UPTAKE AND AMINOACYLATION OF EXOGENOUS TRANSFER RNA BY MOUSE LEUKEMIA CELLS

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SUMMARY

Effective uptake of exogenous ^{3}H-labelled tRNA into the active metabolic tRNA pool of mouse L1210 cells was demonstrated by detecting aminoacyl-(^{3}H)tRNA synthesized in the intact, pulsed cells.

INTRODUCTION

Our laboratory previously reported that E. coli transfer RNA can be "taken up" by a mouse leukemic cell line (L1210), by a human lymphoblastic cell line (NC-37), and by fresh and phytohemagglutinin stimulated human peripheral blood lymphocytes (1,2). Subcellular fractionation and autoradiographic studies indicated that radioactivity from incorporated tRNA was present in nuclei, lysosomes, mitochondria and, predominantly, the "cell sap." Although considerable degradation was noted, approximately 20% of the incorporated E. coli tRNA could be recovered from L1210 cells in an intact, functional state, after a 30-minute incubation period. Recently, Crooke, et al. similarly reported the uptake of homologous tRNA by Novikoff hepatoma ascites cells, which was markedly augmented by the use of the polycation, DEAE-dextran (3).

Although the above studies clearly demonstrate that exogenous tRNA may be assimilated by cells, they do not show that the tRNA has been effectively incorporated in terms of being able to function in the host cells. In fact, with the exception of purified RNA from RNA viruses we are unaware of any conclusive demonstration of a specific biochemical function of exogenously pulsed RNA molecules in receptor cells (Cf. ref. 4 for a comprehensive review). In the present experiments effective

incorporation of homologous tRNA into the active metabolic tRNA pool of L1210 cells is shown by demonstrating aminocylation of pulsed tRNA by intact cells.

MATERIALS AND METHODS

Preparation of ^{3}H-labelled tRNA. L1210 cells, 0.5-1.0 x 10^{6} cells/ml, were incubated with ^{3}H-uridine (New England Nuclear Corp.; specific activity 25.4 mCi/mMole), 20 μCi/ml, for 16 hours at 37°C. The ^{3}H-tRNA was extracted and purified according to the method of Yang and Novelli (5). Two different preparations of ^{3}H-tRNA had specific activities of 0.18 μCi/μg and 0.20 μCi/μg, using a conversion factor of 20 A_{260} units per mg tRNA in aqueous solution.

Cell culture and tRNA uptake methods. L1210 cells were grown in spinner cultures at 37°, using RPMI 1630 and 20% fetal calf serum. The cells were harvested and washed, as previously described (1), and used for uptake studies if at least 90% cell viability was demonstrated by trypan blue dye exclusion. Washed cells were suspended at 3 x 10^{6} cells/ml in RPMI 1630 containing ^{3}H-tRNA (1-5μg/ml) and incubated at 37°C for 30 minutes. In some experiments DEAE dextran (DEAE-D; Pharmacia, Uppsala, Sweden), 50 μg/ml, was incubated with the cells for 30 minutes at 37°C; in these instances, the cells were re-washed twice with RPMI 1630 before incubating with the ^{3}H-tRNA. In one experiment, actinomycin D (Sigma Corp. St. Louis, Mo.), 5 μg/ml was added to the culture 10 minutes before the ^{3}H-tRNA.

Aminoacylation and processing of ^{3}H-labelled tRNA. tRNA was aminoacylated in cell-free experiments using partially purified amino-acyl-tRNA synthetases from L1210 cell, according to previously reported methods (6). The conditions necessary to achieve saturation of tRNA with amino acids was determined by acylating with a mixture of ^{14}C-labelled amino acids (New England Nuclear Corp.). For recovery of amino-acyl-(^{3}H)-tRNA from intact, pulsed cells the method of Yang and Novelli

was employed (5). In some experiments, a mixture of ^{14}C-labelled amino acids was incubated with the ^{3}H-tRNA pulsed cells 5 minutes prior to harvest and the ^{14}C-aminoacyl-tRNA was extracted from the cells and processed in common with aminoacyl-(^{3}H)-tRNA. Deacylation was preformed by incubating at 37°C for 1 hour in 0.3 M Tris, pH 8.0 (7); deacylation was greater than 94% complete. Aminoacyl-tRNA was phenoxyacetylated with phenoxyacetic acid N-hydroxysuccinimide ester (Schwarz Bioresearch, Orangeburg, N.Y.), according to Marmor, et al. (8); recovery exceeded 95%.

Benzoylated Diethylaminoethylcellulose chromatography. A 1x12 cm BD-cellulose column (Schwarz Bioresearch) was prepared according to the method of Gillam and Tener (9). Samples were applied in 5 ml of Buffer A (0.05 M sodium acetate, pH 5.0, 10 mM $MgCl_2$), containing 0.5 M NaCl. Elution (25°C; 2 ml/minute) was performed initially with 200 ml of Buffer A, containing 1.5 M NaCl, followed step-wise by 100 ml of Buffer A, containing 1.5 M NaCl and 20% ethanol. Ten milliliter fractions were collected at 4°C, mixed with 2 A_{260} of carrier DNA, precipitated with 0.6 ml of cold 100% trichloroacetic acid and processed for liquid scintillation counting as reported (6); recovered, precipitable radioactivity exceeded 87%.

RESULTS AND DISCUSSION

The experimental approach used in testing for aminoacylation of pulsed exogenous tRNA by intact cells is illustrated in Fig. 1. Essentially, the detection of aminoacylated tRNA in these experiments depended upon the following two factors: 1) only aminoacylated tRNA is specifically phenoxyacetylated by phenoxyacetic acid N-hydroxysuccinimide ester and 2) N-phenoxyacetyl-aminoacyl-tRNA is tightly bound to BD-cellulose, requiring ethanol for elution, whereas most non-aminoacylated tRNA's can be eluted from BD-cellulose with saline solution (9).

When ^{3}H-uridine-labelled tRNA from L1210 cells was maximally aminoacylated in cell-free experiments with all twenty amino acids and then phenoxyacetylated, 41% of the ^{3}H-tRNA required ethanol for elution from

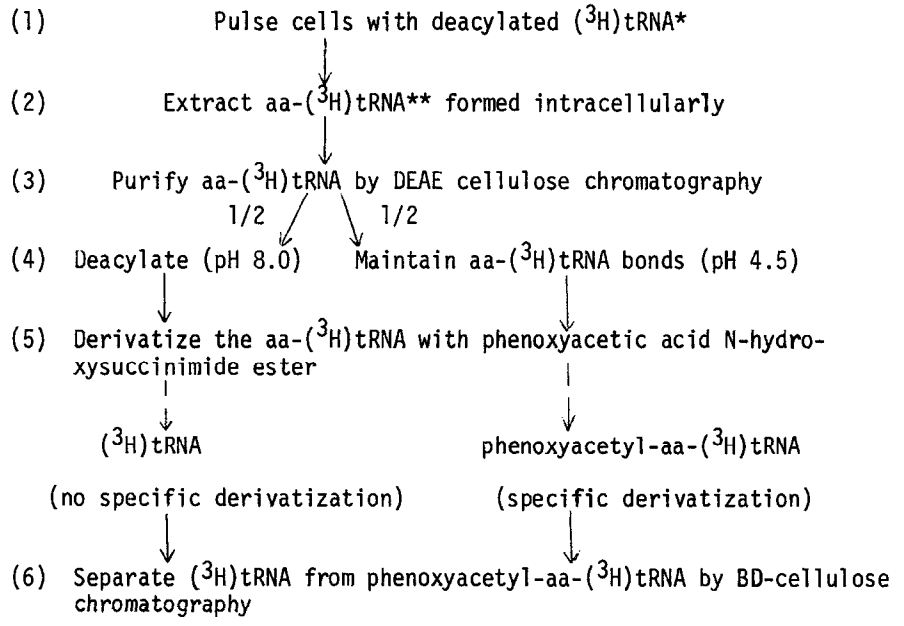

Figure 1. Outline of experimental design to demonstrate intracellular aminoacylation of pulsed, homologous (^{3}H)-tRNA by L1210 cells.
* Details of each step of the procedure are described in MATERIALS AND METHODS.
** aminoacyl-(^{3}H)-uridine-labelled transfer RNA.

BD-cellulose (Table 1). After identical treatment but followed by deacylation, only 16% of the ^{3}H-tRNA required ethanol for elution. The observation that 59% of ^{3}H-tRNA which was subjected to aminoacylation and phenoxyacelylation procedures failed to bind firmly to BD-cellulose (Table 1) may be partly attributed to incomplete purification of the tRNA and partly to the presence of inactive tRNA (which may account for a major fraction of purified eukaryotic cell tRNA; 10). Conversely, the tight binding of 16% of deacylated ^{3}H-tRNA to BD-cellulose is primarily related to the intrinsic properties of certain tRNA species and to a lesser degree to non-specific phenoxyacetylation and possible incomplete deacylation (9). Since the only difference between the aminoacylated and the deacylated ^{3}H-tRNA preparations was exposure of the latter to a deacylation procedure, we

Table 1.

The Effect of Cell-free Aminoacylation on the Fractional Recovery from BD-cellulose of L1210 Cell (^{3}H)tRNA*

	Percent of Radioactivity Eluted	
	1.5 M NaCl	1.5 M NaCl + 20% ethanol
Acylated**	59.0 ± 0.9***	41.1 ± 0.9
Deacylated	83.7 ± 0.7***	16.4 ± 0.7

*Processed as outlined in Fig. 1 from Step 3.

**Aminoacylated to the maximum acceptance capacity of the L1210 cell (^{3}H)tRNA with all twenty amino acids as described in MATERIALS AND METHODS.

***Average values of two experiments ± standard error.

conclude that the differences in BD-cellulose elution are specifically related to differences in aminoacylation (7,9 and vide infra).

Similar to the cell-free aminoacylation results, ^{3}H-tRNA which was recovered from pulsed L1210 cells, according to the procedure outlined in Fig. 1, showed an increased fraction requiring ethanol for elution from BD-cellulose compared to an equal portion of the recovered ^{3}H-tRNA from each pulse experiment which was subjected to deacylation (Table 2). Qualitatively similar results were obtained for the three different pulse conditions shown in Table 2.

Pre-incubation of the cells with DEAE-D markedly increased the amount of pulsed ^{3}H-tRNA which becomes cell-associated (Fig. 2 and ref. 3). Similarly, DEAE-D pre-incubation increased the fraction of pulsed ^{3}H-tRNA which binds tightly to BD-cellulose (Table 2). These data indicate that DEAE-D augments not only cell association of tRNA but also the effective

Table 2.

The Effect of Deacylation on the Fractional Recovery from BD-cellulose of Pulsed ^{3}H-tRNA After Extraction from L1210 Cells*

tRNA Pulse Conditions	Deacylation Performed	Percent of Radioactivity Eluted 1.5 M NaCl	1.5 M NaCl + 20% ethanol
tRNA	-	77.9	22.1
	+	86.0	14.0
tRNA + DEAE-D preincubation	-	71.75 ± 1.32**	28.25
	+	82.90 ± 1.03**	17.10
tRNA + DEAE-D preincubation + actinomycin D	-	69.8	30.2
	+	80.9	19.1

*Processed as outlined in Fig. 1.

**Averages of four experiments ± standard error. The probability is <0.005 that the reported mean values are, in fact, not different according to Null hypothesis.

incorporation of the tRNA into metabolic sites resulting in aminoacylation. Further, since 41% was the highest achieveable binding value for maximally aminoacylated ^{3}H-tRNA (Table 1), the 28% binding noted for pulsed (^{3}H)-tRNA after DEAE-D pre-incubation versus 17% binding for comparable, deacylated (^{3}H)-tRNA (Table 2) suggests that a substantial proportion of the incorporated ^{3}H-tRNA molecules were aminoacylated intracellularly under these conditions.

The following experiments provided confirmatory evidence that the above observations were due to aminoacylation of the pulsed ^{3}H-tRNA and not

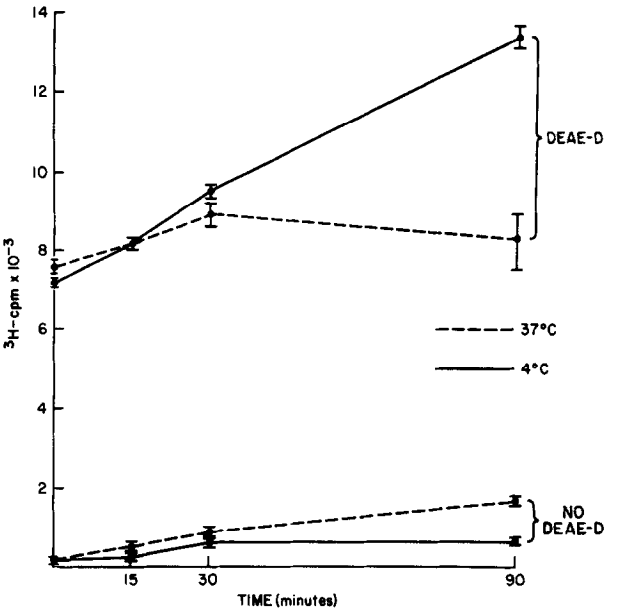

Figure 2. The effect of temperature and DEAE-D preincubation on the cell association of homologous (^{3}H)-tRNA with L1210 cells. Three x 10^6 cells were pulsed with ^{3}H-tRNA, 2 µg/ml, as described in MATERIALS AND METHODS. After the appropriate time, 3 volumes of RPMI 1630 containing a 50 fold excess of non-radiolabelled tRNA was added, and the samples were processed as described by Crooke, et al. (3). The points represent the mean values ± standard error of triplicate cultures.

to adventitious factors. (1) Virtually identical results were obtained with two different preparations of ^{3}H-uridine labelled tRNA. (2) In several experiments, cells were pulsed with ^{14}C-labelled amino acids and the ^{14}C-aminoacyl-tRNA was recovered and processed with the aminoacyl-(^{3}H)-tRNA. The ^{14}C-labelled product served as a marker for following intact aminoacyl-tRNA and for determining the effectiveness of deacylation, derivatization and elution from BD-cellulose (see MATERIALS AND METHODS). (3) No aminoacyl-tRNA was found in the cell culture medium after ^{3}H-tRNA pulsing, eliminating the possibilities that the results might be due to either leakage of aminoacyl-tRNA synthetases into the culture medium leading to extra-cellular aminoacylation or that extra-cellular, non-specific binding of amino acids to ^{3}H-tRNA occurred. (4) Repeated

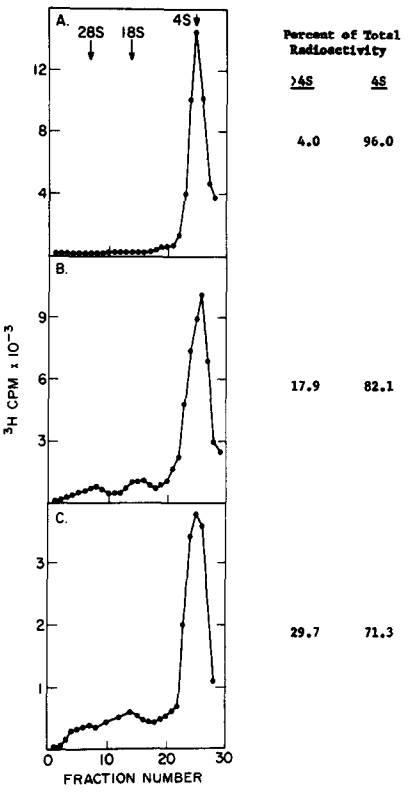

Figure 3. Sucrose density gradient analysis of unpulsed (^{3}H)-tRNA and of ^{3}H-labelled RNA recovered from ^{3}H-tRNA pulsed L1210 cells. Ten ml of cell suspension, 3 x 10^6 cells/ml, was pulsed with ^{3}H-tRNA, 2 µg/ml, for 30 minutes at 37°C. Whole cellular RNA was extracted by the hot phenol -SDS method and applied to a linear sucrose gradient, 5-40%, containing 0.1 M NaCl; 0.001 M EDTA; and 0.01 M sodium acetate, pH 5.0. The samples were centrifuged in a SW41 rotor at 83,000 x g for 18 hours. External markers were provided by (^{14}C) uridine-labelled RNA from L1210 cells. (A) Unpulsed L1210 cell tRNA; (B) ^{3}H- RNA extracted from DEAE-D treated cells; (C) ^{3}H-RNA extracted from non-DEAE-D treated cells.

exposure of ^{3}H-tRNA to the mild alkaline condition used in deacylation did not affect the chromatographic or functional (capacity for aminoacylation) properties of the tRNA (7). (5) In experiments with DEAE-D (Table 2; Fig. 2), complexing of ^{3}H-tRNA and the DEAE-D which might then dissociate during the deacylation procedure could conceivably affect BD-cellulose binding. However, this seems unlikely since treatment of the recovered

^{3}H-tRNA to disrupt such complexes, including extensive DEAE cellulose chromatographic washing and treatment with the strong cationic detergent cetyltrimethylammonium bromide had no effect on BD-cellulose chromatography of the tRNA. (6) Incubation of the cells with actinomycin D (5 μg/ml) for 10 minutes prior to ^{3}H-tRNA pulsing did not alter the results (Table 2). Therefore, we are not detecting aminoacylation of endogenously synthesized ^{3}H-tRNA which has become radiolabelled through re-cycling of the label from degraded, exogenous ^{3}H-tRNA. As reported by others (4), actinomycin D did not affect the uptake of pulsed RNA. The significance of radiolabel recycling is further discounted by the observation that less degradation of ^{3}H-tRNA occurred in DEAE-D treated cells than in untreated cells (Fig. 3). (7) Finally, as discussed in detail elsewhere (2), the uptake of exogenous tRNA is a complex process which is not entirely energy dependent. On the other hand, aminoacylation of tRNA is stongly temperature dependent. Therefore, the finding that the ^{3}H-tRNA is cell-associated to a similar degree at either 4° or 37°C (Fig. 2) but only acquires the BD-cellulose binding properties of aminoacylated tRNA at 37°C (Table 3), provides additional evidence that aminoacyl-tRNA is being detected.

The possibility that cellular differences in isoaccepting species of tRNA may be important regulatory factors in cellular differentiation and neoplasia has been extensively reviewed (11), and there is some evidence to suggest that specific isoaccepting species of tRNA may be involved in the phenotypic expression of specific cellular proteins (6,12,13). By demonstrating that pulsed tRNA can be utilized effectively by receptor cells, the present work supports the feasibility of altering cellular gene expression by achieving optimal intracellular concentrations of appropriate tRNA species.

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Table 3.

The Effect of Pulse Temperature on the Fractional Recovery of ^{3}H-tRNA from BD-cellulose*

Pulse** Temperature	Percent of Radioactivity Eluted	
	1.5 M NaCl	1.5 M NaCl + 20% ethanol
(A) 37°C - 37°C	73.16 ± 1.40***	26.84 ± 1.40
(B) 4°C - 4°C	80.40 ± 1.47***	19.60 ± 1.47
(C) 4°C - 37°C	74.80 ± 0.15***	25.20 ± 0.15

*Processed as outlined in Fig. 1.

**The cells were pulsed with ^{3}H-tRNA, 2 ug/ml, for 20 minutes at the initial temperature. Then the cells were diluted with 10 volumes of RMPI 1630 containing a 100x excess of non-radiolabelled tRNA, and the incubation was continued at the second temperature for an additional 10 minutes.

***Averages of 3 experiments ± standard error. The values reported for (A) and (C) are not significantly different. (A) and (C) are different from (B) with greater than 95% confidence.

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