Changes in Transfer Ribonucleic Acids Accompanying Encystment in *Acanthamoeba castellanii*

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Transfer ribonucleic acid (tRNA) from exponentially growing cells (trophozoites) and from precysts of *Acanthamoeba castellanii* were examined by reversed-phase column (RPC-2) chromatography. This system gave excellent resolution of isoaccepting species of tRNA. The tRNAs for 12 amino acids were studied. A comparison of trophozoite and precyst tRNA elution profiles revealed no apparent differences in the number of isoaccepting species of alanyl-, arginyl-, asparaginyl-, glycyl-, leucyl-, lysyl-, methionyl-, phenylalanyl-, tryptophanyl-, or valyl-tRNAs. Seryl-tRNAs from trophozoites were eluted as three components, whereas precyst seryl-tRNAs were eluted as only two components. Precharged trophozoite and precyst isoleucyl-tRNAs were both eluted as single components; however, post-chromatography charging of trophozoite tRNA resulted in three components of activity for tRNA_{ie} and only one component for precyst tRNA_{ie}. None of the observed changes could be attributed to differences in synthetases or to the presence of altered tRNA lacking the CCA terminus or partially degraded by nuclease. The possible significance of these observations is discussed.

It has been postulated that alterations in species of transfer ribonucleic acid (tRNA) reflect a level of control in cellular development. Numerous studies have been made in a variety of differentiating systems to elucidate differences in the complement of isoaccepting species of tRNA. This was the subject of an extensive review by Sueoka and Kano-Sueoka (21).

Trophozoites of *Acanthamoeba castellanii*, a small, free-living soil amoeba, have been shown (12) to be capable of differentiating into cysts when transferred from a nutrient-rich to a nutrient-poor medium. In recent years, there have been several structural (3, 4, 15) and physiological (6) studies of encystment in axenic cultures of *Acanthamoeba* spp. Analysis of cyst cell walls revealed the presence of at least two new classes of molecules not found in trophozoites: an alkali-insoluble polysaccharide with properties similar to those of cellulose, and a protein with a high phosphorus content (13). Furthermore, it has been demonstrated in unagitated cultures (5, 16) that, as these organisms approach the stationary phase of growth prior to encystment, there is increase of both ribosomal and transfer RNAs. When this accumulation was prevented by actinomycin D, encystment failed to occur. A recent report (19) demonstrated that, in aerated cultures of *A. castellanii* undergoing experimentally induced encystment, transcription rates of RNA are closely coordinated with encystment. The appearance of compounds peculiar to cysts and alterations in total RNA content immediately prior to encystment indicate clearly that changes in the specificity of translation are associated with the morphogenetic events observed in this organism.

This paper (part of a dissertation submitted by J. McMillen, University of Kansas, Kansas City, 1971) describes the isolation and characterization of the tRNAs for 12 amino acids from trophozoites and precysts of *A. castellanii*. Secondly, it reports differences observed in the elution profiles for serine and isoleucine tRNAs between trophozoites and precysts.

**MATERIALS AND METHODS**

**Organisms.** A strain of *Acanthamoeba* sp. was originally isolated from monkey kidney tissue cultures (8) and was adapted to grow under conditions similar to those described (7) for *A. castellanii* JBM. The strain has since been identified as *A. castellanii* and is designated here as *A. castellanii* Vc.

**Culture conditions.** The growth medium contained (in 1 liter of distilled water): mycological peptone (Oxoid), 20 g; yeast extract (Difco), 20 g; glucose, 18 g; NaCl, 120 mg; MgCl$_2$, H$_2$O, 3 mg; Na$_2$HPO$_4$, 142 mg; KH$_2$PO$_4$, 136 mg; CaCl$_2$, 3 mg;
and FeSO₄, 3 mg. The medium was sterilized by being autoclaved for 15 min at 121 C. The unadjusted pH of the sterile medium was 5.6. Exponentially growing cultures were inoculated into 2-liter silicon-treated (Siliclad, Clay-Adams) flasks containing 200 ml of growth medium. The inoculum was adjusted to obtain 2.0 × 10⁸ to 3.0 × 10⁹ organisms per ml. The cultures were incubated on a rotary shaker (New Brunswick) at 150 rpm and at 37 C.

**Encystment conditions.** The ability of *A. castellani* Vc to encyst in growth media was lost when it was adapted to grow to high population densities and with a short generation time (7). Medium for encystment (encystment saline) was a salt solution containing: NaCl, 7 g; MgCl₂, 3 mg; Na₂HPO₄, 142 mg; KH₂PO₄, 136 mg; CaCl₂, 3 mg; FeSO₄, 3 mg; and 1 liter of distilled water. Final pH of the sterilized solution was 6.8. Organisms from the stationary phase of vegetative growth were inoculated into the encystment medium at a final concentration of 2 × 10⁸ organisms per ml. Encystment was allowed to proceed at 30 C on a rotary shaker until the first wall precyst forms could be seen with a microscope. Within 8 to 10 h, at least 80% of the organisms had reached this stage of encystment.

**Preparation of tRNA: trophozoites.** Late log-phase organisms were harvested and washed three times with encystment saline. The final washed pellet (150 to 200 g wet weight) was suspended in 1 liter of tris(hydroxymethyl)aminomethane (Tris) - acetate buffer (0.025 M Tris, pH 8.0; 0.1 mM sodium-ethylenediaminetetraacetate [EDTA]); 10 mM 2-mercapto-ethanol), and the tRNA was isolated by the procedure of Nazario (11). The final, lyophilized powder was dissolved in water and absorbancy at 260 nm (A₂₆₀) in 0.01 M KOH was determined. Twenty A₂₆₀ units were considered equivalent to 1 mg of tRNA. A major contaminant in the preparation was found to be glycogen, as determined by a modified diphenylamino method (18), which is specific for hexoses and shows no interference by nucleic acids. Removal of the glycogen was accomplished by chromatography of the tRNA on a diethylaminoethyl (DEAE)-cellulose column. A column (2 by 30 cm) was prepared by suspending washed DEAE-cellulose (Bio-Rad Cellex-D, 0.78 mg/g) in 0.1 M Tris-chloride buffer (pH 7.5), pouring the slurry into the column, and equilibrating the column at 18 C by washing with 1 liter of 0.1 M Tris-chloride (pH 7.5) made 0.2 M in NaCl. Up to 100 mg of tRNA was applied to the column, which was then eluted with a 300-ml linear gradient of 0.2 to 1.0 M NaCl in 0.1 M Tris-chloride. Fractions (5 ml each) were collected, and absorbance at 260 nm was determined. The fractions containing the nucleic acids were pooled and precipitated with 0.1 volume of 2 M potassium acetate (pH 5.0) and 2.5 volumes of cold 95% ethanol. After 15 h at −20 C, the RNA was collected on a membrane filter (Millipore type HA, pore size 0.45 μm), washed with 70% ethanol, and eluted off of the filter by being shaken at 4 C for 60 min in 0.05 M NaCl-0.01 M magnesium acetate-0.1 mM EDTA. The absorbance at 260 nm was determined, and the RNA was stored at −20 C in small volumes. Yield of RNA from trophozoites averaged 1.4 mg/g wet weight.

**Preparation of tRNA: precysts.** *A. castellani* Vc was allowed to encyst to the first wall stage (precyst). The precysts were collected by centrifugation and washed and resuspended in a small volume of encystment saline. The suspension was frozen, thawed, and centrifuged to lyse and remove any remaining trophozoite forms. The final pellet was suspended in a small volume of tRNA extraction buffer (see above) and allowed to freeze to a semisolid paste. Ice-cold phenol was added, and the resulting mixture was treated by sonic oscillation (Branson Sonifier) for six 30-s bursts at 5 mA. Appropriate amounts of extraction buffer (scaled down to the same proportions used for the isolation of trophozoite tRNA) and phenol were added to the broken precysts, and isolation of the tRNA was completed by the same procedure used for the trophozoites.

**Preparation of the aminoacyl-tRNA synthetases.** The synthetases were prepared by a modification of the method of Muench and Berg (10). All operations were performed at 4 C. Late log-phase organisms were harvested, washed three times in amoeba saline, and suspended to a thick slurry in buffer made of 0.01 M Tris-acetate (pH 8.0), 0.01 M MgCl₂, and 10% glycerol (vol/vol). The suspension was homogenized by hand in a Dounce homogenizer. The homogenate was brought to 100 ml with the same buffer and centrifuged at 29,000 × g for 20 min. The supernatant was then centrifuged at 100,000 × g for 2 h in a Spinco model L centrifuge. The resulting supernatant was applied to a DEAE-cellulose column (2 by 36 cm) which had been equilibrated with initial buffer (0.02 M potassium phosphate buffer [pH 7.5], 0.02 M 2-mercaptoethanol, 1 mM MgCl₂, and 10% glycerol). The column was washed overnight with 400 ml of initial buffer. The column was eluted with 300 ml of elution buffer consisting of 0.25 M potassium phosphate buffer (pH 6.5), 0.02 M 2-mercaptoethanol, 1 mM MgCl₂, and 10% glycerol. Fractions (10 ml each) were collected, and the absorbance at 280 nm was monitored. The fractions containing maximal protein were pooled and concentrated by dialysis against several changes of 1 mM potassium phosphate buffer (pH 6.8) containing 1 mM reduced glutathione and 50% glycerol. The resulting enzyme preparation was stored at −20 C and found to be stable for at least 6 months. This enzyme preparation had very little synthetase activity for charging alanine, asparagine, glycine, and methionine onto their respective tRNAs. The source of these synthetases was a G-25 Sephadex (Pharmacia) filtrate of cell extract prepared fresh each time.

Preparation of the precyst synthetases was accomplished in the same manner as for trophozoite synthetases, except that the precysts were disrupted by shaking with glass beads for 3 to 5 min using a mechanical mixer (Vortex).

**Determination of amino acid-accepting activity.** In a final volume of 0.5 ml, the reaction mixture contained: Tris-acetate buffer (pH 8.0), 50 μmol; MgCl₂, 15 μmol; KCl, 5 μmol; adenosine triphosphate
(ATP), 1.5 µmol; 3H- or 14C-labeled amino acids, 5 to 10 nmol; 2 to 10 A100 units of tRNA; and 0.25 µg of synthetase protein (as determined by the method of Lowry [9]). The reactions were started by the addition of radioactive amino acid. Incubations were at 30 C for appropriate time intervals. Blanks consisted of a reaction mixture without tRNA. The reactions were terminated by the addition of 2 ml of cold 10% trichloroacetic acid. After 15 to 20 min in an ice bath, the samples were collected on glass-fiber filters (Reeve Angel, 934 AH), washed three times with cold 5% trichloroacetic acid, and dried in a 60 C oven. In the case of tryptophan and phenylalanine, the filters were washed with 95% ethanol to reduce otherwise high blank levels. The dried filters were placed in 10 ml of a toluene-based scintillation fluid, and radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer, model 314 EX, with a counting efficiency of 18% for 3H and 77% for 14C.

**Reversed-phase column chromatography.** The reversed-phase chromatographic technique of Weiss and Kelmers (25) was used with the minor modifications reported previously (11). Briefly, the solution employed for equilibration and elution of the column contained 10 mM sodium acetate (pH 4.6) and 10 mM MgCl2 (buffer A) plus the appropriate NaCl concentration (see below). For post-chromatography charging, 500 to 600 A260 units of tRNA were applied to the column which was then washed with 150 ml of buffer A containing 0.1 M NaCl. The column was eluted with a 2-liter linear gradient of 0.1 to 0.5 M NaCl. Fractions (10 ml each) were collected and monitored at A260. The tRNA from appropriate fractions was collected by precipitating each fraction with 2.5 volumes of 95% ethanol. The precipitate was collected by filtration onto a membrane filter (Millipore type HA, pore size 0.45 µm), and the tRNA was eluted as described above. The eluate was then assayed for its amino acid-accepting activity.

For pre-chromatography charging, 20 A260 units of tRNA was incubated in a reaction mixture under conditions described for determination of amino acid-acceptor activity. The reaction was terminated by the addition of 0.2 ml of 1 M Na acetate (pH 4.6) and 1 ml of water-saturated phenol followed by vigorous mixing for 1 min. Yeast tRNA (1 to 2 mg) was added as carrier, the solution was mixed again, and the aqueous phase was separated after centrifugation. The phenol layer was re-extracted with 1 ml of buffer A, and the combined aqueous layers were precipitated with 2.5 volumes 95% ethanol. The precipitate was collected, washed twice with NaCl-saturated 70% ethanol and once with 70% ethanol and dissolved in 1 ml of buffer A. A minimum of 50,000 counts each of [3H]- and [14C]jarniacyl-tRNA per min were mixed and applied to a column. The column was eluted with a 2-liter linear gradient of 0.25 to 0.50 M NaCl in buffer A for isoleucyl-tRNA and 0.30 to 0.50 M NaCl in buffer A for seryl-tRNA. Fractions (10 ml each) were collected, and the radioactivity in each fraction was determined by adding 1 to 2 mg of yeast tRNA and 1 ml of ice-cold 100% trichloroacetic acid containing 0.1 M lanthanum nitrate; the resulting precipitate was collected on glass-fiber filters (Whatman GF/C), washed three times with ice-cold 5% trichloroacetic acid, and dried. The filters were counted in a toluene-based scintillation fluid in a Packard Tricarb scintillation spectrometer set for 3H.14C discriminations. The recovery of input radioactivity was always greater than 90%. The total counts of 3H and 14C per min eluted from the column were determined, and each fraction tested was expressed as a percentage of the total eluted 3H and 14C radioactivity, respectively.

**Chemicals.** The following tritium-labeled amino acids were purchased from New England Nuclear Corp. (the specific activity is expressed as millicuries per millimole): leucine, 5,000; arginine, 741; glycine, 5,300; asparagine, 1,100; serine, 1,700; and isoleucine, 405. The following were purchased from Amersham/Searle (specific activity: millicuries per millimole): valine, 1,500; isoleucine, 250; phenylalanine, 500; tryptophan, 500; alanine, 90; and methionine, 100. 14C-labeled serine (161) and isoleucine (312) were purchased from Amersham/Searle. When necessary, the specific activities of the radioactive amino acids were reduced by addition of unlabeled compounds.

Tricaprylylmethylammonium chloride (Aliquat 336) was a gift from General Mills, Inc.; tetachlorotetrafluoropropane (Freon 214) was a gift from E. I. DuPont deNemours & Co.; Chromosorb W (100 to 200 mesh; acid-washed and dimethylchlorosilane-treated) was purchased from Chemical Research Service, Inc., Addison, Ill.

**RESULTS**

**Amino acid-acceptor ability.** The optimal conditions required for maximal acylation with trophozoite and precyst synthetases were found to be the same with respect to pH, temperature, Mg2+/ATP ratio, and protein concentration. The rate of acylation of trophozoite and precyst tRNAs was constant for 10 to 20 min, but charging ceased after a maximum of 20 min. No loss in charged tRNA occurred after maximal charging levels were attained, suggesting that the enzyme preparations were essentially free from ribonucleases.

The profiles of accepting activities for 12 amino acids, obtained by post-chromatography charging of trophozoite tRNA, are shown in Fig. 1. It can be seen that this column gave good resolution of isoaccepting tRNAs from this organism. Tryptophan showed a single component, whereas multiple components were observed for the 11 other amino acids studied. Four components of activity could be detected for tRNAArg and tRNAAsu. Three components each were found for tRNAs accepting lysine, serine, phenylalanine, isoleucine, and glycine, whereas tRNAVal, tRNA Met, tRNA Ala, and tRNAAn each were eluted in two components.

Figure 2 shows the RPC-2 elution patterns.
ENCYSTMENT OF A. CASTELLANII

Fig. 1. RPC-2 chromatography of A. castellanii trophozoite tRNAs, as detected by post-chromatography charging.

obtained for the same 12 amino acids with tRNA from A. castellanii precysts. With the exception of tRNA_1le and tRNA_1mr, the number of isoaccepting species for the precyst tRNAs was the same as that observed for the trophozoite tRNAs. Precyst tRNA contained two components of acceptor activity for serine, as compared with three components of serine acceptor activity present in the trophozoite tRNA. Transfer RNA_1le from the precyst was eluted in a single component, whereas trophozoite tRNA_1le was resolved into three components. These differences were consistently observed when several different preparations of tRNAs from either precyst or trophozoite were used.

In comparing the elution profiles of tro-

Fig. 2. RPC-2 chromatography of A. castellanii precyst tRNAs, as detected by post-chromatography charging.
phozoite and precyst tRNAs, it was noted that the precyst tRNA eluted from the column at a slightly higher NaCl concentration. To facilitate comparison, results from chromatographic columns were plotted so that the point at which effluent A260 initially increased was taken as the zero effluent volume.

**Comparison of trophozoite and precyst serine-accepting tRNAs.** The elution patterns of tRNA<sub>ser</sub> from trophozoites and precysts, as detected by post-chromatography charging, were compared by plotting the amino acid-accepting activity versus effluent volume (Fig. 3). The first isoaccepting component of trophozoite tRNA<sub>ser</sub> was eluted in 28 fractions and could be charged with a total of 2.5 nmol of serine. The first precyst tRNA<sub>ser</sub> component was eluted in the same position, spanned 30 fractions, and had a total acceptance capacity of 2.0 nmol of serine. Component two of trophozoite tRNA<sub>ser</sub> was eluted in 20 fractions and could accept 1.26 nmol of serine. The second component of precyst tRNA<sub>ser</sub> was eluted in 22 fractions and could accept 1.14 nmol of serine. Trophozoite tRNA contained a third component of serine-accepting tRNA which could be charged with a total of 0.7 nmol of serine.

Figure 4 shows the profiles resulting from cochromatography of precharged seryl-tRNA from trophozoite and precyst. The two isoaccepting species of precyst seryl-tRNA were eluted from the column at approximately the same position as trophozoite seryl-tRNA species 1 and 2. The second precyst component trailed into the position of the third trophozoite seryl-tRNA component. These observed differences were confirmed when the labeled amino acids were reversed.

The trophozoite and precyst tRNAs were each charged in separate reactions with either the homologous or heterologous synthetase preparations. Cochromatography of the resulting seryl-tRNAs is shown in Fig. 5. There was no detectable difference in the trophozoite elution profile (Fig. 5, left panel) resulting from attaching serine to trophozoite tRNA with either the trophozoite or the precyst synthetase. Similarly, the elution pattern of precyst seryl-tRNA (Fig. 5, right panel) was the same when either enzyme was used to charge the tRNA.

Trophozoite and precyst tRNAs were acylated with serine, using their respective synthetases, in a reaction mixture containing 0.1 μmol

![Graph showing comparison of trophozoite and precyst tRNA elution profiles](http://jb.asm.org/)
of cytosine triphosphate (CTP). There was no difference in the charged levels of either tRNA when CTP was present in the reaction mixture, as compared to control assays without CTP.

The trophozoite seryl-tRNA profile was investigated further by heating the tRNA in 0.01 M Mg²⁺ at 85 °C for 5 min, fast cooling, and acylating with [³H]serine. Another portion of the same tRNA preparation, but not heated, was acylated with [¹⁴C]serine. Cochromatography of the two seryl-tRNAs indicated that there was no alteration in the elution profile due to the heat treatment.

RPC-2 fractions containing seryl-tRNA peak three from trophozoite tRNA were pooled, and the tRNA was precipitated by the addition of cold 95% ethanol. The precipitate was dissolved in glycine-sodium hydroxide buffer (pH 9.1) and incubated at 37 °C for 60 min to remove the attached [³H]serine. The tRNA₃₉₅ was then precipitated and assayed for its ability to be acylated with [¹⁴C]serine. It was found that the “stripped” tRNA₃₉₅ component 3 could be reacylated with [¹⁴C]serine to its maximal capacity of 16% (calculated from Fig. 3) of the total serine-accepting activity.

Comparison of trophozoite and precyst isoleucine-accepting tRNAs. Figure 6 presents the results of comparing the RPC-2 elution profiles of tRNA₁₀e from trophozoites and precysts by adjusting the zero point of effluent volume to the initial rise in A₆₀₀ readings. Trophozoite tRNA₁₀e gave three well-resolved peaks of amino acid-accepting activity, whereas precyst tRNA₁₀e had only one peak of activity.

Fig. 5. Left panel: Cochromatography of trophozoite seryl-tRNA when charged by trophozoite synthetase or precyst synthetase. Twenty A₆₅₀ units of tRNA were charged with [³H]serine by using trophozoite synthetase, and 20 A₆₅₀ units of tRNA were charged with [¹⁴C]serine by using precyst synthetase. The charged tRNAs were mixed, applied to a RPC-2 column (1 by 90 cm), and eluted. Ninety-seven percent of the [³H]counts per minute and 97% of the [¹⁴C]counts per minute were eluted from the column. Each fraction is expressed as a percent of the total [³H] or [¹⁴C] counts eluted. Right panel: Cochromatography of precyst seryl-tRNA when charged by trophozoite or precyst synthetase. Experimental details as for left panel. Ninety-six percent of the [³H] counts per minute and 94% of the [¹⁴C] counts per minute were eluted from the column. Symbols: ●●●, trophozoite synthetase; ●●●●, precyst synthetase.

Fig. 6. A comparison of the RPC-2 chromatographic profiles of trophozoite and precyst tRNA₁₀e by adjusting the profiles from Fig. 1 and 2 according to effluent volume, as described in Fig. 3. Symbols: ●●●●, trophozoite tRNA₁₀e; ●●●●, precyst tRNA₁₀e.
This peak eluted in the same position as the first trophozoite peak.

Cochromatography of trophozoite and precyst isoleucyl-tRNA on RPC-2 columns shows that both of these charged tRNAs elute from the column as single peaks and at the same position (Fig. 7). Reversal of the labeled amino acids or the synthetase to charge the tRNA did not alter the elution pattern of either trophozoite or precyst isoleucyl-tRNA.

Figure 8 displays the elution profiles of precharged isoleucyl-tRNA from trophozoites and precysts after separate chromatography on RPC-2 columns. Again, both isoleucyl-tRNA preparations were eluted from the column in a single component.

Trophozoite tRNA was eluted from an RPC-2 column, and fractions from component 1 and component 3 (as indicated in Fig. 9, left panel) were each pooled separately. The tRNA from each component was precipitated, charged with labeled isoleucine, and cochromatographed as described for Fig. 9 (right panel). As can be seen in this figure, the combined components recovered from post-chromatography charging were eluted as a single component after prechromatography charging. The amount of isoleucyl-tRNA eluting from the column was that expected from combining the two components of amino acid-accepting material.

Trophozoite tRNA was heated at 85°C for 5 min, fast-cooled, and chromatographed on an RPC-2 column. When the fractions were assayed for isoleucine-accepting activity by post-chromatography charging, three isoaccepting species of activity could still be detected, and there was not a significant decrease in the accepting activities of any of the three components.

It seemed possible that during the incubation an enzymatic modification of the tRNA could occur. Thus, the following experiment was done. Trophozoite tRNA was incubated under conditions identical to those for pre-chromatography charging, except that radioactive isoleucine was omitted from the reaction mixture.

![Fig. 7. Cochromatography of trophozoite and precyst isoleucyl-tRNA. Twenty A260 units of trophozoite tRNA were charged with [3H]isoleucine by using trophozoite synthetase. Twenty A260 units of precyst tRNA were charged with [14C]isoleucine by using precyst synthetase. The charged tRNAs were mixed and applied to and eluted from an RPC-2 column. Ninety-four percent of the 3H counts per minute and 92% of the 14C counts per minute were recovered from the column. Symbols: O—O, trophozoite isoleucyl-tRNA; ——O, precyst isoleucyl-tRNA.

![Fig. 8. RPC-2 chromatography of trophozoite isoleucyl-tRNA (A) and precyst isoleucyl-tRNA (B). Precharged isoleucyl-tRNA from trophozoite and from precyst were eluted on separate columns as described in Materials and Methods. Recovery of input radioactivity was 97% in both instances.](http://jb.asm.org/)
This "sham-acylated" tRNA was loaded onto an RPC-2 column and eluted. By post-chromatography charging, it was determined that tRNA\textsubscript{\textit{le}} could now be fractionated into only two isoaccepting species (Fig. 10). The major component represents 88% of the total isoaccepting tRNA recovered from the column, whereas the second component of tRNA\textsubscript{\textit{le}} represents only 12%. This compares with percentages of 43, 34 and 22 for isoaccepting species 1, 2 and 3, respectively (data taken from Fig. 6), usually observed for "untreated" trophozoite tRNA.

**DISCUSSION**

The results presented in this study show that there are differences between trophozoite and precyst tRNAs of \textit{A. castellanii}. There is a third isoaccepting species of seryl-tRNA unique to trophozoites which is evident by both post- and pre-chromatography charging. To assure that this difference was real and not due to technical artifacts, it was determined that charging the tRNA with either trophozoite or precyst synthetase did not alter the results. Reversal of labeled amino acids also ruled out any possibility of charging with a labeled contaminant of the radioactive amino acids. Furthermore, the elution profiles of trophozoite and precyst seryl-tRNA were not altered by heating to 85°C prior to acylation and chromatography. This suggests that the observed tRNA profile differences were not products of partial nuclease digestion (14), since such tRNA molecules are irreversibly denatured after this treatment.

The initial observation by post-chromatography charging that trophozoites contained three components of tRNA\textsubscript{\textit{le}} where precysts had only one component was not confirmed with pre-chromatography charging. This was an unexpected finding, and attempts to explain this result by technical artifacts were not successful. Several explanations for this result were considered. One was that the conditions for pre-chromatography charging might lead to aggregations of the three components. Sueoka et al. (22) reported interconversions of two forms of tryptophanyl-tRNA as observed on methylated albumin kieselguhr (MAK) columns of \textit{Escherichia coli} tRNA. They suggested that these two forms could be the result of alterations by phenol in conventional extraction procedures. They also suggested that rearrangements in secondary structure due to concentration differences in ATP or Mg\textsuperscript{2+} could lead to aggregation of the two forms. Since the precharged tRNAs have an additional phenol extraction step and since there is an increased Mg\textsuperscript{2+} concentration in the assay reaction during pre-chromatography charging, it seems possible that the results observed for \textit{A. castellanii} tro-
phagosome isoleucyl-tRNA could be due to aggregation of the three components. It is also possible that during precharging, the enzyme preparation modifies the tRNA in such a way as to alter its chromatographic properties. An obvious modification, of course, would be the addition of the CCA terminus, or part of it, onto tRNA molecules partially degraded at the 3' end. Based on levels of charging only, after incorporation of CTP into the assay, this does not seem to be a likely explanation. The sham acylation experiment (Fig. 10) revealed that 12% of the isoleucine-accepting tRNA was not modified by preincubation. This is an unexplained observation, since this component of tRNA is less than any given component normally occurring on a chromatographic profile of tRNA. To explain the results reported for tRNA, it will be necessary to work with purified tRNA and purified isoleucyl-tRNA synthetase.

The frequent finding of chromatographic profile variations of seryl-tRNA (2, 23, 24, 26) during differentiation and neoplastic development suggests that these tRNA species may be important to these processes.

In A. castellanii, there are alterations in RNA synthesis immediately before encystment (16, 19); in addition, considerable turnover of RNA and changes in the specific activity of RNA polymerase during the early hours of encystment have been reported (17). It is tempting to speculate that the altered patterns of seryl-tRNA found in encysting A. castellanii may be related to the initiation of the encystment process. Thus, the findings reported here could be interpreted in terms of the “adaptor modification” hypothesis proposed by Sueoka and Kano-Sueoka (20). This hypothesis suggests that, as a consequence of the modification of specific tRNA molecules, the function of some of the genes which are transcribed can be shut off and the rest of the genes kept functional at the translational level. By modifying the third seryl-tRNA, the function of certain vegetative genes could be turned off, thus initiating the encystment process. Alternatively, a quantitative difference in a certain tRNA species, as suggested by Anderson (1), might regulate the rate of synthesis of certain proteins. If the third peak of seryl-tRNA found in trophozoites becomes the rate-limiting tRNA required for synthesis of a critical protein found in trophozoites, it could conceivably be the trigger for the initiation of encystment.

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LITERATURE CITED