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Identification of Bacteriophage T4-Specific Precursor tRNA by Using a Host Mutant Defective in the Methylation of tRNA

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A mutant of *Escherichia coli* K-12 that is defective in the synthesis of 5-methyluridine (ribothymidine) in tRNA was used to identify precursors to phage T4-specific tRNA. The precursor molecules, isolated by gel filtration, were more than twice the size of tRNA. This method is suitable for isolation of rather large amounts of such precursor molecules.

Maturation of bacterial and phage-specific tRNA's involves both cleavage of larger precursor molecules and modification of certain nucleosides. Multimeric tRNA precursor molecules have been recognized by the fact that they contained the primary sequence of mature tRNA's (1-4, 12). This paper presents an alternative method of identifying precursor tRNA molecules by utilizing a tRNA modificationless mutant. Bacterial mutant *trmA*, which lacks 5-methyluridine (ribothymidine, m^5U), has a normal level of that nucleoside in rRNA. Another mutant, *rrmA*, which lacks 1-methylguanosine (m^1G) in rRNA, has a normal level of m^1G in tRNA (7). Thus, mutants lacking a modified nucleoside in one kind of RNA are useful tools for recognizing the corresponding precursor molecules, provided that the modified nucleoside normally is present in the precursor molecule. We have utilized this specificity of the bacterial RNA methyltransferase to identify a T4-specific tRNA precursor molecule(s). Furthermore, our isolation method is suitable for purification of rather large amounts of multimeric tRNA molecules. Some of these results have been discussed briefly before (5, 6).

Escherichia coli strains AB1932-5-466 (*metA*, *trmA*$^+$) and AB1932-5-451 (*metA*, *trmA5*) were infected with phage T4 Bc$^+$. Cells were labeled with L-[*methyl*-^{3}H]methionine between 2 and 12 min after phage infection. Figure 1a shows a Sephadex G200 chromatogram of total RNA originating from the *trmA*$^+$ strain infected with phage T4. Labeled material appeared in three different regions. Radioactivity in rRNA probably originated from uninfected cells and from infected cells containing unmaturated rRNA when phage was added. In the tRNA region, more than 90% of the label is T4-specific tRNA (8). Labeled material also eluted just after the rRNA (called "unknown"). The same chromatographic pattern, including the presence of the unknown methyl-labeled material, was also obtained with total RNA from a T4-infected *trmA5* strain. The unknown radioactive material from both strains was alkaline labile, RNase sensitive, and DNase resistant. Thus, this radioactive material is RNA.

The distribution of methylated components in the three different kinds of RNA is given in Table 1. rRNA, tRNA, and unknown RNA from *trmA*$^+$ cells contained m^5Up. In phage-infected *trmA5* cells, m^5Up was present in rRNA but absent in unknown RNA and tRNA. These results, together with the fact that the tRNA (m^5U)-methyltransferase (EC 2.1.1.35) only modifies tRNA, strongly imply that the unknown RNA is related to tRNA. The position of the unknown RNA in the G200 gel chromatogram indicates that it is larger than tRNA. If the unknown RNA is a precursor to tRNA, it should be labile and disappear during a chase with unlabeled methionine. Figure 1a shows that the unknown RNA was present after a 10-min labeling period but disappeared when the labeling was followed by a chase for 20 min in the presence of cold methionine (Fig. 1b). Unknown RNA was not observed when uninfected *E. coli* cells were labeled under the same conditions as for phage T4 infection (Fig. 1a). Taken together, these results show that the unknown methylated material is a precursor molecule(s) to T4-specific tRNA.

To characterize the precursor tRNA with respect to chain length, we compared the chromatographic behavior of the ^{3}H-methylated precursor tRNA with [^{32}P]RNA components of known chain length (4, 13). Our ^{3}H-methylated precursor tRNA eluted only 5 ml before component A (chain length about 175 nucleotides) (Fig. 2). Cochromatography of the methylated precursor tRNA and component A on 10% polyacrylamide gels suggested that both had the same chain length (data not shown). Thus,

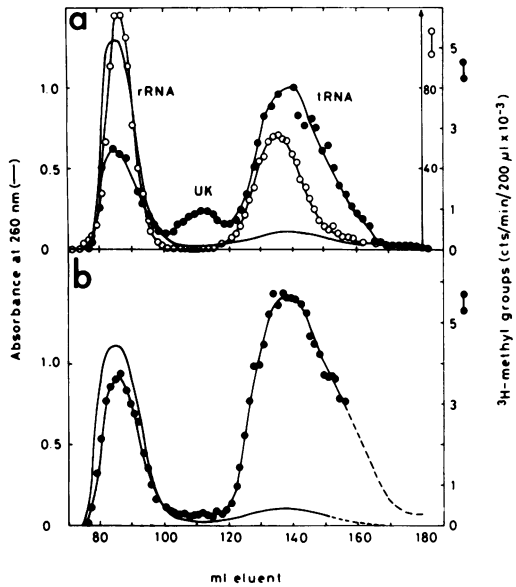

FIG. 1. *Sephadex G200 molecular sieve chromatography of ^{3}H-methylated RNA from uninfected and phage-T4 infected cells. Strain AB1932-5-466 (argH$^+$, trmA$^+$, metA) was grown in glucose-salt medium (11) supplemented with* L*-methionine (6.6 µg/ml), adenine (30 µg/ml), and thiamine (1 µg/ml). The methionine concentration was chosen to support growth to an optical density (420 nm) of 2.6. At an optical density (420 nm) of 2.0, dialyzed phage T4Bc$^+$ was added at a multiplicity of 6.5, and 2 min later 91 µCi of* L*-[methyl-^{3}H]methionine per ml (5.2 Ci/mmol) was added. After 10 min of labeling, the culture was poured on ice and cells were collected. Total RNA was prepared as described previously (8), and applied to a Sephadex G200 column (1.9 by 99 cm; total volume 252 ml). This procedure separates rRNA and tRNA (9). Radioactivity (a, ●) was measured in different fractions. Fractions eluting at about 85, 115, and 140 ml contain rRNA, unknown RNA (UK), and tRNA, respectively. Part of the culture was chased for 20 min in the presence of 1.6 mg of cold* L*-methionine per ml (b, ●). In an experiment where phage T4 was omitted, the labeling was performed for 10 min at the same cell density as before (a, ○).*

the methylated precursor tRNA is more than twice the chain length of mature tRNA.

The distribution of methylated compounds in the precursor tRNA was quite different from the one in mature tRNA (cf. Table 1). The dimeric precursor tRNA's isolated by McClain and co-workers (4, 12) contained 2 mol of m^{5}Up (4, 12). Since the precursor tRNA isolated by us was at least twice the size of tRNA, we used m^{5}Up as an internal standard and assumed that each precursor molecule contains two m^{5}Up. Table 2 shows that the levels of m^{1}Gua and m^{7}Gua, expressed as mole/mole, were higher in the precursor tRNA than in the mature tRNA. The level of m^{1}Gua was about 10 times higher than in the average tRNA population. Since rRNA is known to contain m^{1}G, degradation of rRNA might influence the level of m^{1}G in the

TABLE 1. *Distribution of methylated compounds in different RNA fractions labeled in vivo with* L*-[methyl-^{3}H]methionine between 2 and 12 min after T4 infection*[a]

Methylated compound	AB1932-5-466 (*trmA*$^+$)			AB1932-5-451 (*trmA5*)		
	rRNA	UK	tRNA	rRNA	UK	tRNA
m^{6}Ade	1.1[b]	1.0	1.8	1.6	1.4	1.6
m^{2}Ade	0.2	0.7	8.2	0.2	0.6	8.1
m^{7}Gua	2.1	51	36	1.5	68	38
m^{1}Gua	0.3	50	12	0.4	57	11
mUp	6.2	92	244	4.9	1.6	3.1

[a] In these experiments, strains AB1932-5-466 (*argH*$^+$, *trmA*$^+$, *metA*) and AB1932-5-451 (*argH*$^+$, *trmA5*, *metA*) differ only in the allelic state of *trmA*. They were constructed by P1 transduction, using strain IB5 (*argH*$^+$, *trmA5*) (7) as donor and strain AB1932 (*argH*, *trmA*$^+$, *metA*) as recipient and selecting for *argH*$^+$ transductants. Transduction frequency between *argH*$^+$ and *trmA5* is about 65% (Björk, unpublished data). The distribution in vivo of the methylated bases in the tRNA and rRNA of these two strains has been determined, and the only difference observed was the complete lack of m^{5}U in tRNA of the *trmA5* derivative (Björk and Neidhardt, unpublished data). The strains were grown and labeled, and the different RNA species were separated as described in the legend to Fig. 1. Pooled fractions (rRNA, unknown RNA [UK], and tRNA [cf. Fig. 1]) were concentrated and hydrolyzed for 30 min at 100 C in 1 M HCl. Portions were subjected to two-dimensional thin-layer chromatography as described previously (8). Part of such an analysis is shown in the Table. Methylated uridylic acid (mUp) migrates just ahead of uridylic acid. Probably all of mUp is m^{5}Up, since only m^{5}Ura and no m^{3}Ura was found in tRNA hydrolyzed by $HClO_4$. The values obtained with strain AB1932-5-466 (*trmA*$^+$) as host are the average of two independent experiments. Between 5,000 and 11,000 counts/min was applied to each chromatogram, and the recovery was 78 to 100%. Values within boxes are regarded as significantly different from the corresponding value in the wild-type strain (AB1932-5-466).

[b] Picomoles per 100 µg of RNA.

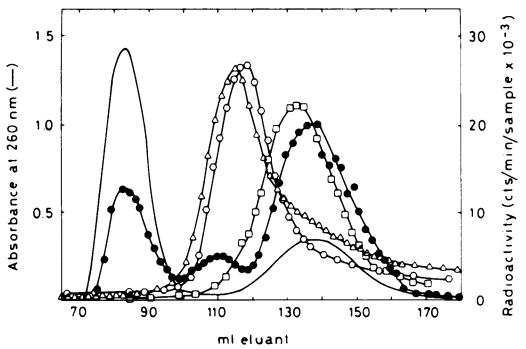

FIG. 2. *Sephadex G200 molecular sieve chromatography of ^{3}H-methylated RNA from phage T4-infected cells and ^{32}P-labeled components A, C, and 2. Unlabeled total RNA from strain AB1932 (internal control) was mixed with ^{32}P-labeled component A (Δ, counts/minute per milliliter, dimeric precursor to tRNA^{Pro} and tRNA^{Ser}, 175 nucleotides in chain length [4]), with component C (○, counts/minute per 0.2 ml, 140 nucleotide chain length [13]), and with component 2 (□, counts/minute per 0.5 ml of tRNA^{Leu}, 85 nucleotides in chain length [13]). Samples were chromatographed separately on the same column as used in the experiment in Fig. 1 and with the same flow rates and fraction volumes. The elution volumes for the two internal controls, E. coli rRNA and tRNA, were exactly the same for all three samples and were identical to the elution volumes obtained for E. coli rRNA and tRNA from ^{3}H-methylated T4-infected cells (●, counts/minute per 0.2 ml).*

TABLE 2. *Calculated levels of methylated compounds in precursor tRNA after T4 infection in rrmA$^+$ and rrmA10 hosts*[a]

Methylated compound	precursor tRNA		mature tRNA	
	rrmA$^+$-host	*rrmA10*-host	*rrmA$^+$*-host	*rrmA10*-host
m^{7}Gua	1.1[b]	0.9	0.15	0.12
m^{1}Gua	1.1	1.3	0.05	0.06
m^{5}Up	2.0	2.0	1.0	1.0

[a] Data from Table 1 (*rrmA$^+$*, host), calculated with m^{5}Up as an internal standard, using the value 2.0 for the precursor tRNA as described in the text and 1.0 for mature tRNA as suggested from the results obtained by Björk and Neidhardt (8). The values obtained with the *rrmA10* host (i.e., a host strain unable to make m^{1}G in its rRNA) are averaged from two independent experiments.

[b] Moles per mole of RNA.

precursor tRNA region (7, 10). This was ruled out by analyzing the level of m^{1}Gua in a T4-specific precursor tRNA isolated in a bacterial host unable to synthesize m^{1}G in its rRNA (Table 2). If we assume that both tRNA counterparts of a dimer contain m^{5}U but only one of them contains m^{1}Gua and m^{7}Gua, the expected level (expressed as mole/mole) of these methylated bases should be 2:1:1, respectively. The data in Table 2 suggest that such a relation holds for precursor tRNA, which also agrees with the ratios in component A, the precursor to tRNA^{Pro} and tRNA^{Ser} (4). One explanation of our results is that the precursor tRNA fraction was enriched with a certain kind of T4 tRNA precursor molecules.

We have shown earlier that phage T4 uses host tRNA (m^{5}U)-methyltransferase to synthesize m^{5}U in its specific tRNA (8). This, together with the results presented here, indicates that this host-modifying enzyme is able to work on a large T4 precursor tRNA molecule. However, lack of this modification does not influence the processing of the precursor tRNA molecules since precursor molecules, as well as mature tRNA, were also found in a *trmA5* strain. Our isolation procedure for precursor tRNA molecules allows the purification of larger quantities of different types of precursors than methods used by others (1, 3, 4, 12). Such bacterial precursors might be used to establish the substrate requirements for the bacterial tRNA methyltransferases and to elucidate a possible sequential maturation of tRNA molecules.

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LITERATURE CITED

1. Altman, S. 1971. Isolation of tyrosine tRNA precursor molecules. Nature (London) New Biol. **229**:19-21.
2. Altman, S. 1975. Biosynthesis of transfer RNA in *Escherichia coli*. Cell **4**:21-29.
3. Altman, S., and J. D. Smith. 1971. Tyrosine tRNA precursor molecule polynucleotide sequence. Nature (London) New Biol. **233**:35-39.
4. Barrel, B. G., J. G. Seidman, C. Guthrie, and W. H. McClain. 1974. Transfer RNA biosynthesis: the nucleotide sequence of a precusor to serine and proline transfer RNAs. Proc. Natl. Acad. Sci. U.S.A. **71**:413-416.
5. Björk, G. R. 1973. Methylation of phage T4 specific tRNA and its precursor, p. 176. 9th International Congress of Biochemistry, 1 to 7 July 1973. Royal Academy of Science, Stockholm.
6. Björk, G. R. 1974. Bacterial RNA methylase mutants: tools in studying the biosynthesis of phage T4tRNA, p. 181. 9th FEBS Meeting, August 1974. Hungarian Biochemical Society, Budapest.
7. Björk, G. R., and L. A. Isaksson. 1970. Isolation of mutants of *Escherichia coli* lacking 5-methyluracil in transfer ribonucleic acid or 1-methylguanine in ribosomal RNA. J. Mol. Biol. **51**:83-100.
8. Björk, G. R., and F. C. Neidhardt. 1973. Evidence for the utilization of host tRNA(m^{5}U)methylase to modify tRNA coded by phage T4. Virology **52**:507-519.
9. Boman, H. G., and S. Hjerten. 1962. "Molecular sieving" of bacterial RNA. Arch. Biochem. Biophys. Suppl.

1:276–282.
10. Fellner, P. 1969. Nucleotide sequences from specific areas of the 16S and 23S ribosomal RNAs of *E. coli*. Eur. J. Biochem. **11**:12–27.
11. Fraenkel, D. G., and F. C. Neidhardt. 1961. Use of chloramphenicol to study control of RNA synthesis in bacteria. Biochem. Biophys. Acta **53**:96–110.
12. Guthrie, C., J. G. Seidman, S. Altman, B. G. Barrel, J. D. Smith, and W. H. McClain. 1973. Identification of tRNA precursor molecules made by phage T4. Nature (London) New Biol. **246**:6–11.
13. McClain, W. H., C. Guthrie, and B. G. Barrel. 1972. Eight transfer RNA induced by infection of *Escherichia coli* with bacteriophage T4. Proc. Natl. Acad. Sci. U.S.A. **69**:3703–3707.