Aminoacylation of Transfer Ribonucleic Acid *in Vitro* during the Mitotic Cycle of *Physarum polycephalum*†

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ABSTRACT: *In vitro* aminoacylation of purified transfer RNA was studied during the mitotically synchronous growth phase of the myxomycete *Physarum polycephalum*. Transfer RNA was prepared from early-cycle (1-hr postmitosis), mid-cycle (4-hr postmitosis), and late-cycle (1–2 hr premitosis) stationary plasmodia, and aminoacylated with homologous and heterologous synthetase. The relative amount of tRNA/unit amount of total nucleic acid did not vary during the cycle. The total *in vitro* amino acid acceptor activity/A_{260} unit of tRNA remained constant at 75% (picomoles of amino acid/nanomole of tRNA), and the relative acceptor activity for each amino acid was strikingly uniform throughout the cycle. The data show that in terms of *in vitro* aminoacylation ability neither the tRNAs nor the synthetase enzymes change during the mitotic cycle of *Physarum* and demonstrate a tight quantitative control on the synthesis of tRNA during the mitotically synchronous growth phase of the organism.

The possibility that tRNA[1] may be involved in the control of protein synthesis at the translational level has received much consideration (Novelli, 1967; Sueoka and Kano-Sueoka, 1970; Gauss *et al.*, 1971). Data in support of this possibility have been obtained by comparing the amino acid acceptor activities and/or isoaccepting tRNA chromatographic profiles from different tissues within the same organism (Hatfield and Portugal, 1970; Ortwerth, 1971), different organelles in the same tissue (Guderian *et al.*, 1972), from normal and leukemic lymphoblasts (Gallo and Pestka, 1970), normal liver and Novikoff hepatoma (Baliga *et al.*, 1969), between various animal and human cells (Taylor *et al.*, 1967; Holland *et al.*, 1967), and during phage infection (Waters and Novelli, 1967, 1968; Sueoka and Kano-Sueoka, 1964). Similar studies have also been carried out in developmental systems (Zeikus *et al.*, 1969; Bagshaw *et al.*, 1970; Caston, 1971; Chou and Johnson, 1972), and during sporulation in *Bacillus subtilis* (Chuang and Doi, 1972). In almost all instances some quantitative changes in either the amount of aminoacylation and/or in the relative amounts of the isoacceptors have been found.

In contrast to the information available from systems such as those cited above, very little data have been reported concerning changes in aminoacylation levels or in isoaccepting tRNAs during the mitotic or cell cycle. Agarwal *et al.* (1970) showed that during the G_1 phase of the partially synchronous cell cycle of regenerating rat liver no change in aminoacylation levels or isoaccepting profiles occurred when compared to SHAM operated animals. Tidwell *et al.* (1972) investigated the same system throughout G_1 and into early and late S period. Similar results were obtained in that no differences were found in the relative percentages of amino acid acceptance or isoaccepting profiles for the tRNAs reported.

It has recently been shown (Melera and Rusch, submitted for publication) that the amount of total nuclei acid per milligram dry weight and the amount of sRNA per unit amount of total nucleic acid do not significantly change during the mitotic cycle of *Physarum polycephalum*, a myxomycete whose mitotic cycle contains no G_1 period and whose 2–3-hr DNA synthetic period directly follows mitosis. Since during its growth phase this simple eucaryote displays naturally synchronous and recurrent mitotic divisions with a doubling time (mitotic cycle time) of approximately 8 hr (Mohberg and Rusch, 1969) it was decided to analyze its tRNA population to see if any changes in the total amino acid acceptor activity or in the individual amino acid acceptor activities could be detected during this highly synchronous growth phase.

Materials and Methods

Maintenance of Cultures. Stationary cultures of *Physarum polycephalum* subline M_3C7 were grown as described previously (Mohberg and Rusch, 1969), except that the large pan cultures used here were set up on Whatman 576 filter paper instead of Millipore paper, and 2 ml of a 1:1 (v/v) dilution of packed microplasmodia in sterile water was used as inoculum.

Preparation of tRNA and Aminoacyl tRNA Synthetase. The basic techniques used here are those described by Yang and Novelli (1971) for the analysis of mammalian tRNA except that the high salt precipitation of total nucleic acid and the stripping of the final tRNA product were eliminated. Also, rotory evaporation at 30° (pH 7.0) was used to concentrate the tRNA preparations after both DEAE and Sephadex G-100 chromatography. Total nucleic acid was prepared from lyophilized plasmodia with a three-step phenol–sodium dodecyl sulfate–bentonite technique described previously (Melera and Rusch, submitted for publication). The final ethanol-washed precipitate was drained well and solubilized in total nucleic acid buffer (0.01 M Tris-HCl (pH 7.5), 0.05 M NaCl, 0.001 M $MgCl_2$, 0.001 M disodium EDTA, and 0.001 M dithiothreitol) and the tRNA was purified by DEAE and Sephadex G-100 chromatography. The final product (30–50 A_{260} units/ml) was assayed for purity by uv absorption and analysis for protein (Lowry *et al.*, 1951), DNA (Burton, 1956), and carbohydrate (Fairbairn, 1953).

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[1] Abbreviations used are: tRNA, transfer RNA; sRNA, 4S plus 5S RNA; uv, ultraviolet; DEAE-cellulose, diethylaminoethylcellulose; A_{260} unit, the amount of nucleic acid required to give an optical density reading of 1 in a 1-cm light path at 260 mμ.

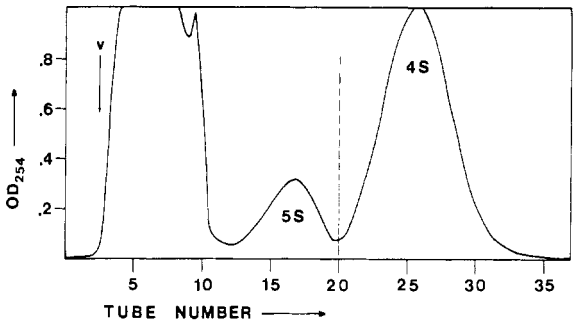

FIGURE 1: Sephadex G-100 profile of *Physarum* total nucleic acid after DEAE-cellulose chromatography. Approximately 100 OD units of nucleic acid was chromatographed on a 1.5 cm × 180 cm G-100 Sephadex column at a pressure head of 30–50 cm. Four-milliliter fractions were collected and tubes 20–35 pooled and then concentrated by rotary evaporation: (—), OD_{254}.

Aminoacyl-tRNA synthetase was prepared by homogenizing fresh tissue in 0.01 M potassium phosphate (pH 7.5), 0.005 M KCl, 0.001 M $MgCl_2$, 0.002 M dithiothreitol, and 15% glycerol in a Duall homogenizer with Teflon pestle. The homogenate was centrifuged at 105,000*g* for 2 hr and the supernatant fraction passed through a 2.5 cm × 100 cm Sephadex G-100 column adapted for ascending chromatography. The fractions containing tRNA synthetase activity were pooled and then concentrated in an Amicon Diaflow with PM-10 membrane. The concentrated enzyme solution was centrifuged at 10,000*g* for 15 min to remove insolubles and the resulting supernatant was adjusted to 50% glycerol, and 10^{-4} M ATP (pH 7.0), before being stored at −25°.

Aminoacylation Assay Conditions. The reaction mixture contained, per milliliter, 50 μmol of Tris-HCl (pH 7.5), 50 μmol of KCl, 2 μmol of dithiothreitol, 4 μmol of ATP (pH 7.0), ~1.0 A_{260} unit of tRNA, 20 nmol of [^{14}C]amino acid, 20 nmol of the remaining 19 unlabeled amino acids, and approximately 0.50 mg of synthetase protein. The magnesium concentration for each reaction was the same as that described by Yang and Novelli (1971) and varied between 3 and 20 μmol, depending upon the amino acid charged. Unless otherwise stated, all charging assays were carried out in 0.5-ml reaction mixtures at 30° with the filter paper disk technique (Bollum, 1968; Mans and Novelli, 1961).

Results

tRNA and Synthetase Preparation. Figure 1 shows a typical G-100 chromatogram of *Physarum* total nucleic acid after DEAE chromatography. Electrophoresis of an aliquot of the 4S fraction in 7.5% acrylamide gel for 5 hr before and after heating the sample at 85° and then quick cooling in ice showed the material to be homogeneous in molecular size and, within the limits of the method, to contain no heat-denaturable nicked molecules. It was also determined that of the sRNA fraction recovered from the G-100 column, 12% was 5S RNA and 88% was 4S RNA. Assuming 100% recovery of both RNAs, these amounts accounted for 1.4 and 10.6% of *Physarum* total nucleic acid, respectively; the percentages did not change during the mitotically synchronous growth phase. Estimation of the true recovery of tRNA from total nucleic acid was based upon the recovery of 5S RNA (see Discussion) and indicated that approximately 85% of the total extracted tRNA had been recovered as purified tRNA.

The acceptor activity of the purified tRNA for various amino acids was measured before and after heating to 85° and the results were consistent with the electrophoretic data above, in that no loss in amino acid acceptor activity was observed after the heat treatment. It was also demonstrated that the acceptor activity for five amino acids was not altered by stripping the purified tRNA of any endogeneously charged amino acid by incubation in 0.3 M Tris-HCl (pH 8.0) at 37° for 30 min. This observation suggested that the purification procedure had allowed discharging of *in vivo* esterified amino acids, a result probably due in large measure to the 30° rotory evaporation steps at pH 7.0.

TABLE I: Effects of Preincubation on tRNA Charging Levels.[a]

Preincubation Time (min)	^{14}C cpm Incorporated at Plateau Level for		
	Ser	Ala	Leu
0 (control)	991	1620	3100
10	820	1320	2674
20	786	1460	2412
40	812	1367	2736

[a] Purified tRNA was aminoacylated normally as a control (0 time). Preincubation was carried out by incubating tRNA with the reaction mixture and synthetase protein without [^{14}C]amino acid for various periods of time at 30°. At the end of the preincubation, 5 μl of 0.001 M ATP (pH 7) and the appropriate amount of [^{14}C]amino acid were added and the kinetics of ^{14}C incorporation followed. The values shown in the table are plateau cpm's for 50-μl samples of the respective aminoacylation reaction. Different tRNAs and enzyme preparations were used for the various experiments.

Ultraviolet absorption analysis (260 mμ/280 mμ, 260 mμ/230 mμ, and 260 mμ/220 mμ ratios of 1.975, 2.046, and 1.300, respectively), Lowry protein analysis, Burton diphenylamine analysis, and anthrone carbohydrate analysis indicated that the purified tRNA preparations were free from contaminating protein, DNA, and carbohydrate.

The Sephadex G-100 enzyme concentrate was found to contain synthetase activity for 20 amino acids. Further purification by DEAE chromatography resulted in a quantitative loss of alanyl and glycyl activity and was abandoned. The presence of tRNA-CMP-AMP pyrophosphorylase in the synthetase preparations was confirmed by the method of Hurwitz and Firth (1966) with bakers yeast tRNA as substrate, except that the assay was run in the tRNA charging reaction mixture used in this paper. It was also observed that addition of CTP to the *Physarum* tRNA charging reaction did not alter the aminoacylation levels of five different amino acids, suggesting that the *Physarum* tRNA had intact-CCA termini.

Estimation of Nuclease Contamination in the Synthetase Preparations. Crude tRNA was prepared by high salt precipitation of total nucleic acid isolated from a shake flask culture of *Physarum* which had been labeled for 6 hr with 1 μCi/ml of [^{14}C]uridine. When incubated at 30° for various lengths of time with 1 mg of synthetase protein no ^{14}C radioactivity was rendered acid soluble suggesting that the G-100 concentrate contained little appreciable exonuclease activity. In an attempt to demonstrate that the G-100 synthetase preparation did not contain a more subtle endonuclease activity which might have been undetectable in the above experiment, purified tRNA was preincubated with enzyme for various

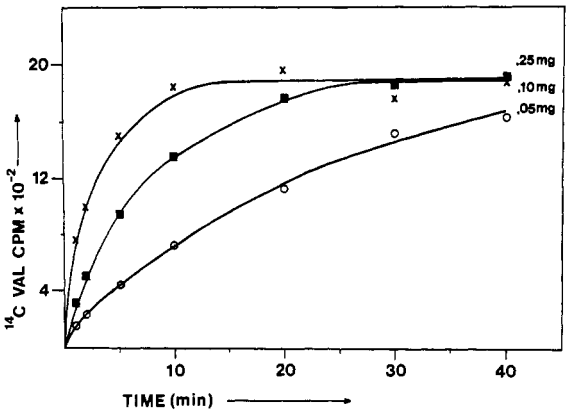

FIGURE 2: Effect of enzyme concentration on the aminoacylation of a fixed amount of purified tRNA. tRNA (0.4 A_{260} unit) was aminoacylated under standard conditions with [^{14}C]valine in the presence of 0.05 mg of synthetase protein (○), 0.1 mg of synthetase protein (■), and 0.25 mg of synthetase protein (×).

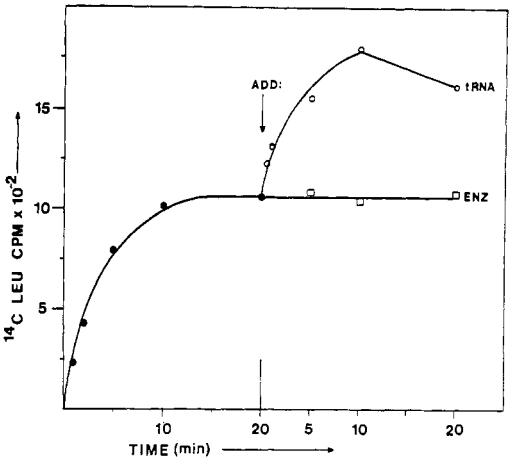

FIGURE 3: Effect of the addition of tRNA and enzyme on the extent of aminoacylation. tRNA (0.27 A_{260} unit) was aminoacylated with [^{14}C]leucine to plateau level (20 min) in each of two separate reaction mixtures shown above as one line (●). To one mixture, 0.1 mg of synthetase protein was added (at 20 min) and 5-, 10-, and 20-min time points were taken (□). To the other mixture, another 0.27 A_{260} unit of tRNA was added (at 20 min) and 1-, 2-, 5-, 10-, and 20-min time points were taken (○).

periods of time before charging (Muench and Berg, 1966). After the preincubation, a small amount of ATP and the [^{14}C]amino acid of choice were added to the reaction mixture containing the preincubated tRNA and enzyme, and the kinetics of ^{14}C incorporation followed. The data (Table I) show that the length of preincubation did not drastically alter the observed plateau level for the different charging reactions when compared to controls, strongly suggesting the absence of any untoward effect of enzyme on tRNA acceptor activity. All the reactions gave an initial 20% drop in acceptor activity during the first 10 min of preincubation. If this had been due to enzymatic degradation of tRNA, it would have been expected that the loss would have increased with longer preincubation time since substrate tRNA for the postulated nuclease would have been present and under those conditions the formation of product, presumably degraded tRNA, should have continued. This was not the case and a firm explanation for the initial decrease in acceptor activity has not as yet been established.

Characterization of the Aminoacylation Reaction. The data of Figure 2 indicate that when the tRNA concentration is fixed the time required for the aminoacylation reaction to reach plateau value is dependent upon the amount of synthetase protein present. Other experiments showed that the initial rate of aminoacylation increased linearly with increasing amounts of enzyme. Assaying each of the 20 acceptor activities indicated that plateau levels for all could be reached within 30 min using approximately 0.25 mg of synthetase protein and 0.5 OD unit of tRNA in a 0.5-ml reaction mixture. To demonstrate that the observed plateau levels were tRNA and not enzyme concentration dependent, the experiment shown in Figure 3 was carried out. After the [^{14}C]leucine aminoacylation reaction had reached its plateau at 20 min, the addition of more enzyme (in one experiment) or tRNA (in another experiment) was made to the reaction mixtures and incorporation of ^{14}C followed. The data show that the addition of more enzyme did not alter the plateau level, whereas the addition of tRNA to double the amount originally present essentially doubled the plateau level. Similar addition experiments, data not shown, consisted of adding more [^{14}C]amino acid, ATP, or magnesium to the reaction mixture. In no case was the plateau level increased, strongly suggesting that under the conditions used the only limiting factor in the aminoacylation reaction mixture was the tRNA concentration. It was then demonstrated (Figure 4) that the extent of the reaction was linearly dependent upon tRNA concentration and that the amount of synthetase used linearly acylated up to 1.0 A_{260} unit of purified tRNA.

tRNA Acceptor Activity during the Mitotic Cycle. Transfer RNA and synthetase were prepared from stationary cultures of *Physarum* in the mitotically synchronous growth phase during the interphase time between mitosis 2 and 3, *i.e.*, cycle II (Mohberg and Rusch, 1969). Samples were prepared from 1 hr after mitosis II (early), 4 hr after mitosis II (mid), and 1–2 hr before mitosis III (late). Homologous charging reactions were performed with early tRNA and early enzyme, mid tRNA and mid enzyme, and late tRNA and late enzyme.

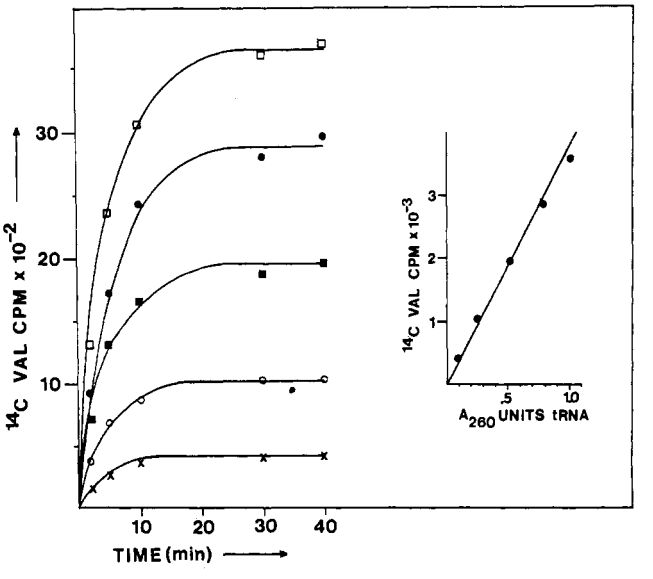

FIGURE 4: Dependence of the extent of aminoacylation upon tRNA concentration. Synthetase protein (0.25 mg) was used to aminoacylate increasing concentrations of purified tRNA with [^{14}C]valine. The kinetics of aminoacylation were followed: 0.105 A_{210} unit (×), 0.263 A_{260} unit (○), 0.525 A_{260} unit (■), 0.789 A_{260} unit (●), and 1.050 A_{260} units (□).

TABLE II: Aminoacylation of *Physarum* tRNA during the Mitotic Cycle.[a]

Amino Acid	Early tRNA+ Early Enzyme			Late tRNA + Late Enzyme			Early tRNA + Late Enzyme			Late tRNA + Early Enzyme		
	pmol	% Total	pmol/ OD	pmol	% Total	pmol/ OD	pmol	% Total	pmol/ OD	pmol	% Total	pmol/ OD
Ala	34.0	5.6	70.4	41.9	6.3	81.2	37.9	5.9	72.2	44.3	6.3	81.3
Arg	39.4	6.5	81.6	43.8	6.6	84.9	40.7	6.3	77.5	43.6	6.2	80.0
Asn	28.7	4.8	59.5	30.5	4.6	59.1	29.2	4.6	55.6	34.5	4.9	63.3
Asp	25.4	4.2	52.7	25.6	3.8	49.6	24.5	3.8	46.7	28.0	4.0	51.4
Cys	32.1	5.3	66.6	31.5	4.7	61.1	32.8	5.1	62.5	33.3	4.7	61.1
Glu	27.5	4.5	57.0	28.2	4.2	54.7	29.3	4.6	55.8	30.1	4.3	55.2
Gln	12.3	2.0	25.5	12.0	1.8	23.3	13.3	2.1	25.3	16.9	2.4	31.0
Gly	47.3	7.8	98.1	48.8	7.3	94.6	50.5	7.9	96.2	53.5	7.6	98.2
His	20.4	3.4	42.3	23.6	3.5	45.7	20.9	3.3	39.8	23.1	3.3	42.4
Ile	30.3	5.0	62.9	34.5	5.2	66.9	32.8	5.1	62.5	37.0	5.2	67.9
Leu	50.1	8.2	103.8	57.5	8.6	111.4	51.5	8.0	98.1	56.6	8.0	103.8
Lys	35.1	5.8	72.7	36.3	5.4	70.3	39.5	6.2	75.2	38.1	5.4	69.9
Met	36.0	5.9	74.6	36.2	5.4	70.2	37.8	5.9	72.0	40.0	5.6	73.4
Phe	27.7	4.6	57.5	27.2	4.1	52.7	24.3	3.8	46.3	28.7	4.1	52.7
Pro	24.0	3.9	49.7	23.5	3.5	45.5	24.1	3.8	45.9	26.2	3.7	48.1
Ser	44.8	7.4	93.0	46.5	7.0	90.1	48.4	7.5	92.2	48.8	6.9	89.5
Thr	34.3	5.6	71.2	38.7	5.8	75.0	36.3	5.7	69.1	42.7	6.0	78.4
Trp	9.1	1.5	18.8	12.1	1.8	23.4	11.5	1.8	21.9	10.7	1.5	19.6
Tyr	17.0	2.8	35.2	20.0	3.0	38.8	17.9	2.8	34.1	21.1	3.0	38.7
Val	32.2	5.3	66.7	49.7	7.4	96.3	39.1	6.1	74.5	51.6	7.3	94.7
Total	607.2	100.0	1259.8	668.1	100.0	1294.8	642.3	100.3	1223.4	708.8	100.4	1300.6

[a] Purified tRNAs from early and late mitotic cycle times were aminoacylated with homologous and heterologous enzymes under the standard conditions outlined in Materials and Methods. The data presented for the homologous reactions are an average of two separate experiments carried out with different tRNA and enzyme preparations. The heterologous reactions represent only one experiment each, but confirm the high reproducibility of the data, *i.e.*, compare the aminoacylation for early and late tRNAs in both homologous and heterologous reactions.

Heterologous charging reactions were carried out between early tRNA and late enzyme, and late tRNA and early enzyme. The results (Table II) showed that the total acceptor activity for both early tRNA and late tRNA was almost identical indicating that the same proportion of tRNA was chargeable *in vitro* regardless of the mitotic cycle time. The data also showed that the relative amount of each amino acid acceptor activity to the total acceptor activity did not change during the cycle. (In only one instance was there any significant change in amino acid acceptor activity, and that occurred with valine tRNA. Appropriate heterologous charging experiments with different enzymes indicated that the change may have been due to both tRNA and enzyme; however, the significance of such a change, even though reproducible, in a single tRNA species over an entire mitotic cycle, is not clear.) The results for the homologous reactions between mid tRNA and mid enzyme are not presented, since they were essentially identical with the results obtained from early and late homologous reactions and showed no differences in total or relative acceptor activity.

The heterologous experiments showed that early tRNA and late tRNA were charged to the same extent by both homologous and heterologous enzyme. Other experiments indicated that mid-cycle tRNAs responded equally well to early and late enzyme, and late and early tRNAs were charged equally well by mid-cycle enzyme. These data indicated that the synthetase enzymes in early, mid, or late cycle did not differ in their ability to acylate homologous or heterologous tRNA *in vitro*, and that tRNAs from early, middle, and late cycle did not differ in their ability to be aminoacylated, *in vitro*, by homologous or heterologous enzyme. Together the data of Table II showed a remarkable consistency in the tRNA population of *Physarum* during the mitotically synchronous growth phase, and suggested the presence of a tight control on the synthesis of these molecules. Also suggested was that any posttranscriptional modifications which occurred in the tRNA, and affected charging, were similar in early, mid, and late cycle, and that these modifications were made close to the time of transcription since at no point studied was the acceptor activity different than at any other point. To confirm that the above results were not applicable just to cycle II, late cycle I and early cycle III tRNAs were purified and charged with homologous enzymes from cycle II. No differences were seen in total or relative acceptor activities when these tRNAs were compared to each other or to those of cycle II, indicating that during optimum growth both inter- and intracycle tRNAs and enzymes were essentially identical.

Discussion

Recovery of tRNA. It has been shown that *Physarum* total nucleic acid is made up of 12% sRNA (4S + 5S RNA), 82% rRNA (54.4% (1.43 × 10^6 daltons) rRNA and 27.5% (0.74 ×

10^6 daltons) rRNA), and 6% DNA (Melera and Rusch, submitted for publication). These data allow a direct measurement of the recovery of sRNA from the G-100 columns (*i.e.*, 85%), and also allow an estimate of the amount of 5S RNA to be expected in total nucleic acid. Assuming 5S RNA to have a molecular weight of 33,000 and assuming it to be in equal molar amounts with the other rRNA, it can be calculated that 1.2% of *Physarum* total nucleic acid is 5S RNA. This number is in very close agreement with the 1.4% calculated on the basis of observed recovery of 5S RNA (see Results) and suggests that the 15% loss of sRNA during purification is due to a preferential loss of tRNA. On the basis of these observations the recovery of tRNA is judged to be approximately 85%.

tRNA Acceptor Activity during the Mitotic Cycle. Assuming the molecular weight of an average tRNA molecule to be 26,000, and using the conversion factor 1 mg of tRNA/ml = 22 A_{260} units/ml, approximately 75% of the purified tRNA can accept amino acid, *i.e.*, 750 pmol of amino acid are accepted per 1000 pmol of tRNA. Several reasons for the 25% discrepancy between the amount of tRNA acylated and the amount of tRNA present are possible and include contamination of the preparations with other RNAs of similar molecular size and the presence of tRNAs which are inactive in the charging reaction. Electrophoretic analysis of purified tRNA showed it to migrate as one band in 7.5% acrylamide for 5 hr at room temperature. If precursor tRNAs such as those reported for the tyrosine tRNA of *Escherichia coli* (mol wt approximately 40,000; Altman, 1971; Altman and Smith, 1971) were present, they would have been detected, as would RNAs similar in size to the nuclear 4.5S RNA of Novikoff hepatomas (*i.e.*, pre-tRNA of mammalian cells; Smellie and Burdon, 1970) which migrates between 4S and 5S RNA on 8% gels (Busch *et al.*, 1971). The presence of smaller unchargeable (precursor?) tRNAs (Mowshowitz, 1970) or smaller low molecular weight RNAs cannot be ruled out, however, and perhaps a combination of these is actually present.

The data presented in Table II show that neither the total amino acid acceptor activity nor the individual amino acid acceptor activities of *Physarum* tRNA quantitatively change *in vitro* during the mitotic cycle. The possibility that changes in isoaccepting tRNAs might occur is now under investigation; however, changes in isoacceptors not withstanding the quantitative control on total and relative amino acid acceptance are quite clear and provide a stable base line against which observed changes in amino acid acceptance, induced by exogenous stimuli, *e.g.*, drugs, radiation, etc., or normal differentiation, can be measured.

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References

Agarwal, M. K., Hanoune, J., and Weinstein, I. B. (1970), *Biochim. Biophys. Acta 224*, 259.

Altman, S. (1971), *Nature (London), New Biol. 229*, 19.

Altman, S., and Smith, J. D. (1971), *Nature (London), New Biol. 233*, 35.

Bagshaw, J. C., Finamore, F. J., and Novelli, G. D. (1970), *Develop. Biol. 23*, 23.

Baliga, B. S., Borek, E., Weinstein, I. B., and Srinivasan, P. R. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 899.

Bollum, F. J. (1968), *Methods Enzymol. 12, Part B*, 169.

Burton, K. (1956), *Biochem. J. 62*, 315.

Busch, H., Ro-Choi, T. S., Prestayko, A. W., Shibata, H., Crooke, S. T., El-khatib, S. M., Choi, Y. C., and Mauritzen, C. M. (1971), *Perspect. Biol. Med. 15*, 117.

Caston, J. D. (1971), *Develop. Biol. 24*, 19.

Chou, L., and Johnson, T. C. (1972), *Biochem. Biophys. Res. Commun. 47*, 824.

Chuang, R. Y., and Doi, R. H. (1972), *J. Biol. Chem. 247*, 3476.

Fairbairn, J. J. (1953), *Chem. Ind. (London)*, 86.

Galibert, F., Larsen, C. J., Lelong, J. C., and Boiron, M. (1965), *Nature (London) 207*, 1039.

Gallo, R. C., and Pestka, S. (1970), *J. Mol. Biol. 52*, 195.

Gauss, D. H., Von der Haar, F., Maelicke, A., and Cramer, F. (1971), *Annu. Rev. Biochem. 40*, 1045.

Guderian, R. H., Pulliam, R. L., and Gordon, M. P. (1972), *Biochim. Biophys. Acta 262*, 50.

Hatfield, D., and Portugal, S. H. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1200.

Holland, J. J., Taylor, M. W., and Buck, C. A. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 2437.

Hurwitz, J., and Furth, J. J. (1966), *in* Procedures in Nucleic Acid Research, Cantoni, G., and Davies, D., Ed., New York, N. Y., Harper and Row, p 347.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Mans, R. J., and Novelli, G. D. (1961), *Arch. Biochem. Biophys. 94*, 48.

McCormick, J. J., Blomquist, J. C., and Rusch, H. P. (1970), *J. Bacteriol. 104*, 1110.

Mohberg, J., and Rusch, H. P. (1969), *J. Bacteriol. 97*, 1411.

Mowshowitz, D. B. (1970), *J. Mol. Biol. 50*, 143.

Muench, K. H., and Berg, P. (1966), *in* Procedures in Nucleic Acid Research, Cantoni, G., and Davies, D., Ed., New York, N. Y., Harper and Row, p 375.

Novelli, G. D. (1967), *Annu. Rev. Biochem. 36*, 449.

Ortwerth, B. J. (1971), *Biochemistry 10*, 4190.

Smellie, E. J., and Burdon, R. H. (1970), *Biochim. Biophys. Acta 213*, 248.

Sueoka, N., and Kano-Sueoka, T. (1964), *Proc. Nat. Acad. Sci. U. S. 52*, 1535.

Sueoka, N., and Kano-Sueoka, T. (1970), *Progr. Nucleic Acid Res. Mol. Biol. 10*, 23.

Taylor, M. W., Granger, G. A., Buck, C. A., and Holland, J. J. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1712.

Tidwell, T., Bruce, B. J., and Griffin, A. C. (1972), *Cancer Res. 32*, 1002.

Waters, L. C., and Novelli, G. D. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 979.

Waters, L. C., and Novelli, G. D. (1968), *Biochem. Biophys. Res. Commun. 32*, 971.

Yang, W. K., and Novelli, G. D. (1971), *Methods Enzymol. 20, Part C*, 44.

Zeikus, J. G., Taylor, M. W., and Buck, C. A. (1969), *Exp. Cell Res. 57*, 74.