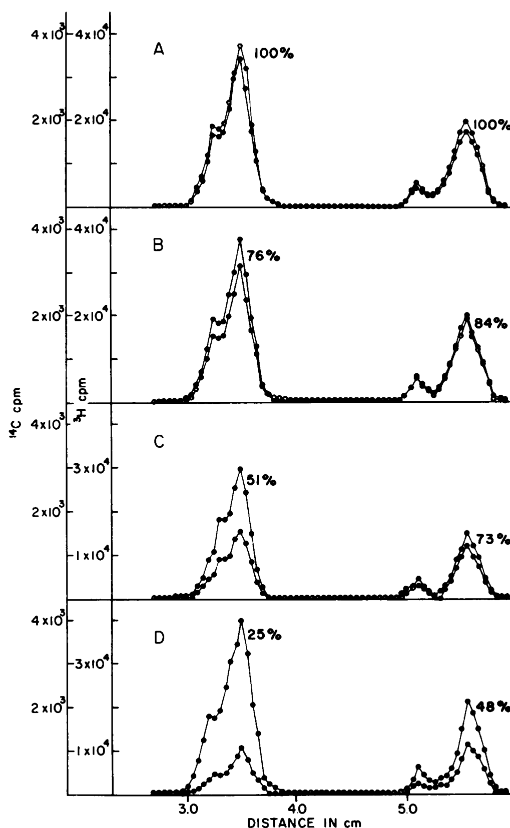

FIG. 1. *Sensitivity of the syntheses of 16S and 23S rRNA to actinomycin D (6-min label). A log-phase culture of B. subtilis was divided into a number of parts. To one part (500 ml), ^{14}C-uridine (final concentration, 0.1 μc/ml) was added and the culture was harvested 2 min thereafter. To each of the remaining parts (100 ml), AMD at the concentrations indicated was added along with ^{3}H-uridine (3.0 μc/ml, final concentration) and the cultures were harvested 6 min thereafter. Predetermined (constant) amounts of ^{14}C-labeled (6-min control) cells were mixed with the cells from each of the ^{3}H-labeled (experimental) cultures, and the resulting mixtures were processed for RNA, which was analyzed by electrophoresis on 2.8% polyacrylamide gels as described in Materials and Methods and previously (1). The figures shows the counts/min of ^{14}C (●), and ^{3}H (○) in each slice of the polyacrylamide gel as a function of position of that slice in the gel, distance being measured from the point of application of the RNA sample. (A) No AMD; (B) AMD, 0.1 μg/ml; (C) AMD, 0.2 μg/ml; (D) AMD, 0.4 μg/ml. The four peaks are p23, m23, p16, and m16 from left to right in that order (5). The profiles shown are from the top portions (2.8%) of "split gels." The numbers next to the 16S and 23S regions indicate the fraction of the control amount of RNA that is synthesized in that general region for that dose of AMD.*

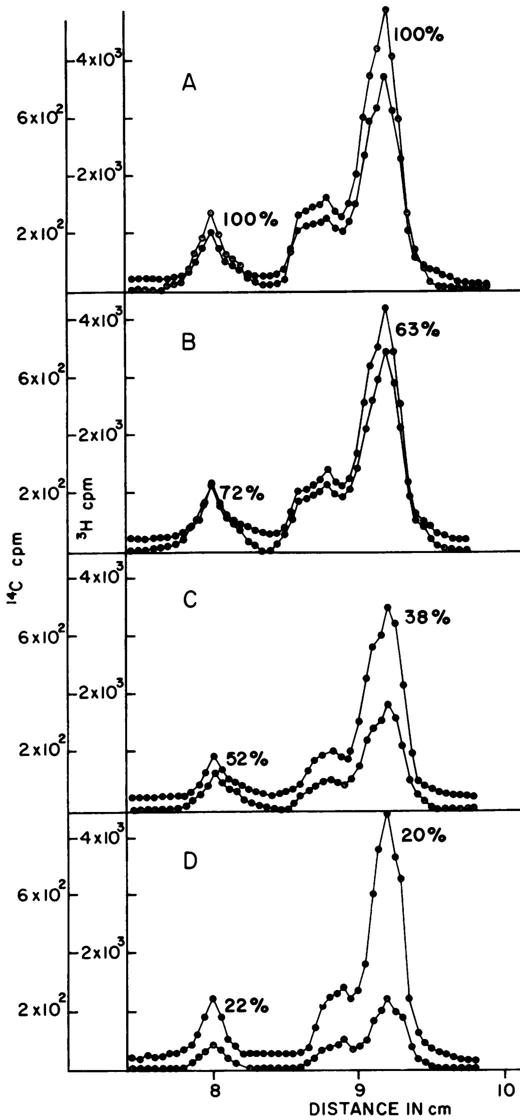

FIG. 2. *Sensitivity of the synthesis of 5S rRNA and tRNA to actinomycin D (6-min label). Same experiment and representation as in Fig. 1. These profiles are from the bottom portions (6% acrylamide) of the same "split gels" represented in Fig. 1. The 5S rRNA peak is to the left, the tRNA peak (a double peak) to the right (6).*

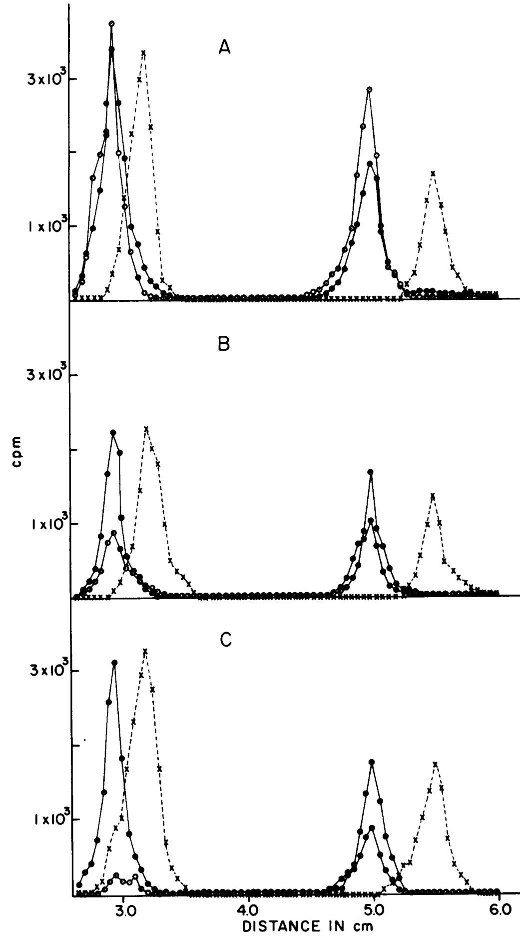

FIG. 3. *Sensitivity of the syntheses of 16S and 23S rRNA to actinomycin D (2-min label). Experiment identical to that described in Fig. 1, except that the labeling time for ^{14}C- (●) and ^{3}H-labeled (○) uridine was 2 min and ^{32}P-labeled (steady-state) cells were included (X). Symbols are as in Fig. 1. (A) AMD, 0.1 µg/ml; (B) AMD, 0.4 µg/ml; (C) AMD, 0.8 µg/ml.*

23*S* rRNA appear when the labeling period is short enough (and negligible amounts of label enter 5*S*- and t-RNA), whereas the more extended labeling periods show the mature forms of 16*S* and 23*S* rRNA, and 5*S*- and t-RNA as well (5, 6). The numbers to the right of the peaks in Fig. 1 and 2 indicate what fraction of the control level is synthesized for each RNA species at each AMD concentration. In Fig. 4, these fractions are shown as a function of AMD concentration, on a semilog plot.

Several interesting points emerge from Fig. 4. (i) 23*S* rRNA synthesis is almost exactly twice as sensitive to AMD as is 16*S* rRNA synthesis. (The 2-min labeling time also yields this twofold difference, and the slopes on a semilog plot for this case are within 10% of those shown in Fig. 4.) (ii) 5*S* rRNA synthesis has a sensitivity to AMD indistinguishable from that characterizing 23*S* rRNA. (iii) tRNA synthesis is surprisingly sensitive to AMD, more so even than is 23*S* rRNA. (iv) While the AMD sensitivity of the rRNA seems approximately "first order"—i.e., a

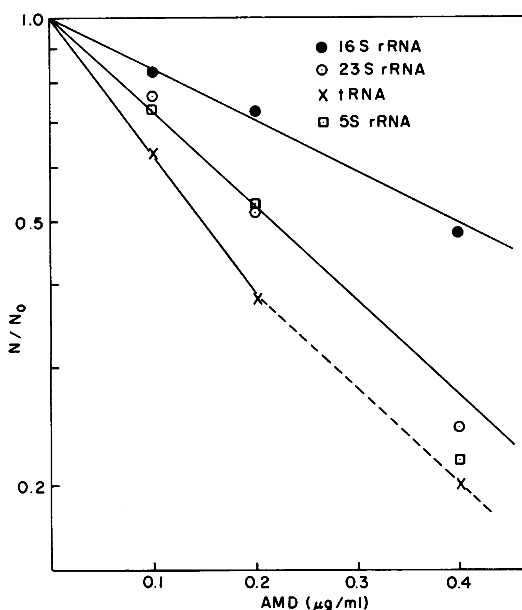

FIG. 4. *Relative sensitivity of the syntheses of rRNA and tRNA to actinomycin D. Fraction of the control level of each RNA species synthesized in the presence of various levels of AMD (6-min labeling time), as a function of AMD concentration in the culture. Ordinate, fractional level of any RNA species; abscissa, AMD concentration in culture. See text and Fig. 1 and 2 captions for details.*

straight line—with AMD concentration, tRNA sensitivity manifests a pronounced "tailing off." (We feel that this "tailing off" of tRNA sensitivity is real in that it was observed in all experiments and occurred in the very same gels that gave no such effect for the 5*S* rRNA.) The significance of these points will be discussed below.

One also wants to know the approximate sensitivities of 16*S* and 23*S* rRNA syntheses relative to those of mRNA molecules of comparable size. Figure 5 shows the results of experiments exactly like those described in Fig. 1–3, except that the electrophoretic analysis was carried out on 2.4% polyacrylamide gels—gels giving less resolution, and so permitting one to detect appreciable levels of the pulse-labeled mRNA species, which is not possible with the 2.8% gels (1). It appears (Fig. 5) that 23*S* and 16*S* rRNA syntheses have about the same sensitivity to AMD as do the syntheses of mRNA species comparable in size; any slight discrepancies that may exist will be discussed below.

The extreme sensitivity to AMD manifested by tRNA synthesis would be expected were tRNA cistrons grouped into one (or more) large operons. Another prediction of such an operonal arrangement is that the synthesis of some tRNA species is far less sensitive to AMD than is that of other tRNA species. As stated above, the nearer to the operator locus a cistron is located, the less

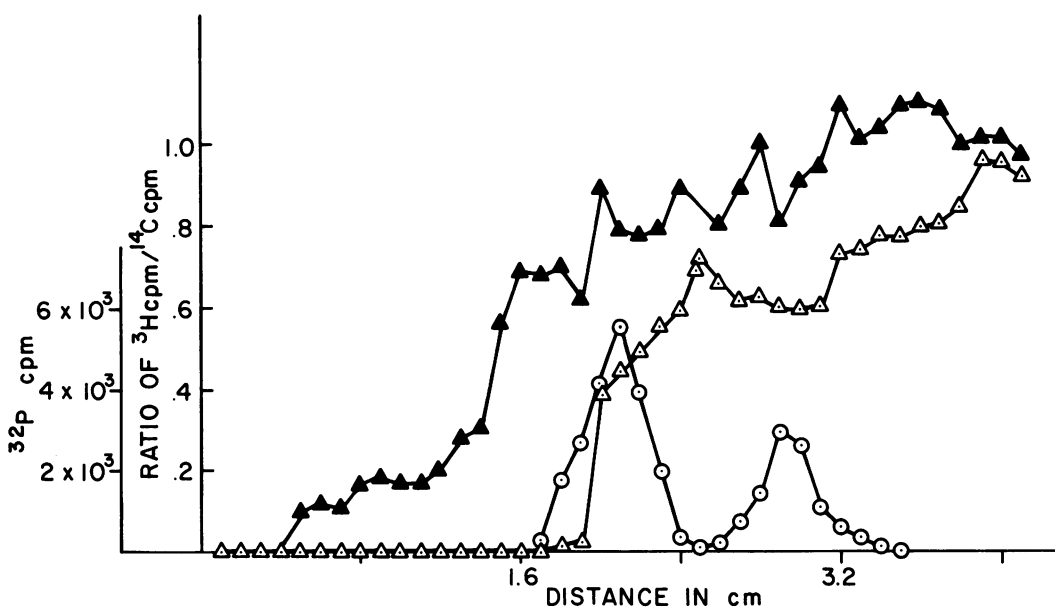

FIG. 5. *Relative AMD sensitivities of the syntheses of 16S rRNA, or 23S rRNA and general pulse-labeled RNA. Experimental protocol identical with that described in Fig. 3, except that the RNA was analyzed on a 2.4% polyacrylamide gel, run for 1.5 hr at 10 ma (50 v). For ^{14}C (2-min control) and ^{3}H (2-min experimental), the $^{3}H/^{14}C$ ratio is plotted instead of the individual ^{3}H and ^{14}C counts/min.* (○) *^{32}P, counts/min;* (▲) *^{3}H to ^{14}C ratio for AMD at 0.1 μg/ml;* (△) *^{3}H to ^{14}C ratio for AMD at 0.2 μg/ml.*

sensitive to AMD its expression becomes (provided that transcription of the complete operon is not a necessary condition for expression of the cistron in question). This sort of effect would produce a "tailing off" in the sensitivity of tRNA synthesis as a function of AMD level. And the data of Fig. 4 are consistent with this prediction, although no "tailing off" is indicated for 16*S*, 23*S*, and 5*S* rRNA from the very same experiment.

While polyacrylamide gel electrophoretic analysis is an excellent method for determining the AMD sensitivity of the synthesis of total tRNA, it is clearly of no use in distinguishing among AMD sensitivities of the syntheses of individual tRNA species. For this purpose, chromatography on MAK columns can be used, though the separations obtained here are far from spectacular. Figure 6 illustrates the separation among tRNA species that can be obtained by use of the MAK column. Figure 7 presents MAK chromatography of tRNA preparations that have been labeled for 6 min in the presence of AMD. The ordinate scales have been adjusted to facilitate comparison of the shapes of the profiles for various AMD levels. The systematic changes in the shape of the profile in Fig. 7 with AMD dose suggests that the AMD sensitivities of the syntheses of all tRNA species are not the same. A comparison of the complete tRNA charging profile data with Fig. 7 indicates that the most resistant tRNA (*see* region I of Fig. 7) is from a group comprising $tRNA_{gly}$, $tRNA_{glu}$, and $tRNA_{ilu}$ (Kondo, Ph.D. Thesis, University of Illinois, 1967). These particular studies are at present being extended in order to quantitate the relative AMD sensitivities for individual tRNA species.

DISCUSSION

A method of "transcriptional mapping" based upon AMD blocking of transcription has been used to reveal the functional organization of the tRNA and the rRNA cistrons in the *B. subtilis* genome. Consider the tRNA cistrons first. Data suggestive of tRNA operons, but not proving the case, have existed for some time now. Incorporation of labeled precursors (e.g., uridine) into tRNA manifests a lag under conditions in which no appreciable lag is seen for entrance of label into total tRNA or mRNA (7). And, many if not all of the tRNA (and rRNA) cistrons in *B. subtilis* (and seemingly in *Escherichia coli* too) map in a restricted region of the genome (3, 4, 9, 10). Neither line of evidence proves polycistronic organization, as we have seen above.

The existence of one or a few enormous tRNA operons would require that the synthesis of tRNA in general be very sensitive to AMD, but that within the class of tRNA species a spectrum of sensitivities could exist, the sensitivitiy being a function of the distance between any particular tRNA's cistron and the operator locus of the operon, so that the more distal cistrons are the more sensitive ones. (This second point is predicated upon the assumption that a blocked, and therefore incomplete, transcription of a large tRNA operon can nevertheless be processed by the cell to yield those tRNA species whose cistrons are operator-proximal to the AMD block.) The above results do indeed show the synthesis of tRNA in general to be extremely sensitive to AMD, exceeding even that of 23*S* rRNA! Further, the data of Fig. 7 indicate that not all tRNA syntheses manifest the same AMD sensitivity.

It is instructive to compare the "AMD inactivation kinetics" of a single cistron (i.e., a unit operon) versus a polycistronic operon—taking the simplest model for AMD action, namely, a "target theory" picture of how it works. In this case we should expect a unit operon to be "inactivated" with first order "hit kinetics"

$$(N/N_0) = e^{-sD} \qquad (1)$$

where s and D, respectively, are the AMD "sensitivity parameter" and the "dose" of AMD (in appropriate units). However, AMD inactivation of a polycistronic operon would produce a different sort of inactivation curve. The mathematical description in this case would be something of the sort:

$$(N/N_0) = e^{-sD} [1 + sD/2! + \overline{sD}^2/3! + \cdots + \overline{sD}^n/(n + 1)!] \qquad (2)$$

where the additional terms take into account the fact that a partial transcription of the operon still leads to production of some functional products (Welton, Bleyman, and Woese, *in preparation*). Although the AMD inactivation of 16*S* and 23*S* rRNA syntheses seems to be approximated by equation 1, the inactivation of tRNA synthesis, which manifests a recognizable "tailing off" in Fig. 4, is approximately what would be expected on the basis of equation 2. From equation 2 and from the 16*S* and 23*S* rRNA sensitivities, one can calculate that the postulated polycistronic tRNA precursor should be at least twice the size of the 23*S* rRNA molecule, and so of the order of 100 tRNA cistron-equivalents in length.

Turning now to the rRNA species, we know from the fact that multiple cistrons for 16*S* and 23*S* rRNA exist in bacteria and from previous mapping studies indicating that 5*S*, 16*S*, and 23*S* cistrons in *B. subtilis* are localized in a small (actually two small) region(s) of the genome, that a number of polycistronic operonal organizations

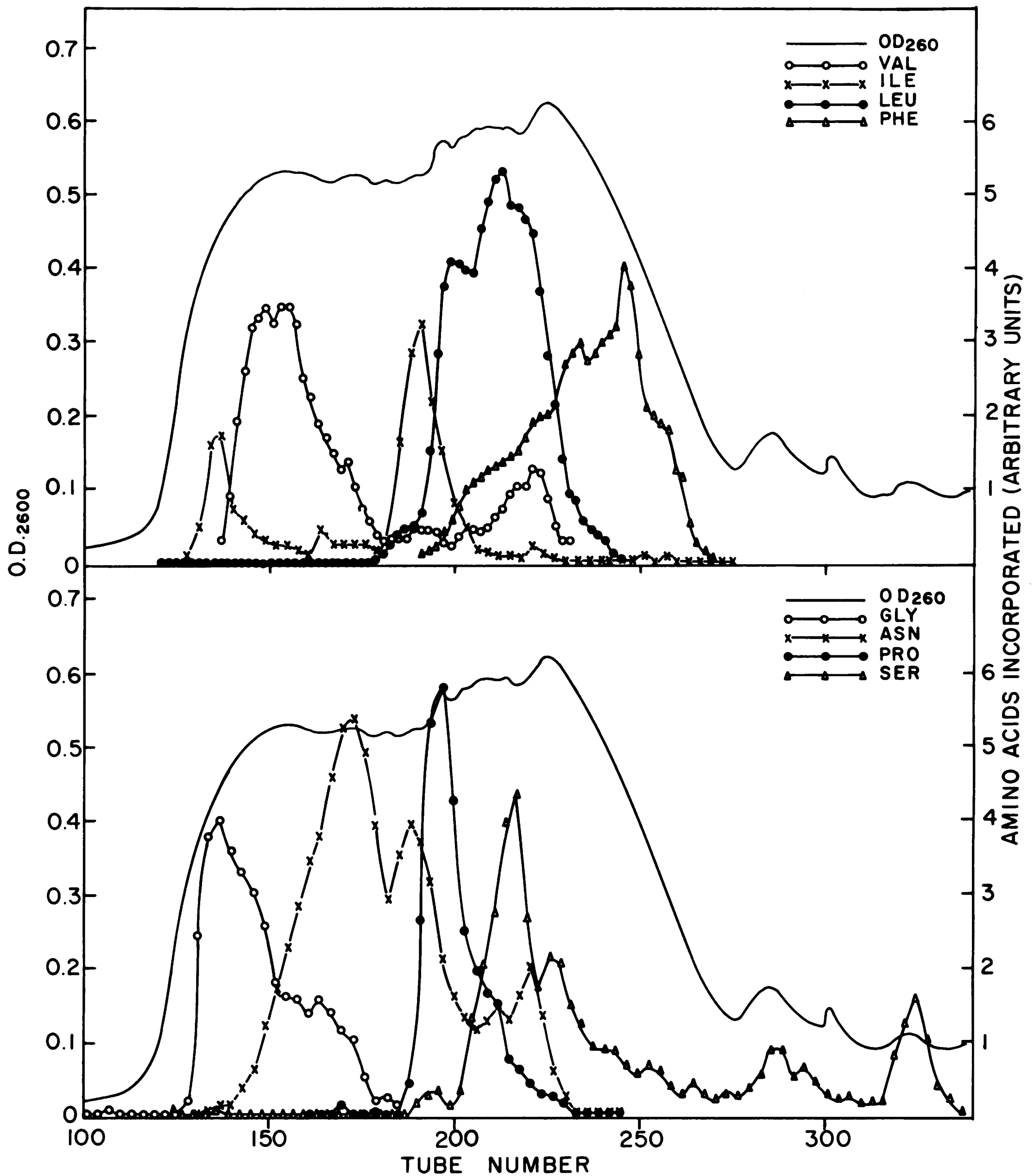

FIG. 6. *Separation of B. subtilis transfer RNA species by methylated albumin column chromatography. tRNA prepared from B. subtilis was fractionated on a methylated albumin one-layer column (14). Elution was accomplished by a linear saline gradient from 0.4 to 0.8 M in 0.05 M sodium phosphate buffer (pH 6.7). The RNA was eluted in a total volume of 1,100 ml, in 3-ml fractions. Portions of each tRNA fraction from the column were then charged separately with each of 19 amino acids, by the procedure of Yamane and Sueoka (14). Charging profiles for a number of amino acids are shown, with amino acid incorporation expressed in arbitrary units in each case. The total optical density profile (260 nm) for the column is also shown.*

are conceivable (1, 3, 4, 9, 10, 13). Table 1 depicts the simpler possibilities for the arrangement of 16*S* and 23*S* cistrons. On the basis of the above data, one can immediately eliminate models *c*, *f*, and *h*, all of which would make synthesis of 16*S* rRNA more sensitive to AMD than that of 23*S* rRNA. In any case, all large operon models seem to be ruled out on the basis that the 16*S* and 23*S* rRNA synthesis is not appreciably more sensitive to AMD than the syntheses of mRNA species of

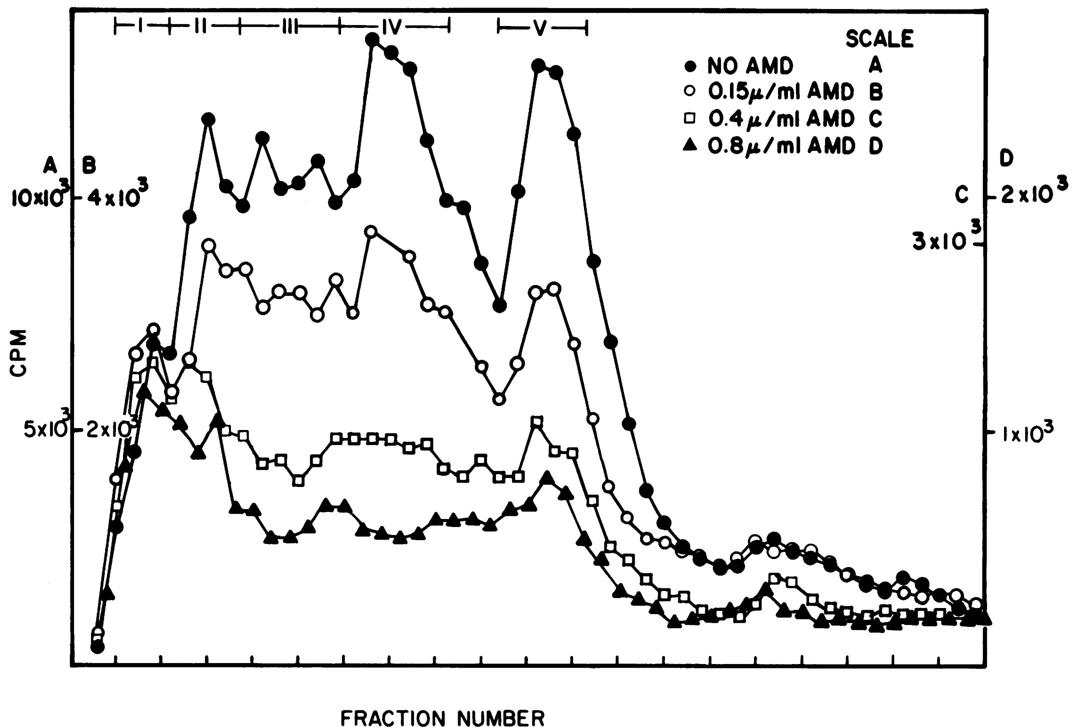

FIG. 7. *Relative sensitivities of the syntheses of various tRNA species to AMD. A 400-ml log-phase culture of B. subtilis carrying a long-term ^{3}H-uridine label was divided into four parts, each of which was treated with AMD at the final concentrations shown, and to each of which was added ^{14}C-uridine (final concentration, 0.3 μc/ml) 30 sec after the AMD. Incorporation was then allowed to continue for a 6-min period, before the cultures were harvested. Total tRNA was extracted by the method of Yamane and Sueoka (14) and chromatographed on a MAK column, as described in Fig. 6. The radioactivity in the tRNA region (trichloracetic acid-insoluble) was determined by the method of Britten et al. (2). All four tRNA preparations were chromatographed on the same column, the column being washed between successive runs with a large volume of the starting buffer. The elution profiles for the long-term label were essentially identical in all four cases, and thus are not shown. The four ordinate scales (A, B, C, and D) apply to the four different AMD levels in increasing order of concentration, respectively.*

comparable sizes (Fig. 5). Polycistronic organization of these cistrons would, of course, have demanded that 23*S* and 16*S* rRNA syntheses be far more sensitive to AMD than synthesis of mRNA species of comparable sizes (which is true for the case of tRNA).

As the reader may have noticed, the data of Fig. 5 indicate the 23*S* and 16*S* rRNA syntheses to be perhaps a bit more sensitive to AMD than are the syntheses of mRNA species of comparable size. The explanation for this, however, we feel has nothing to do with an operonal organization for 23*S* or 16*S* cistrons, but rather to do with either or both of the following. (i) In any given region of the RNA gel profile for AMD-treated cells—excluding for the moment the 16*S* and 23*S* rRNA regions—one finds appreciable quantities of two types of RNA, one, the normal mRNA of that size range, the other, RNA species of that size produced from larger operons, that contain AMD blocks, so that partial transcription of the operon only occurs. These abnormal RNA species in a sense tend to compensate for (to mask) the inactivation by AMD of the synthesis of normal mRNA species in their size range. In the 23*S* or 16*S* regions of a gel, however, so much rRNA is produced that these abnormal partial transcriptions of 23*S* or 16*S* size contribute an inappreciable increment to the already large amount of RNA, and so would not tend to mask the true extent of AMD inactivation of rRNA synthesis—which effect in turn would produce a slight dip in the experiment control ratios of Fig. 5 at that point. Alternatively, (ii) the fact that AMD attaches to DNA in relation to the guanine residues and rRNA and tRNA genes must have $\geq 53\%$ guanine plus cytosine in *B. subtilis*, may contribute to a slightly greater sensitivity to AMD of these genes (1, 8).

Other characteristics one might expect were

TABLE 1. *Possible (simple) operonal arrangements of the rRNA cistrons*

a – individual cistrons (unit operons) 16, 16, 23, 23 etc.
b – multiple operons: one 16*S* preceding one 23*S* in each 16-23, 16-23, 16-23, etc.
c – multiple operons: one 23*S* preceding one 16*S* in each 23-16, 23-16, 23-16, etc.
d – two operons: 16*S* all separate from 23*S* 16-16-16-16-16 23-23-23-23-23
e – one operon: alternating 16-23, with 16*S* first 16-23-16-23-16-23-16-23-16-23
f – one operon: alternating 16-23 with 23*S* first 23-16-23-16-23-16-23-16-23-16
g – one operon: all 16*S* before any 23*S* 16-16-16-16-16-23-23-23-23-23
h – one operon: all 23*S* before any 16*S* 23-23-23-23-23-16-16-16-16-16

rRNA cistrons arranged into large operons; characteristics that are not observed are: a "tailing off" of the AMD inactivation curve, such as is seen for the tRNA case, and a lag in incorporation of label into 16*S* and 23*S* rRNA (5, 6).

This then leaves us with models *a* (unit operons) and *b* (two cistron operons, 16*S* before 23*S*) as the only probable ones. Model *b* would require the AMD sensitivity of 23*S* rRNA synthesis to be three times greater than that of 16*S* rRNA, whereas a twofold difference (that demanded by model *a*) is what is observed. This difference we consider to render model *b* unlikely though not impossible.

It is interesting to note that in a eucaryotic system the synthesis of the 45*S* rRNA precursor is more sensitive to AMD than is the synthesis of mRNA species of comparable size (11). In this case, then, it does appear that polycistronic organization of the rRNA cistrons exists, a conclusion also suggested by the work of Quagliarotti and Ritossa in *Drosophila* (12).

Turning finally to 5*S* rRNA, we again encounter an AMD sensitivity that is not in keeping with the size of the molecule in question. However, this is not unexpected. Hecht et al. (6) have shown that 5*S* rRNA is not a primary transcription product; that is, 5*S* rRNA must be derived (as the mature forms of 16*S* and 23*S* rRNA are) from some macromolecular precursor. Although the precursor forms of the 16*S* and 23*S* molecules have been identified, no clearly recognizable precursor for the 5*S* rRNA has been located to date. For this reason and others, it has been postulated that the precursor form of 23*S* rRNA splits to yield both mature 23*S* rRNA and 5*S* rRNA (6). The present finding that the AMD sensitivity of the synthesis of 5*S* rRNA is indistinguishable from that of the 23*S* rRNA is most encouraging in this respect.

Of course, all that the transcriptional mapping approach can claim in this instance is that the 5*S* cistron should be a part of a larger operon the same size as the 23*S* rRNA cistron. However, if the actual 23*S* cistron were not involved here, one would expect pulse-labeled RNA preparations to contain large amounts of an (unstable) RNA species of about 23*S* size that is not the true 23*S* rRNA. The demonstration that essentially all of the 23*S* rRNA labeled in a short pulse is converted to mature 23*S* rRNA disproves the existence of any other 23*S*-like RNA species present in the cell in detectable amounts (Bleyman, Ph.D. Thesis, University of Illinois, 1969). Therefore, the AMD sensitivity of 5*S* rRNA is an additional indication that this RNA species is closely associated with the 23*S* rRNA. (It might be added here that were all of the 5*S* cistrons thought to be present in the *B. subtilis* genome grouped into one large operon, this operon would still be much smaller than a single 16*S* rRNA cistron, and so the AMD sensitivity manifested by such an operon should be far less than observed (13).)

We shall not consider in detail here which end of the proposed 23*S*-5*S* operon contains the 5*S* cistron. It is likely that complete transcription of such an operon is a necessary condition for the production of 5*S* rRNA (6). Therefore, even were the 5*S* portion located at the operator-proximal extreme, its expression would still be as sensitive to AMD as that of the 23*S* portion.

It should be noted that molecular hybridization studies appear to show that the *B. subtilis* genome contains fewer 5*S* rRNA cistrons than 23*S* rRNA cistrons (13). Although this is incompatible with the above-proposed 5*S*-23*S* relationship, we do not, as stated previously (6), take this as compelling evidence against our hypothesis, in that molecular hybridization methods tend occasionally to be quantitatively imprecise.

In conclusion, we feel a strong case can be made (i) for the grouping of most tRNA cistrons in *B. subtilis* into one (or a few) large polycistronic operon(s) (of the order of 100 cistrons in length), but (ii) for the lack of any such polycistronic organization in the case of the rRNA cistrons, with (iii) the exception that each 5*S* rRNA cistron may be located in the same operon

with (actually may be a part of) a 23*S* rRNA cistron.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Bleyman, M., and C. R. Woese. 1969. Transcriptional mapping: introduction to the method and the use of actinomycin D as a transcriptional mapping agent. Proc. Nat. Acad. Sci. U.S.A. 63: *in press*.
2. Britten, R. J., R. B. Roberts, and E. F. French. 1955. Amino acid incorporation in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 41:863–870.
3. Cutler, R. G., and J. E. Evans. 1967. Relative transcription activity of different segments of the genome throughout the cell division cycle of *Escherichia coli*. The mapping of ribosomal and transfer RNA and the determination of the direction of replication. J. Mol. Biol. 26:91–106.
4. Dubnau, D., I. Smith, and J. Marmur. 1965. Gene conservation in *Bacillus* species. II. The location of genes concerned with the synthesis of ribosomal components and soluble RNA. Proc. Nat. Acad. Sci. U.S.A. 54:724–731.
5. Hecht, N. B., and C. R. Woese. 1968. Separation of bacterial ribosomal ribonucleic acid from its macromolecular precursors by polyacrylamide gel electrophoresis. J. Bacteriol. 95:986–990.
6. Hecht, N. B., M. Bleyman, and C. R. Woese. 1968. The formation of 5S ribosomal ribonucleic acid in *Bacillus subtilis* by posttranscriptional modification. Proc. Nat. Acad. Sci. U.S.A. 59:1278–1283.
7. Midgley, J. E. M., and B. J. McCarthy. 1962. The synthesis and kinetic behavior of deoxyribonucleic acid-like ribonucleic acid in bacteria. Biochim. Biophys. Acta 61:696–717.
8. Müller, W., and D. M. Crothers. 1968. Studies on the binding of actinomycin and related compounds to DNA. J. Mol. Biol. 35:251–290.
9. Oishi, M., and N. Sueoka. 1965. Location of genetic loci of ribosomal RNA on *Bacillus subtilis* chromosome. Proc. Nat. Acad. Sci. U.S.A. 54:483–490.
10. Oishi, M., A. Oishi, and N. Sueoka. 1966. Location of genetic loci of soluble RNA on *Bacillus subtilis* chromosome. Proc. Nat. Acad. Sci. U.S.A. 55:1095–1101.
11. Penman, S., C. Vesco, and M. Penman. 1968. Localization and kinetics of formation of nuclear heterodisperse RNA and polyribosome associated messenger RNA in HeLa cells. J. Mol. Biol. 34:49–70.
12. Quagliarotti, G., and F. M. Ritossa. 1968. On the arrangement of genes for 28S and 18S ribosomal RNAs in *Drosophila melanogaster*. J. Mol. Biol. 36:57–69.
13. Smith, I., D. Dubnau, P. Morrell, and J. Marmur. 1968. Chromosomal location of DNA complementary to transfer RNA, and to 5S, 16S, and 23S ribosomal RNA in *Bacillus subtilis*. J. Mol. Biol. 33:123–140.
14. Yamane, T., and N. Sueoka. 1963. Conservation of specificity between amino acid acceptor RNA and amino acyl-sRNA synthetase. Proc. Nat. Acad. Sci. U.S.A. 50:1093–1100.