to the peripheral vestibular organ, and that there appear to be two groups with different conduction velocities. Moreover, a parallel light- and electron-microscopic study has confirmed the existence of the direct cerebello-vestibular pathway in the bullfrog by showing that section of the eighth nerve extracranially produces degeneration of both climbing and mossy fibers, as well as characteristic retrograde changes in the somas of Purkinje cells (*15*). The latter finding (Fig. 2, top) directly confirms the thesis that Purkinje cells send axons or axon collaterals to the vestibular organ. In addition, if the cerebellar cortex is removed, care being taken not to injure the cerebellar nuclei, one can demonstrate degenerating synaptic boutons in contact with the peripheral vestibular receptor cells (Fig. 2, bottom) (*15*).

The aforementioned results demonstrate the existence of a cerebello-vestibular efferent system in the frog. Previous anatomical and physiological studies have indicated the presence of an efferent system to the peripheral vestibular organ. In the case of the cat, light- (*16*) and electron-microscopical studies (*17*) have revealed efferent terminals on the vestibular receptor cells. Furthermore, direct-current potential changes have been recorded from the surface of the semicircular canals following stimulation of the central nervous system (*18*). In the frog, recent electron-microscopical studies have verified the existence of this efferent system (*19*), which had been suspected on the basis of earlier physiological findings (*20*). The origin of the efferent system was, however, unknown.

Since the cerebellum develops in very close relation with the stato-acoustic system and since a certain component of the vestibular projection to this center is direct in both lower (*21*) and higher vertebrates (*2*), it seems possible that the cerebellum has some direct action upon the peripheral organ. Occasionally we observed direct-current potential changes at the frog vestibular organ following the stimulation of the auricular lobe; however, no clear physiological meaning has so far been attached to this finding.

Although the functional significance of the cerebello-vestibular system has not been ascertained, this system may be inhibitory in nature, as is the olivo-cochlear bundle (*22*), particularly since Purkinje cells inhibit all target cells so far studied (*4*). The demonstration of a direct cerebello-vestibular pathway in the frog implies that, at least in this form, the cerebellum is not involved in motor control exclusively, but has a sensory regulatory role as well.

RODOLFO LLINÀS
WOLFGANG PRECHT
STEPHEN T. KITAI

American Medical Association, Institute for Biomedical Research, Chicago, Illinois 60610

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Erythrocyte Transfer RNA: Change during Chick Development

Abstract. *Radioactive aminoacyl transfer RNA's isolated from erythrocytes in the blood of 4-day-old chick embryos and from reticulocytes of adult chickens were analyzed by chromatography on methylated albumin kieselguhr and freon columns. Embryonic and adult methionyl transfer RNA's showed qualitative and quantitative differences in both chromatographic systems. The patterns for arginyl, seryl, and tyrosyl transfer RNA's in the two cell types were similar, while the leucyl transfer RNA patterns suggested a difference.*

Structural modification of transfer RNA (tRNA) may play a regulatory function in cell differentiation and metabolism (*1*). With methylated albumin kieselguhr (MAK) chromatography, Kano-Sueoka and Sueoka (*2*) demonstrated an alteration in leucyl-tRNA after bacteriophage T2 infection of *Escherichia coli*; this finding was confirmed by Waters and Novelli (*3*), using the reversed-phase chromatography developed by Kelmers *et al.* (*4*). Kaneko and Doi (*5*) found a change during sporulation of *Bacillus subtilis* in the elution pattern of valyl-tRNA from MAK columns.

To look for changes in specific tRNA's during development, we used avian immature red cells as a test system and compared the chromatographic profiles of aminoacyl-tRNA's from red cells present in the blood of 4-day-old chick embryos and of reticulocytes of adult chickens. The techniques in this study were MAK (*2*) and freon columns (*6*). Of the five aminoacyl-tRNA's examined, only the methionyl-tRNA gave a strikingly different elution pattern.

Adult chickens (White Leghorn) were made anemic by daily injection (for 5 days) of 15 mg of phenylhydrazine [in 0.1*M* tris(hydroxymethyl)aminomethane (tris) buffer, *p*H 7.2] and were bled on the 6th day. Eggs from the same strain were incubated at 37°C for 4 days, and blood cells were collected by bleeding the embryos. Blood cells from either source were washed twice with 0.145*M* NaCl, $5 \times 10^{-4}M$ KCl, and 0.0015*M* $MgCl_2$, and once with 0.145*M* NaCl, $5 \times 10^{-4}M$ $FeCl_2$, and 0.01*M* phosphate buffer (*p*H 7.4); 0.12 ml of packed embryonic cells was suspended in the latter medium supplemented with glucose (200 mg/ml) with a total volume of 0.5 ml. For adult cells, 0.5 ml of packed cells was similarly incubated in a total volume of 2.0 ml. To cells previously incubated at 38°C for 10 minutes, ^{14}C- or ^{3}H-labeled amino acid (5 to 10 μc) and the 19 remaining nonradioactive amino acids (1 μmole of each) were added. After incubation for an additional 12 minutes, cycloheximide ($10^{-4}M$) was added to inhibit protein synthesis (*7*) and thus prevent the trans-

fer of amino acids from aminoacyl-tRNA's. After 10 minutes more, the cells were lysed by suspension in 5 volumes of 0.01*M* tris, 0.01*M* KCl, and 0.0015*M* $MgCl_2$ containing 15 mg of purified bentonite per milliliter (*8*) and by repeated freezing (in a mixture of dry ice and acetone) and thawing. The lysate was extracted with a half volume of carbon tetrachloride, and the upper phase was shaken for 15 minutes with an equal volume of twice-distilled, buffer-saturated phenol. The aqueous phase was extracted again with phenol, and traces of phenol were removed with cold, peroxide-free ether. The solution was then adjusted to 0.1*M* with sodium acetate buffer (4*M*, *p*H 6), and then 2 volumes of cold ethanol were added. After several hours at --20°C, the precipitated RNA was collected by centrifugation, washed with cold ethanol, and dried. The yields of tRNA from the above-mentioned volumes of packed cells were 1.3 optical density (O.D.) units (260 mμ) and 0.85 O.D. units for embryonic and adult cells, respectively.

The MAK column (1 by 3 cm) was similar to that of Kano-Sueoka *et al.* (*2*); it contained 1 g of kieselguhr and 0.25 ml of 1-percent methylated albumin, because only small quantities of material were available for analysis. The second change was the use of a shallower gradient of 0.20 to 0.65*M* NaCl (250 ml in total volume).

The freon column used was a modification of the method of Weiss *et al.* (*6*). Radioactive aminoacyl-tRNA's, together with 5 mg of carrier unlabeled tRNA's (*9*), were applied to a column (0.5 by 250 cm). Elution was effected with a concave NaCl gradient containing 0.01*M* sodium acetate and 0.01*M* $MgCl_2$ (*p*H 4.5) at room temperature. Fractions (2 ml) were collected at 12-minute intervals.

Optical density was measured at 260 mμ on alternate fractions. A sample (1 ml) of each fraction was then mixed with 10 ml of Bray's solution (*10*), and the radioactivity was measured in an Ansitron liquid-scintillation counter.

Aminoacyl-tRNA's isolated from erythrocytes of 4-day-old chick embryos and from reticulocytes of adult chickens were compared first by chromatography on the MAK column and then on the freon column. The specific tRNA's examined were: arginine, leucine, methionine, serine, and tyrosine. Typical results of the MAK chromatographs are shown in Fig. 1. With the exception of the methionyl-tRNA, which gave a clear alteration in the elution profiles, there were no significant differences in the profiles between the aminoacyl-tRNA's from the two sources. Under the present chromatographic conditions, both the adult- and the embryonic-cell methionyl-tRNA showed two major peaks in MAK chromatography. However, a dramatic change during development in the proportion of methionyl-tRNA in the two major peaks was observed. The ratio of the areas under the peaks which was 1.1 in the embryonic cells shifted to 3.8 in the adult cells.

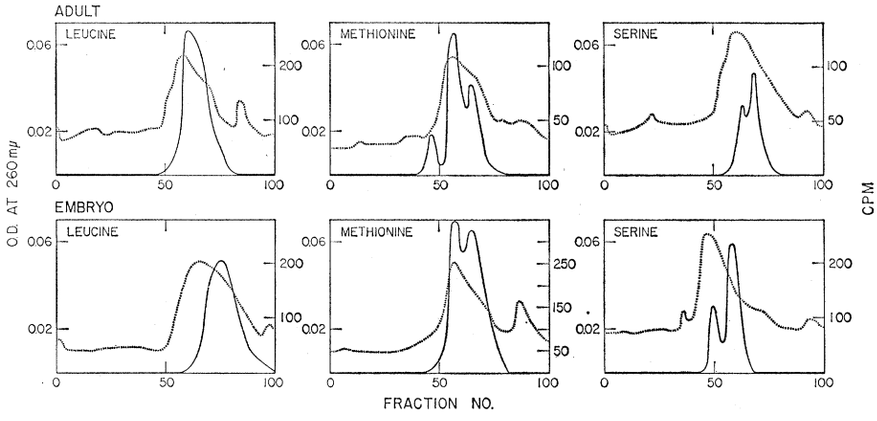

Fig. 1. Elution profiles of aminoacyl-tRNA's of blood cells from embryonic and adult chickens on MAK columns. Dotted lines, optical density at 260 mμ; solid lines, radioactivity. Amino acids labeled with ^{14}C were used in the preparation of all aminoacyl-tRNA's.

Figure 2 shows the elution patterns of the aminoacyl-tRNA's on the freon column. The better resolution of this chromatographic system revealed the presence of multiple peaks for several of the amino acids tested (arginine, leucine, and methionine). No significant difference was observed between the elution patterns of the embryonic and adult cells of the amino acids arginine, leucine, serine, and tyrosine. However, the elution profiles of the methionyl-tRNA's from cells of the two different developmental stages showed a striking alteration. Although both the adult and the embryonic methionyl-tRNA's gave two resolvable peaks, the proportions of the two peaks differed. The ratio of the peaks was 1.3 for the embryonic cells and 2.7 for the adult cells. These results were similar to those found with the MAK column. In addition, the two peaks for the embryonic cells were further apart; a small third peak was observed, but its significance is doubtful.

The chromatograph of leucyl-tRNA from avian reticulocytes differed strikingly from that from *E. coli* B on both

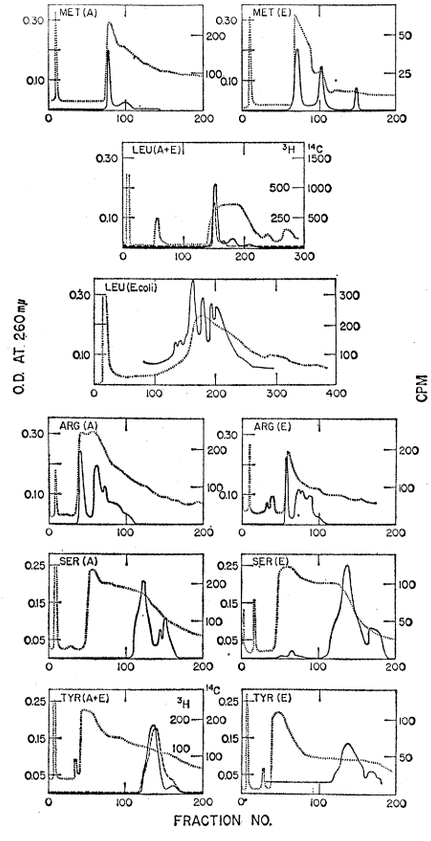

Fig. 2 (right). Elution profiles of aminoacyl-tRNA's on freon columns. Avian aminoacyl-tRNA's were prepared as described. The following samples were prepared with ^{14}C-labeled amino acids: arg (A), arg (E), leu (E), met (A), ser (E), and tyr (A), whereas leu (A), met (E), ser (A), and tyr (E) were acylated with ^{3}H-labeled amino acids. *Escherichia coli* leucyl-tRNA was prepared as described by Kano-Sueoka and Sueoka (*2*). Dotted lines, optical density at 260 mμ; solid lines, radioactivity. (In the double-labeling experiments, ^{14}C is shown by a solid line, and ^{3}H is shown by a dashed line.)

the MAK and the freon columns. A typical elution profile of the bacterial leucyl-tRNA on the latter column is shown in Fig. 3. There were at least four major peaks in the bacterial system, but only two were detected in the avian system. Moreover, the elution profiles of adult and embryonic leucyl-tRNA's appeared to differ slightly. The two embryonic peaks were further apart. However, the significance of this difference is not clear at the moment.

Thus, the analysis of aminoacyl-tRNA's from immature erythrocytes of chick embryos and from reticulocytes of adult chickens by chromatography on the MAK and freon columns has revealed that the elution pattern for methionyl-tRNA changes during development, whereas the patterns for the four other amino acids studied remain essentially unaltered with a possible difference for leucyl-tRNA. However, it is possible that changes in other untested tRNA's may exist. The fact that remarkably similar elution patterns of the other aminoacyl-tRNA's were observed apparently eliminates the possibility of ribonuclease activity as a major factor in the observed change in methionyl-tRNA. In a later experiment, we examined the embryonic methionyl-tRNA with the use of the same ^{14}C-methionine sample as we had used for the adult tRNA in Fig. 2. Because the expected embryonic pattern was observed, it seemed unlikely that the difference illustrated in Fig. 2 is due to contamination in the ^{3}H-methionine. The identical elution profiles obtained either by ^{14}C-, ^{3}H-, double-, or single-labeling techniques (as in the case of tyrosyl-tRNA) demonstrated that these profiles are reproducible and reliable.

The actual mechanism and biological significance of this change are not clear at the moment. The observed modification in methionyl-tRNA during development may be a change of either the tRNA molecules or of the specificities of the aminoacyl-tRNA synthetases involved. The tRNA may also be involved in the regulation of protein synthesis at the translational level (*1*). Moreover, our finding is of particular interest because *N*-formyl-methionyl-tRNA from *E. coli* plays a crucial role in the initiation of protein synthesis in bacteria (*11*). Two methionyl-tRNA's of *E. coli* have been reported (*12*); one of these can be converted to *N*-formyl-methionyl-tRNA, and the other does not accept formyl groups. Our finding that methionyl-tRNA's are modified during development agrees with the expectation based on the model of regulation that a change in tRNA molecules may lead to an alteration in their functional capacity and thus may affect translation.

JOHN C. LEE
VERNON M. INGRAM
Department of Biology,
Massachusetts Institute of Technology,
Cambridge 02139

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A Mutagenic Effect of Visible Light Mediated by Endogenous Pigments in Euglena gracilis

Abstract. *Mutant cells lackng chlorophyll, chloroplasts, and chloroplast DNA were produced by irradiating* Euglena gracilis *in aerobic conditions with visible or red light (greater than 610 nanometers) of an intensity equivalent to that of direct sunlight. The photosensitizer is apparently the endogenous chlorophyll present in the chloroplasts. These mutants are comparable to those induced by ultraviolet light, x-rays, heat, or streptomycin. Our findings indicate that visible light can serve as a mutagenic agent in the absence of exogenous photosensitizers, thus directly effecting the course of evolution of organisms containing chlorophyll.*

In the presence of a suitable exogenous photosensitizing dye, cells exposed to visible light and air display a photodynamic action, which can be lethal (*1*) or mutagenic (*2*) in nature. In the latter case the photosensitizer can be preferentially bound to nucleic acids (*3*) and may act directly as a "photomutagenic" agent, whereas, in other cases, the mutagenic mechanism is not clearly understood (*4*). In the absence of an exogenous photosensitizer, it has not been possible to demonstrate mutagenesis with visible light under normal physiological conditions. Kaplan and Kaplan (*5*) have reported the appearance of *S*-mutants of *Serratia marcescens* from cells which had been initially dehydrated and then rehydrated and exposed to visible light. Except for this experiment, it is generally held that light with wavelengths above 300 nm has primarily lethal action but very little mutagenic action (*6*), although there is no doubt that near-visible light (320 to 400 nm) can be mutagenic (*7*). The conditions which limit mutagenesis with visible light include the absence of endogenous photosensitizers or the development of suitable protective mechanisms such as those which operate to prevent the damage caused by aerobic photosensitivity (*8*). We report here the induction of a mutation (that is, a stable, heritable change expressed in the phenotype) by visible light in *Euglena gracilis* in the absence of exogenous photosensitizers.

Euglena gracilis var. *bacillaris* was cultivated on Hutner's medium (*p*H 3.5) with continuous shaking under visible light (275 lumen/m^2) (*9*). Cells taken during the logarithmic phase of growth were transferred to flat-sided tissue-culture flasks and immersed in an aquarium tank maintained at 23°C. Air was bubbled through the flasks for the duration of the experiment, both to insure adequate aeration and to avoid settling of the cells. Illumination was by means of a Sylvania Sun Gun II (650 watts), and the intensity was determined with a Yellow Springs Industry radiometer, Model 65. For those experiments involving red-light irradiation, a Corning C.S. No. 2-61 glass filter which transmits light only above 610 nm, was placed in front of the culture flask. Illumination was continued for as long as 6 hours, during which time the temperature within the flask, monitored with an electronic thermometer, was