DIFFERENCES IN METHIONYL- AND ARGINYL-tRNA'S OF LARVAL AND ADULT BULLFROGS

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Summary: Several transfer RNA's from blood cells of larval and adult Rana catesbeiana have been examined by chromatography on methylated albumin-kieselguhr columns. Striking differences between the larval and adult organisms were noted in both arginyl- and methionyl-tRNA's.

The switch in synthesis from larval to adult hemoglobin which occurs during metamorphosis in Rana catesbeiana has been extensively studied at both the biochemical and cellular levels (1-5). However, the mechanisms involved in the control of hemoglobin biosynthesis during metamorphosis remain largely unexplained. Many workers have proposed that transfer RNA may be implicated in the control of protein synthesis at the translational level (6-8). Recent work on the role of methionyl-tRNA in the initiation of protein synthesis (9-12) has further suggested the validity of this approach. In a preliminary effort to elucidate some information on the regulation of hemoglobin synthesis during metamorphosis, I have examined transfer RNA from red blood cells of larval and adult bullfrogs by

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chromatography on methylated albumin-kieselguhr columns. The results demonstrate striking qualitative and quantitative differences in methionyl- and arginyl-tRNA profiles.

In all experiments, RNA was extracted from pooled mixtures of separately-labelled larval and frog red blood cells in order to eliminate variability arising from the extraction procedure. Washed blood cells were labelled *in vitro* at 30°C by a method similar to that used by Lee and Ingram (13). Tadpole cells were incubated in amphibian Ringer's with 2-5 uc of tritiated amino acid; the corresponding C^{14}-labelled amino acid was used to label frog cells. Labelling was terminated by the addition of twenty volumes of cold Ringer's, and contaminating white cells were removed by filtering the suspensions through small columns packed with lamb's wool. The purified red cells were resuspended in 5 volumes of cold 0.01 M KCl, 0.0015 M $MgCl_2$, 0.01 M Tris, pH 7,2 containing 10 mg/ml of washed bentonite (14). The tadpole and frog cells were mixed together and quickly disrupted by freezing and thawing. RNA was extracted at 0°C using buffer-saturated phenol (15). The washed RNA precipitate was resuspended in 0.1 M NaCl, 0.05 M sodium phosphate buffer, pH 6,7 prior to fractionation on methylated albumin-kieselguhr (MAK) columns (16).

Typical MAK profiles of double-labelled amino acyl-tRNA's for leucine, arginine and methionine are shown in Figure 1. Although there appears to be no significant alteration of leucyl-tRNA (Figure 1A), both arginyl- and methionyl-tRNA profiles show significant differences (Figure 1B & C). The profile obtained for methionyl-tRNA is particularly striking, since a new amino acyl-tRNA component appears in the adult red blood cell which is not present, or only in very minor amounts, in

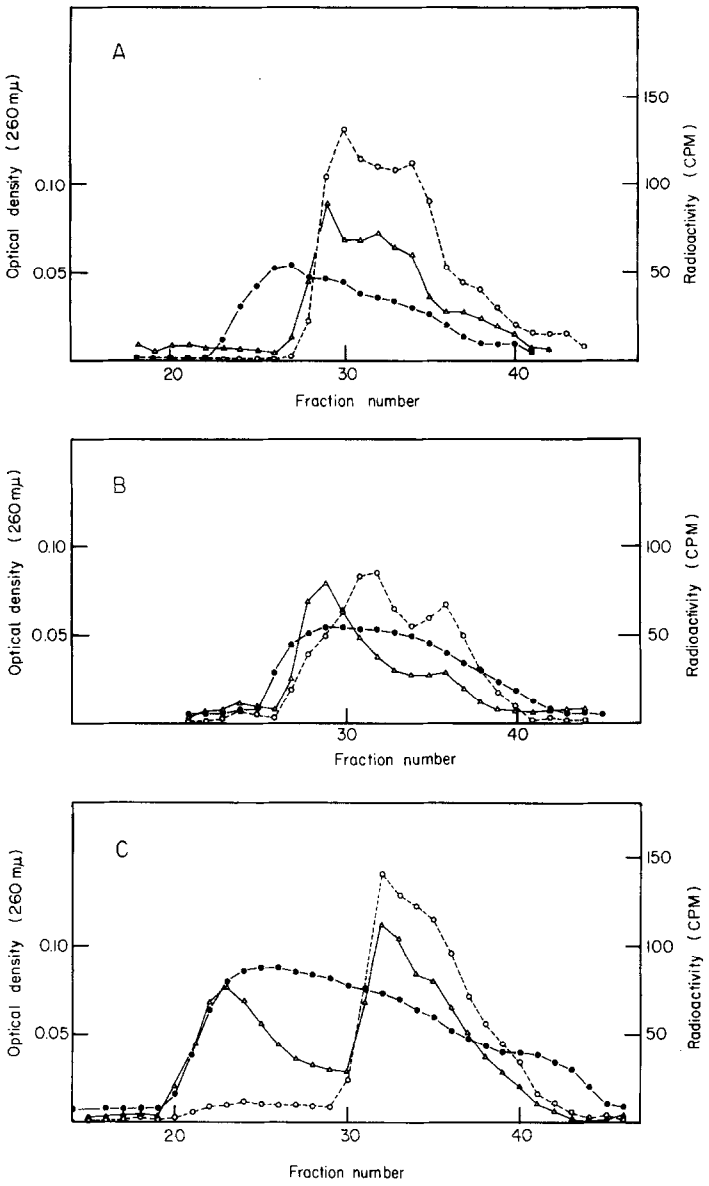

Figure 1. Elution profiles of (A) leucyl-, (B) arginyl- and (C) methionyl-tRNA's from red blood cells of larval and adult Rana catesbeiana. Methylated albumin-kieselguhr chromatography was employed (16) using a linear gradient consisting of 100 ml of 0.2 M NaCl, 0.05 M sodium phosphate buffer, pH 6.7 and 100 ml of 0.8 M NaCl, 0.05 M sodium phosphate buffer, pH 6.7. Two-ml fractions were collected and assayed for optical density at 260 mu (solid circles) and for radioactivitiy by precipitation with cold 10% trichloroacetic acid, as described previously (5). Open circles represent larval amino acyl-tRNA; triangles represent amino acyl-tRNA from adult frogs.

the larval cell. The observed differences do not appear to be artifacts for the following reasons: (1) all results are reproducible, and hence RNA breakdown by ribonuclease is unlikely; (2) superimposable profiles are obtained when either frog or larval red cells are incubated with both H^3- and C^{14}-labelled amino acids under the conditions of the experiment; and (3) identical results are obtained if the labelling is reversed, so that contamination of radioisotopes is unlikely.

Since only three amino acids were examined in these experiments, it is quite possible that additional alterations occur in transfer RNA's for other amino acids. The changes I have observed in tRNA profiles may involve structural changes in tRNA molecules, changes in the specificities of the amino acid activating enzymes, or both. Thus, they may play an important function in the regulation of protein synthesis at the translational level. In this regard, recent work in E. coli has demonstrated that the rate of protein synthesis in vitro can be regulated by the concentration of tRNA species present in the reaction mixture (17).

Tonoue, et al. (18) have reported quantitative changes in leucyl-tRNA's of liver, kidney, tail and gills following the administration of triiodothyronine to bullfrog tadpoles. I have not observed similar differences in leucyl-tRNA of blood cells from larval and adult organisms.

Lee and Ingram (13) have examined amino acyl-tRNA's for arginine, leucine, methionine, serine and tyrosine in embryonic and adult avian red cells. They have reported possible changes in leucyl-tRNA, as well as quantitative and qualitative alterations in methionyl-tRNA. In view of the importance of methionyl-tRNA in the initiation of protein synthesis, it

is tempting to speculate that such changes in methionyl-tRNA may represent an important step in the switch from embryonic to adult hemoglobin that occurs during development in a variety of organisms.

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