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Transfer Ribonucleic Acid Methylase Deficiency Found in UGA Supressor Strains

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Extracts of recessive UGA suppressor strains, designated *supK*, are deficient in transfer ribonucleic acid (tRNA)-methylating activity when compared to wild-type extracts. Moreover, the tRNA from suppressor strains is methyl deficient when compared to wild-type tRNA. This deficiency is due to the lack of a single tRNA methylase activity in suppressor strains. UGA suppressor activity may be caused by the miscoding of one or more methyl-deficient tRNA's in *supK* strains.

The isolation and genetic analysis of a recessive UGA suppressor from *Salmonella typhimurium*, designated ***supK***, was reported by Reeves and Roth (21). The *supK* gene, located near *serA*, controls the production of a vital function, and suppressor activity apparently results from only partial loss of this gene function. Some *supK* mutants also have suppressor activity for certain frameshift mutations as well as UGA mutations (1).

The most interesting aspect of this suppressor is its recessivity. Merodiploids carrying both the wild-type (*supK*$^+$) and suppressor (*supK*) genes display no suppressor activity. Long doubling times also characteristic of the suppressor strains are not observed with the heterozygous merodiploids. Recessive mutations usually are the result of complete or partial loss of a gene function and not due to generation of a new or altered function. Most nonsense suppressor mutations that have been tested are dominant and are presumed to be due to the generation of an altered function, such as a transfer ribonucleic acid (tRNA) with an altered codon response (13, 26). Two examples of this type of altered tRNA are the *sup3* tyrosine tRNA (8) and the tryptophan tRNA responsible for UGA suppression (11). The *sup3* tyrosine tRNA allele is dominant to the wild-type allele for suppressor activity (8, 24), and the dominance of the tryptophan-inserting UGA suppressor gene has recently been demonstrated (28, 33). The recessivity of the *supK* UGA suppressors, therefore, indicates that its gene product is not a tRNA with an altered codon response. Suppressor activity seems to result from the lack, or reduced activity, of the *supK* gene product.

This report will show that tRNA isolated from *supK* strains is methyl deficient when compared to tRNA from wild-type strains. This deficiency in tRNA methyl groups is not due to a general reduction in tRNA-methylating activity such as might result from a deficiency of *S*-adenosylmethionine (SAM) synthesis, but is due to the reduced activity of a single tRNA methylase. This methylase acts on at least two tRNA species. This is demonstrated by in vitro methylation of *supK* tRNA with labeled methyl groups from SAM followed by fractionation of the labeled tRNA on a benzoylated diethylaminoethyl (DEAE)-cellulose column. These results suggest that the *supK* gene product is a tRNA methylase. The partial loss of this tRNA methylase results in undermodified tRNA's, one or more of which may act to suppress UGA mutations.

MATERIALS AND METHODS

Bacterial strains. The strains used as sources of tRNA or tRNA methylase extracts are given in Table 1. The methods of isolation and strain construction are described by Reeves and Roth (21).

Media and growth of strains. Strains were grown at 37 C in E medium (32) supplemented with 0.1 mM of each of the required L-labeled amino acids, except for L-serine which was added to a final concentration of 10 mM. The medium was also supplemented with a 0.05 mM thiamine for the growth of strains TR1273 and TR1276. For the preparation of enzyme extracts, 1-liter cultures were grown with rapid shaking. For tRNA preparations the desired strain was grown with vigorous aeration in a 20-liter carboy at 37 C. Cells were always harvested in late log phase (at an absorbancy at 650 nm [A_{650}] of 0.6 to 0.9).

For the preparation of methyl-deficient tRNA from the *rel*$^-$ strain TR1350, cells were grown in E medium supplemented with 0.1 mM L-histidine and 0.03 mM L-methionine. At this level of methionine the culture reaches an A_{650} of about 0.7. After 6 h of methionine starvation (the A_{650} tends to drop slightly in this time

TABLE 1. *Bacterial strains*[a]

Strain no.	Genotype
TR149	*aroD5 hisT1529 hisG200*(UGA)
TR226	*aroD5 hisT1529 hisG200*(UGA) *supK584*
TR1142	*lys554 serA790 hisI570*(UGA)
TR1168	*lys554 hisG200*(UGA) *supK584*
TR1175	*lys554 serA790 his01242 hisB3744*(UGA)
TR1273	*hisI570*(UGA) *supK1175*
TR1276	*lys554 hisI570*(UGA) *supK1175*
TR1280	*lys554 hisI570*(UGA) *supK1175*/F'116 *supK*$^+$
TR1284	*lys554 serA790 hisI570*(UGA)/F'116 *supK*$^+$
TR1350	*hisT1504 hisOGDCBH2253 met850 rel1*
TR1359	*lys554 serA790 his01242 hisB3744* (UGA)/F'116 *supK*$^+$
TR1407	*lys554 serA790 his01242 hisB3744* (UGA)/F'116 *supK1188*
TR1408	*lys554 serA790 his01242 hisB3744* (UGA)/F'116 *supK1189*

[a] Suppressor mutation *supK584* arose spontaneously in strain TR149. The *supK1175* mutation was induced in *hisI570* by diethyl sulfate and was transduced into nonmutagenized *hisI570*. TR1273 is the resulting transductant. Strain TR1350 is a Met$^-$ mutant of a relaxed control strain obtained from R. Martin; *met850* was induced with diethyl sulfate.

interval), the cells were harvested and used to extract tRNA.

tRNA preparation, aminoacylation, and benzoylated DEAE-cellulose column chromatography. The preparation of tRNA and its aminoacylation and fractionation by benzoylated DEAE-cellulose chromatography were described by Riddle and Roth (22).

The aminoacylation of tryptophan tRNA was carried out with approximately 100 A_{260} units (an A_{260} unit is that amount of tRNA in 1.0 ml with an absorbance of 1.0 at 260 nm. This corresponds to about 55 μg of tRNA. Measurement of A_{260} units is done at approximately pH 7.5 in the presence of 10 mM $MgCl_2$.) of crude tRNA incubated at 25 C with 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.0, 10 mM $MgCl_2$, 1 mM sodium ethylenediaminetetraacetic acid (EDTA), 5 mM sodium adenosine 5'-triphosphate, 0.04 mM L-[^{3}H]-tryptophan (Schwarz/Mann, 250 Ci/mol) and 100 to 200 μg of enzyme protein in a total volume of 2.5 ml. After 60 min, the reaction was stopped by the addition of 0.5 ml of 1 M sodium acetate, pH 4.5. The tRNA was recovered from the reaction mixture by DEAE-cellulose chromatography and ethanol precipitation as described by Riddle and Roth (22).

The source of the activating enzyme was a crude protein extract from *S. typhimurium* LT2 obtained by a method adopted from Hoskinson and Khorana (14). Cells (50 g [wet weight]) grown in E medium were harvested in late log phase and resuspended in 50 ml of 100 mM Tris-hydrochloride, pH 7.0, 10 mM $MgCl_2$, and 2 mM mercaptoethanol. The cells were disrupted by sonication for a total of 3 min by six 30-s pulses with the large probe of the Sonifier cell disrupter model W140 at full intensity. The cellular debris was removed by centrifugation for 30 min at 15,000 × *g*. Barium chloride, streptomycin sulfate, and ammonium sulfate precipitations were performed on the supernatant as described by Hoskinson and Khorana (14). After the final 80% saturated ammonium sulfate precipitation, the protein, disolved in 50 ml of the above Tris buffer plus 50 ml glycerol, was stored at −20 C. Activity for seryl-, glycyl-, phenyl-, alanyl-, and tryptophanyl-tRNA synthetases remained high for up to 1 year. Just before use the protein was passed through a Sephadex G-25 column (1.5 by 20 cm) previously equilibrated with 25 mM Tris-hydrochloride, pH 7.0, and 2.5 mM sodium EDTA.

tRNA methylase extracts. Cells harvested from a 1-liter culture (about 2 g [wet cells]) were collected by centrifugation and resuspended in 10 ml containing 40 mM triethanolamine-hydrochloride, pH 7.8, 10 mM $MgCl_2$, 1 mM sodium EDTA, 1 mM mercaptoethanol, and 10% glycerol (vol/vol). This buffer is used throughout the enzyme preparation. The cells were sonicated for a total of 90 s in six 15-s pulses at the maximum setting of a Sonifier cell disrupter model W140. The temperature of the protein extract was kept between 0 and 4 C during sonication and all subsequent steps. The cellular debris was removed by centrifugation for 2 h at 30,000 × *g*. For some tRNA methylase assays this crude preparation was applied to a small Sephadex G-25 column equilibrated with the above buffer, and the effluent, freed of small-molecular-weight material, was used as the source of enzyme. For assays of tRNA methyl acceptance and for DEAE-cellulose column chromatography of the methylases, the crude extract was treated with streptomycin sulfate at a final concentration of 20 mg/ml to remove nucleic acid. The white precipitate was removed by centrifugation. The remaining protein was freed of streptomycin and other small-molecular-weight material by chromatography on Sephadex G-25. This protein extract was used for both tRNA methylase assays and for DEAE-cellulose column chromatography. Protein was assayed by the biuret method or by the ultraviolet absorption method after nucleic acids were removed (6).

DEAE-cellulose chromatography of the tRNA methylases. The protein solution from above containing up to 40 mg of protein was slowly applied to a DEAE-cellulose column (1 by 10 cm). The column was previously equilibrated with the triethanolamine buffer from above. After adding the protein extract the column was washed with 10 ml of the equilibrating buffer. The methylases were eluted with a 100-ml linear gradient from 0 to 0.4 M NaCl contained in the same triethanolamine buffer. Protein elution was monitored by A_{280} and the tRNA methylases in each 2-ml fraction from the column were assayed directly by the method below.

In vitro tRNA methylation. All assays were carried out by the method of Hurwitz et al. (15) with some modifications. The methyl donor was *S*-adenosyl-L-[^{14}C-*methyl*]methionine (Amersham/Searle, 50 Ci/mol) at a final concentration of 20 μM. All assay mixtures also contained 40 mM triethanolamine-hydrochloride, pH 7.8, 10 mM $MgCl_2$, and nonradioactive L-methionine at a concentration of 2 mM. Small amounts of glycerol, less than 10% (vol/vol), had no effect on the enzyme activity. Three in vitro methylation procedures were used. For measuring the

extent of methylation of a tRNA preparation, the assay contained approximately 1 A_{260} unit of tRNA and 200 μg of enzyme protein in a final volume of 250 μl. For determining the specific activity of a tRNA methylase preparation, 3 to 5 A_{260} units of tRNA were used each with two different levels of enzyme protein, usually at final protein concentrations of about 20 and 100 μg/ml. For the large scale methylation of tRNA, 100 A_{260} units of tRNA were incubated with 2 to 5 mg of protein in a final volume of 2.5 ml. After large scale methylation the tRNA was recovered from the reaction mixture by DEAE-cellulose chromatography and ethanol precipitation as described by Riddle and Roth (22) for aminoacylated tRNA.

Time course assays were always performed except when monitoring tRNA methylase activity from DEAE-cellulose column effluents. At various times during the reaction 25-μl aliquots were withdrawn and applied to 2.2-cm Whatman 3MM filter disks (18). The disks were washed in trichloroacetic acid solutions and prepared for scintillation counting as described by Reeves et al. (20). One necessary modification of the washing procedure was to extend the trichloroacetic acid washing times to 20 min each. The trichloroacetic acid-precipitable ^{14}C counts were determined in a Packard liquid scintillation spectrometer.

SAM synthetase assay. The level of SAM synthetase was measured by the methionine-dependent release of [^{32}P]orthophosphate from [α-^{32}P]ATP (kindly provided by M. Chamberlain). The assay conditions were similar to those of Greene et al. (9). The assay contained 150 mM Tris-hydrochloride, pH 8.5, 100 mM KCl, 15 mM $MgCl_2$, 20 mM L-methionine, 4 mM 2-mercaptoethanol, 8 mM [α-^{32}P]ATP (60 counts/min per nmol) and about 300 μg of protein in a total volume of 250 μl. After 10 to 30 min at 37 C, the reaction was stopped with 0.05 ml of ice cold 4 N HCl. The unreacted, radioactive adenosine 5'-triphosphate was adsorbed to charcoal in the following way: to the acidified assay mixture was added 0.1 ml of 25% acid-washed Norit A in water plus 0.1 ml of 0.025 M tetrasodium pyrophosphate, 0.025 M disodium phosphate, and 5 mg of bovine serum albumin per ml. After shaking for 15 min at room temperature, the charcoal was centrifuged for 10 min at 10,000 $\times$ g. Radioactivity was determined on 0.2 ml of the supernatant pipetted onto an aluminum planchet and counted in a Nuclear Chicago gas flow counter.

The enzyme protein was prepared from 0.5 g of LT2 cells harvested in late log phase from E medium. The cells were resuspended in 50 mM Tris-hydrochloride, pH 7.5, and 10 mM 2-mercaptoethanol, and then sonicated. Cellular debris was removed by centrifugation at 30,000 $\times$ g for 30 min. The supernatant was passed through a Sephadex G-25 column equilibrated with the same Tris-hydrochloride, 2-mercaptoethanol buffer. The protein-containing fractions were used for the SAM synthetase assay after the protein concentration was determined by the biuret method (6).

RESULTS

Behavior of supK mutants. The phenotype of some of the UGA suppressor strains, as well as the map position of *supK*, suggested that the affected gene might be responsible for the production of SAM synthetase. Some of the very slow-growing *supK* strains (this includes *supK1175* strains) display an unusual sensitivity to purines and purine nucleosides. Their growth is completely inhibited by 0.4 mM adenine. However, even after 18 h in the presence of adenine, growth will resume immediately after the addition of 0.01 mM thiamine. Pantothenate or a combination of methionine plus lysine also overcomes the sensitivity to purines. This phenotype was described by Dalal et al. (5). They examined several unmapped but unlinked *S. typhimurium* mutants displaying this phenotype and attributed the adenine sensitivity to defects in one-carbon metabolism. The *supK* gene is co-transducible with *serA* and *lys* and the gene for SAM synthetase (*metK*) is also linked to *serA* although it is apparently not co-transducible with *lys* (12, 16). SAM synthetase is a key enzyme in one-carbon metabolism. It catalyzes the synthesis of SAM which in turn is required for the enzymatic methylation of tRNA. The behavior of the *supK* mutants and the map position of *supK* suggested that these strains might be deficient in SAM synthetase.

supK is not the SAM synthetase gene. If *supK* were the gene responsible for the production of SAM synthetase, extracts from *supK* mutants should be deficient in SAM synthetase activity. Chater (3) has shown that the SAM synthetase gene (*metK*) is carried by the *Escherichia coli* episome F'116, and that *metK* is recessive to its wild-type allele. We have shown this to be the case for *supK* with the same episome (21). Therefore, various haploid and diploid strains for *supK* were used to assay the level of SAM synthetase. Using F'116, we constructed the following *supK* genotypes: $supK^+$ (wild type), *supK*, $supK^+/supK^+$ $supK^+/supK$.

Table 2 gives the results of SAM synthetase assays from these strains. These data indicate that the SAM synthetase gene is carried by the F'116 episome, as shown by Chater (3) for *metK*, but that *supK* is not this gene. Haploid strains, whether $supK^+$ or *supK*, have nearly identical specific activities. The *supK* strains may even have slightly more SAM synthetase activity. The merodiploid strains regardless of their *supK* genotype have double the level of SAM synthetase activity over their haploid counterparts. Thus, these results confirm the location of the SAM synthetase gene near *serA* by a gene dosage effect, but the *supK* gene product is not responsible for the production of SAM.

Methyl deficiency of tRNA from supK

TABLE 2. *SAM synthetase activity from suppressor and nonsuppressor strains*

Strain	Suppressor genotype	SAM synthetase activity[a]
LT2	*supK*$^+$	14.2
TR1141	*supK*$^+$	9.7
TR1142	*supK*$^+$	12.1
TR1175	*supK*$^+$	11.9
TR1168	*supK584*	16.3
TR1276	*supK1175*	16.4
TR1359	*supK*$^+$/*supK*$^+$	27.2
TR1407	*supK*$^+$/*supK1188*	30.6
TR1408	*supK*$^+$/*supK1189*	33.5

[a] SAM synthetase activity was determined on crude extracts as described in Materials and Methods. All values are averages of at least two determinations. Specific activities are nanomoles of [^{32}P]orthophosphate released (from [α-^{32}P]ATP) per milligram of protein in 10 min at 37 C.

strains. While carrying out assays for SAM synthetase, *supK* strains were also checked for a possible methyl deficiency of their tRNA. The extent of methylation of tRNA from *supK* strains in vitro was found to be two to three times higher than the extent of methylation of wild-type tRNA. The methyl acceptance of tRNA extracted from various suppressor and nonsuppressor strains is given in Table 3. Note especially the methyl acceptance of the tRNA from strains TR1142, TR1276, TR1280, and TR1284. The latter three strains all have the genetic background of TR1142, but only the *supK1175* haploid strain, TR1276, is methyl deficient. It is methylated in vitro to about three times the level of the other three strains all of which carry at least one wild-type *supK*$^+$ gene. This result correlates well with the recessivity of *supK*. If the *supK* gene product is a tRNA methylase and suppressor activity results from lack of this enzyme, only the tRNA from the *supK* haploid strain should be methyl deficient, as is observed with strain TR1276.

Since the early work of Srinivasan and Borek (29) and Gold et al. (7) it has been generally assumed that a tRNA methylase preparation is not able to methylate in vitro its own tRNA, i.e., there is no methylation of tRNA with enzymes from a homologous species. It has been assumed that in vivo the methylation of tRNA is complete under normal growth conditions so that no additional methylation of this tRNA can be detected in vitro with an homologous enzyme preparation. However, methylation of tRNA by an homologous methylase preparation is apparent from the data in Table 3. The methylating enzyme used to assay methyl acceptance was in all cases isolated from strain TR1142. This enzyme is able to carry out to some extent the methylation of its own tRNA in vitro. A level of methylation of about 20 pmol of methyl group accepted per A_{260} unit of tRNA has been observed with this and a number of other homologous crude tRNA methylase and tRNA preparations from various *S. typhimurium* strains. When the *supK* enzyme is purified, the methyl acceptance of homologous tRNA may increase to about 150 pmol per A_{260} unit of tRNA (W. T. Pope and R. H. Reeves, unpublished data). The reason for the lower methyl acceptance values reported in Table 3 is not completely understood, but these values are consistently obtained when crude extracts are used as a source of the methylating enzyme.

TABLE 3. *tRNA methyl acceptance and tRNA methylase activity from suppressor and nonsuppressor strains*

Strain	Suppressor genotype	Methyl acceptance[a] (picamoles of methyl accepted per A_{260} unit of tRNA)	Methylase activity[b] (picamoles of methyl accepted per milligram of protein)
LT2	*supK*$^+$	14	76
his570	*supK*$^+$	23	
TR149	*supK*$^+$	18	
TR1142	*supK*$^+$	15	75
TR1284	*supK*$^+$/*supK*$^+$	19	80
TR1280	*supK1175*/*supK*$^+$	19	74
TR1273	*supK1175*	50	
TR1276	*supK1175*	46	31
TR226	*supK584*	42	
TR1168	*supK584*	37	45

[a] Methyl acceptance of the tRNA from the strains indicated was carried out as described in Materials and Methods using a TR1142 extract as the source of the methylating enzyme.

[b] The tRNA methylase activities were determined on crude protein extracts from the strains indicated using *supK1175* tRNA as methyl acceptor. Methyl acceptance was linear for at least 30 min. Each specific activity reported is the average of determinations on at least two separately prepared extracts. The values for TR1142 and TR1276 are the averages from determinations on five separate extracts. Specific activities are picamoles of methyl accepted per milligram of protein in 10 min.

tRNA methylase activities from supK strains. The total tRNA methylase activity found in *supK* strains was about twofold lower than that found in wild-type extracts. The same strains which were examined for tRNA methyl-

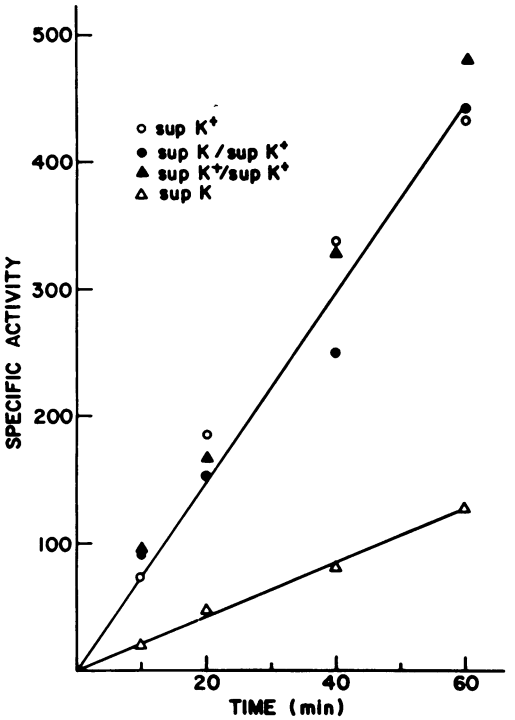

FIG. 1. *The in vitro methylation of supK tRNA using extracts from four supK strains. The tRNA used as methyl acceptor was from the suppressor strain, TR1273. The extracts were made from TR1142, supK+; TR1280, supK/supK+; TR1284, supK+/supK+; and TR1276, supK. The methylation was carried out as described in Materials and Methods.*

ation levels were also screened for the specific activity of their tRNA methylases. The tabulation of tRNA methylase activities from several strains is given in Table 3. The rate of tRNA methylation is shown in Fig. 1. The tRNA used as substrate for the methylase extracts was prepared from *supK1175* strains and should be deficient in methyl groups normally added by the *supK* methylase. Note that methylase activity is more than twofold reduced in the *supK1175* strain, TR1276, when compared to the wild-type *supK*+ strains. Some of the remaining methylase activity seen in TR1276 is possibly due to tRNA methylases other than the one specified by the *supK* gene. However, our recent results indicate that the remaining methylase activity in supK strains is due to the same tRNA methylase observed in wild-type strains. We have found that most if not all the tRNA methylase activity observed in both *supK*+ and *supK* extracts using supK (or wild type) tRNA as methyl acceptor is due to one enzyme. This activity chromatographs on both DEAE-cellulose and on phosphocellulose columns in the same position as the *supK* methylase activity (Pope and Reeves, unpublished data).

One result shown in Table 3 is difficult to explain. The *supK*+/*supK*+ strain, TR1284, was expected to have somewhat more methylase activity than the wild-type haploid strains due to double the wild-type gene dose for *supK*+. At present we have no completely satisfactory explanation for the absence of a gene dosage effect with this strain.

Analysis of the supK tRNA methylase. The *supK* tRNA methylating activity and the total tRNA methylase activities from *supK*+ and *supK* extracts were compared after chromatography on DEAE-cellulose. Figures 2a and 2b show the elution profiles of these activities. The total methylase activity is assayed using undermethylated tRNA from a methionine-starved *rel*- strain of *S. typhimurium*. The *supK* tRNA methylase activity is assayed with tRNA iso-

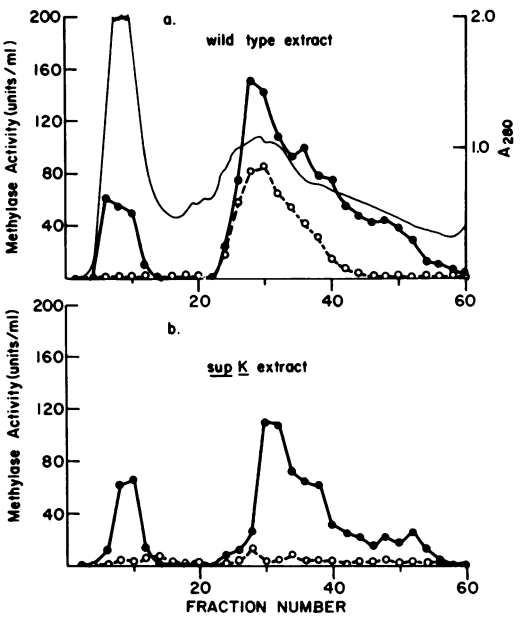

FIG. 2. *DEAE-cellulose chromatography of tRNA methylase extracts. (a) Wild-type extract from strain TR1142. Protein was monitored by* A_{280} (——). *Methyl acceptance was assayed in every other fraction using methyl-deficient tRNA from the rel- strain TR1350* (●) *and using tRNA from the UGA suppressor strain, TR1273* (○). *One unit of methylase activity is 1 pmol of methyl group accepted by tRNA in 10 min at 37 C. (b) Extract from the UGA-suppressor strain, TR1276. Methyl acceptance was assayed by using methyl-deficient tRNA* (●) *and using tRNA from the UGA suppressor strain, TR1273* (○). *For details see Materials and Methods.*

lated from a *supK* strain. Figure 2a shows the activity profile obtained from the chromatography of wild-type extract. Note the peak of *supK* tRNA-methylating activity at fraction 30. This peak of activity is completely absent in profiles of a *supK* extract shown in Fig. 2b. The total methylase activity around fraction 30 also is reduced (Fig. 2b). This is especially apparent from the shift in position of the leading edge of the main methylase peak. These results have been obtained with several different extract preparations from *supK* and *supK*$^+$ cells. If the fractions from a DEAE-cellulose column of wild-type extract as in Fig. 2a are assayed using wild-type tRNA, the same profile as that for the *supK* tRNA is observed. The maximum level of methylase activity, however, is usually reduced about twofold using wild-type tRNA as methyl acceptor. These results confirm the results found with crude extracts from *supK*$^+$ and *supK* strains. Extracts from wild-type strains contain a tRNA methylase activity that is apparent when assayed with *supK* tRNA; *supK* extracts are deficient in this activity. Although the profiles in Fig. 2b indicate a complete absence of this tRNA methylating activity in *supK* extracts, other chromatographic profiles obtained from *supK* strains show a small peak of activity at about fraction 30. It appears that the methylase from *supK* strains is reduced in activity and is somewhat unstable to DEAE-cellulose chromatography.

This *supK* tRNA methylating activity also binds to phosphocellulose and can be separated from the bulk of the methylating activities by elution with a linear salt gradient. Extracts from *supK*$^+$ and *supK* strains have been compared also by phosphocellulose chromatography (not shown). As with DEAE-cellulose chromatography a single tRNA methylase peak is absent or reduced in extracts of *supK* strains. This activity from wild-type cells has been purified more than 1,000-fold using several chromatographic techniques, and behaves as a single tRNA methylating enzyme (Pope and Reeves, unpublished data).

Nature of the methylated tRNA. The tRNA isolated from either wild-type or suppressor strains will accept *methyl*-^{14}C-labeled groups in vitro, although as described above, the tRNA from the *supK1175* strain, TR1276, accepts up to three times more methyl groups than does the parent *supK*$^+$ strain, TR1142. The methylated tRNA from a *supK* strain was examined by benzoylated DEAE-cellulose chromatography. Approximately 5 mg (100 A_{260} units) of tRNA was methylated using about 2 mg of the partially purified enzyme and [*methyl*-^{14}C]SAM as the methyl donor. This tRNA, reisolated from the methylase reaction, was applied to benzoylated DEAE-cellulose. The chromatographic profile of the labeled tRNA is shown in Fig. 3a. The profile reveals that at least two tRNA species are accepting methyl groups in vitro. However, it is likely that several different tRNA species make up the radioactive peak eluting in the salt gradient.

Since the *supK* tRNA is more methyl deficient than wild-type tRNA and the *supK* strain contains UGA suppressor activity, an obvious model for the action of the suppressor would be one in which a methyl-deficient tRNA species is abnormal in its codon recognition and mispairs with the UGA triplet. Tryptophan tRNA (responds to codon UGG) is a likely candidate because it recognizes a closely related codon and because it can mutate to an efficient UGA suppressor by a non-anticodon base change (10, 11). To test whether tryptophan tRNA is one of the methyl-accepting species, a *supK* tRNA preparation was first methylated in vitro with [*methyl*-^{14}C]SAM followed by aminoacylation

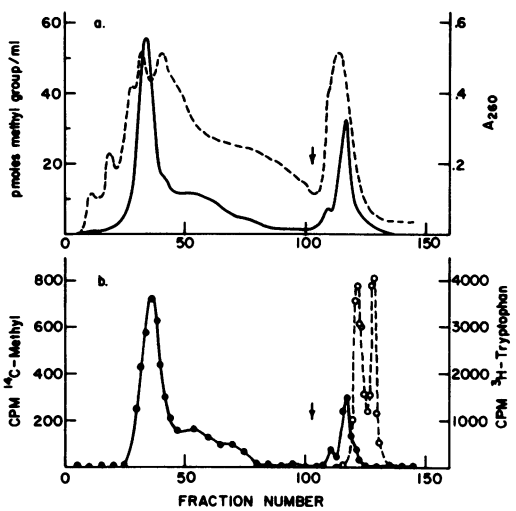

FIG. 3. *Benzoylated DEAE-cellulose chromatography of in vitro methylated supK tRNA. (a) Chromatography of TR1276 tRNA (approximately 114 A_{260} units). The dotted line is absorption at 260 nm. The solid line is picamoles of methyl group per milliliter. (b) Chromatography of methyl-^{14}C-labeled tRNA charged with [^{3}H]tryptophan. The A_{260} profile (not shown) is essentially the same as in (a). Counts per minute per milliliter of [methyl-^{14}C] (●). Counts per minute per milliliter of [^{3}H]tryptophan (○). For details of BD-cellulose column chromatography see Riddle and Roth (22). The arrows indicate the start of the ethanol gradient.*

with [^{3}H]tryptophan. This double-labeled material was chromatographed on benzoylated DEAE-cellulose. The two radioactive profiles do not have peaks that coelute (Fig. 3b). Tryptophanyl-tRNA reproducibly elutes as two peaks near the end of the ethanol gradient as shown. The ^{14}C-containing peak due to tRNA methylation that also elutes in the ethanol gradient does not coincide with tryptophanyl tRNA. If suppressor activity is due to a methyl deficient tRNA in the *supK* strains, these results tend to exclude tryptophan tRNA as the suppressor tRNA species.

DISCUSSION

The *supK* strains of *S. typhimurium* have been genetically characterized as recessive UGA suppressors. Biochemical evidence is presented here which suggests that suppressor activity is due to an undermethylated tRNA species. The *supK* strains tested are methyl deficient when compared to wild-type, nonsuppressor strains. This deficiency is due to the reduced activity of a single tRNA methylase and not due to a general reduction of tRNA methylation. The recessivity of the *supK* gene is consistent with the idea that suppression is due to a deficient tRNA methylase (reduced level of a function) instead of the generation of a new function such as an altered codon of a tRNA.

As previously reported the weak UGA suppressor activity observed in wild-type *S. typhimurium* may also be due to the *supK* gene product, or rather, due to deficiency of the *supK* function even in wild-type cells (21). This weak suppressor activity is consistent with the finding that in wild-type cell extracts of *S. typhimurium* there is undermethylated tRNA which can be detected only with the *supK* methylase, i.e., the *supK* methylase is capable of methylating homologous tRNA. Possibly one or more of the undermethylated tRNA species in wild-type cells displays weak UGA suppressor activity. In *supK* strains even more undermethylated tRNA is produced due to the deficiency in this methylase. The result is UGA suppression with about 10% efficiency, and possible loss of normal coding properties for the undermethylated tRNA which would account for the slow growth rate of these strains. We have shown that complete absence of the *supK* gene product is a lethal event (21). The complete absence of the *supK* methylase may result in complete loss of one or more coding functions. Thus, three different alleles at the *supK* locus have quite different results on the cell. The wild-type *supK*$^+$ allele gives normal growth rates for the cell and weak UGA suppressor activity. The *supK* allele results in long doubling times for the cell and UGA suppressor activity with an efficiency of about 10% (and at least in some strains, purine sensitivity). What we earlier designated at the *supK*^{0} allele (e.g., a frameshift mutation in *supK*) results in cell death (21).

To date one other tRNA modification deficient mutant class has been isolated in which the unmodified tRNA results in altered cell physiology. The *hisT* mutants were isolated as histidine regulatory mutants by Roth et al. (23). Singer et al. (27) showed that in *hisT* strains histidine tRNA contains two uridine residues which replace two pseudouridine residues found in the wild-type tRNA sequence. The *hisT* gene product is apparently a tRNA-pseudouridylating enzyme which is altered or absent in *hisT* strains (4). The absence of the two pseudouridine modifications results in a histidine tRNA defective in its role in the repression of the histidine operon. In this case the unmodified tRNA is apparently normal or almost normal in carrying out its role in protein synthesis, but is unable to function in its regulatory role of controlling the level of the histidine biosynthetic enzymes.

No such methylation-deficient mutants, however, have been found which affect cell physiology, except possibly the *trmB* mutant (*E. coli* strain GM1) reported by Marinus et al. (17). Four classes of tRNA-methylating enzyme mutants have been isolated, three in *E. coli* (2, 17), the other in yeast (19). Only the *trmB* mutant appears to have any observable effect on cell physiology. The presence of *trmB* may result in less efficient suppression of amber mutants by *supE*. However, the authors point out that all their *trm* strains were heavily mutagenized, and altered physiological effects may be due to second site mutations (17). The *trmA* strains of *E. coli* have been extensively studied, and the growth of these strains appears to be perfectly normal (31). The tRNA from these strains is completely lacking in ribothymidine, but by several in vitro tests it is indistinguishable from wild-type tRNA. There is evidence, however, that methyl-deficient tRNA's can be potentially lethal to the cell. Even though most undermethylated tRNA's behave in vitro like their fully methylated counterparts, there are a few cases reported where differences in aminoacylation or in fidelity of translation have been observed (for a review see reference 30). Shugart et al. (25) have demonstrated poor amino acid acceptance for undermethylated phenylalanine, leucine, ty-

rosine, and histidine tRNA's, and in the case of the phenylalanine and histidine species, in vitro methylation can restore normal amino acid acceptance. Lack of the methylating activity or activities involved in these cases could conceivably be a lethal event for the cell. These undermethylated tRNA species were obtained by methionine starvation of a relaxed control *E. coli* strain. All methyl groups in these tRNA's are probably deficient, and the one or more methylated nucleosides responsible for restoration of activity for these tRNA's have not been characterized.

With the *supK* mutants the reduction of a single tRNA methylating activity apparently results in pronounced phenotypic changes. The cells grow slowly and become UGA suppressors. Complete lack of this methylating activity appears to be lethal for the cells. Upon further examination of the tRNA from *supK* strains, we hope to identify the methylated nucleoside that is deficient, and we hope to find how this deficiency results in altered coding properties for the tRNA.

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