*MODIFICATION IN TRANSFER RNA DURING THE DIFFERENTIATION OF WHEAT SEEDLINGS**

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Mechanisms involved in the regulation of the synthesis of macromolecules have been investigated primarily in bacterial systems. Various schemes have been proposed which implicate transcription of the genetic message as the initial site for the regulation of enzyme synthesis. Recently, attention has been focused on the steps involved in translation of the ribonucleic acid message as the level at which regulation occurs. It has been suggested that certain transfer RNA molecules are involved in the regulation of cell metabolism and cell differentiation at the translation level.[1, 2] If such conjecture were true, one should find evidence of changing tRNA populations when the physiologic processes of an organism are profoundly altered. Changes in the distribution of certain tRNA species have been reported in bacterial cells undergoing sporulation,[3, 4] in phage-infected bacteria,[5, 6] and in virus-infected animal cells.[7]

In the present investigation we have examined the aminoacylated-tRNA's of wheat embryos and wheat seedlings. The purpose was to learn whether or not detectable changes occur in the distribution of tRNA's during differentiation. In this report we show changes in at least three aminoacyl-tRNA's and discuss their possible role in differentiation.

Materials and Methods.—The organism studied was *Triticum aestivum* (var. *Pawnee*). *Pawnee* wheat seed was obtained from the Champaign (Illinois) County Seed Co.

Radioactivity was determined by liquid scintillation spectroscopy on 1.5- to 2-ml fractions in Bray's dioxane fluid. The samples were cooled 8 hr before they were counted.

Seeds were germinated in trays on paper towels saturated with water containing 100 μg dihydrostreptomycin sulfate per milliliter. The trays were covered with plastic film (Saran Wrap) and left at room temperature in room lighting. Nongerminated embryos were isolated by the method of Johnston and Stern.[8]

The partial purification of aminoacyl-tRNA synthetases from wheat embryos, the isolation of wheat embryo and wheat seedling sRNA, and the characterization of the *in vitro* aminoacylation of wheat tRNA will be reported elsewhere.[10]

The methylated albumin kieselguhr column of Mandel and Hershey[9] was used throughout. Soluble RNA samples from embryos (0 hr) and seedlings (48 hr) were separately charged *in vitro* with a given C^{14} or H^3 amino acid. The reaction mixture contained a C^{14} amino acid (sp. act. of 50 mc/mmole) or a H^3 amino acid (sp. act. between 0.25 and 4.8 c/mmole) at a concentration of at least 10^{-6} mmoles amino acid/reaction; 250 or 500 μg sRNA; 0.1–2.0 mg protein (amino-acyl-RNA synthetase fraction); 50 μmoles Tris, pH 8.0 at 18°C; 5 μmoles $MgCl_2$; 5 μmoles adenosine 5′-triphosphate (ATP); 1 μmole reduced glutathione; 0.15 μmole cytidine 5′-triphosphate (CTP); and glass-distilled water to a total volume of 0.5 ml. The reaction mixture was incubated at 18°C until a level of saturation was reached.

Results.—(a) *Cochromatography of individual species of tRNA's from embryos and 48-hour seedlings:* Radioactivity and optical density profiles for embryo and seedling aminoacyl-tRNA coeluted from MAK are given in Figure 1*A–H*. Profiles for valyl-, lysyl- and prolyl-tRNA are shown in Figures 2, 3*A*, and 4*A*.

The second optical density peak observed with wheat sRNA corresponds to the slowly descending tail observed with *E. coli* sRNA.[5, 11] The chromatographic patterns for the aminoacyl-tRNA's of wheat and *E. coli* are similar for phenyl-alanyl-, glycyl-, threonyl-, leucyl-, seryl-, and prolyl-tRNA's but are dissimilar for valyl-, histidyl-, glutamyl-, arginyl-, and lysyl-tRNA's.

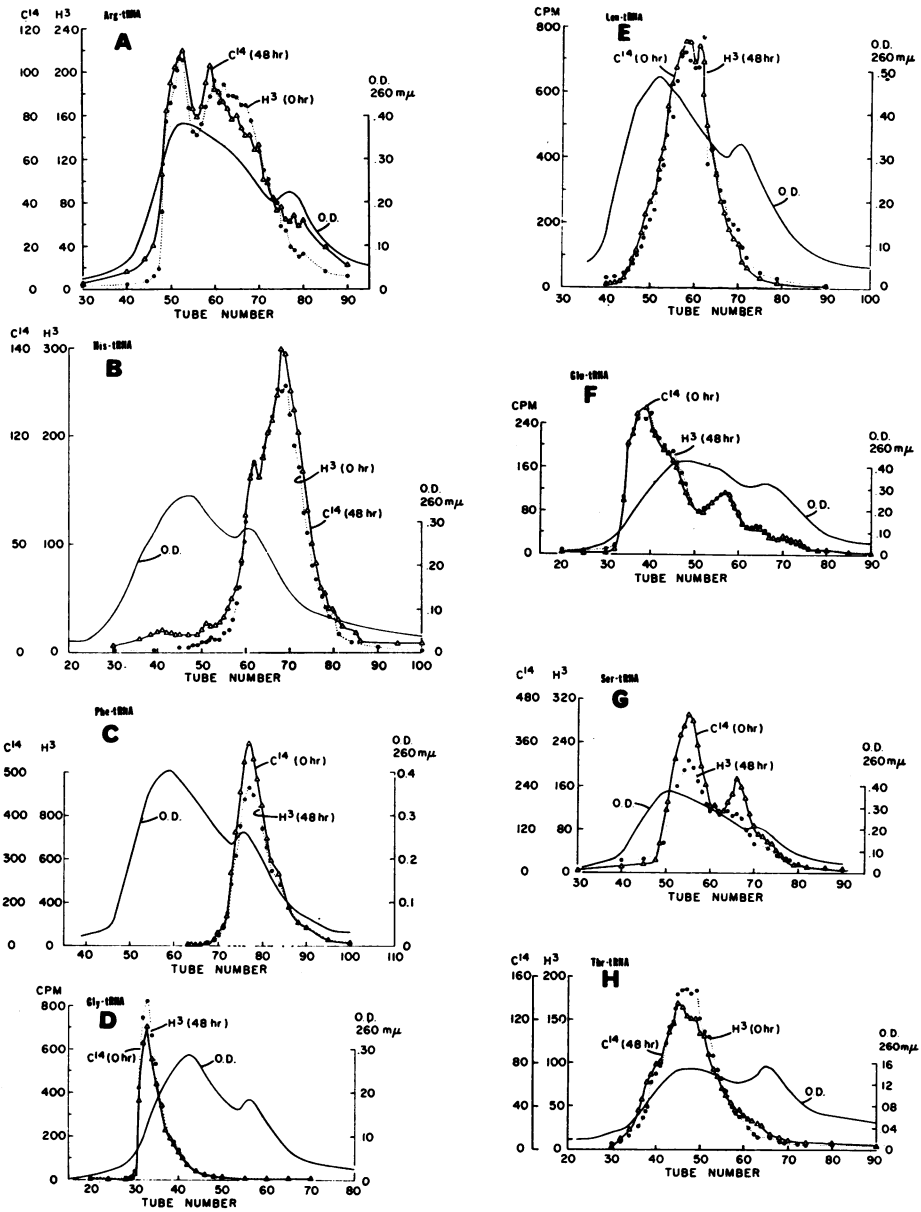

FIG. 1.—Elution of aminoacyl-tRNA's from columns of methylated albumin kieselguhr. The columns were loaded with sRNA which was isolated from an *in vitro* charging system. sRNA was prepared from wheat seed embryos and from 48-hr seedlings, charged with C^{14}-or H^3-amino acid, mixed and extracted.

The purpose of the cochromatography of 0-hour and 48-hour aminoacyl-tRNA's was to investigate possible quantitative changes in the species of any aminoacyl-tRNA. In order to determine differences in isotopic profiles, the amount of aminoacyl-tRNA in each of two peaks was compared. The treatment of the data presented here is similar to that used by Kaneko and Doi[3] to express the difference in isotopic profiles which they found after MAK chromatography of valyl-tRNA from sporulating *Bacillus subtilis*.

First, a peak ratio is calculated to compare the area under each radioactivity peak for the same amino acid:

$$\text{Peak ratio} = \frac{\text{Total cpm in peak 1}}{\text{Total cpm in peak 2}}.$$

Next, the difference was taken between the ratios for 0-hour and 48-hour aminoacyl-tRNA in each peak and expressed in per cent as the factor f:

$$f = \frac{|\text{Peak ratio 0 hour} - \text{Peak ratio 48 hour}|}{\text{Highest peak ratio}} \times 100.$$

Estimations of the magnitude of variation in f necessary to be greater than random variations in the technique were made by computing f for: (1) three separate runs of 0-hour tRNA charged only with C^{14}-valine (e.g., Fig. 2), and

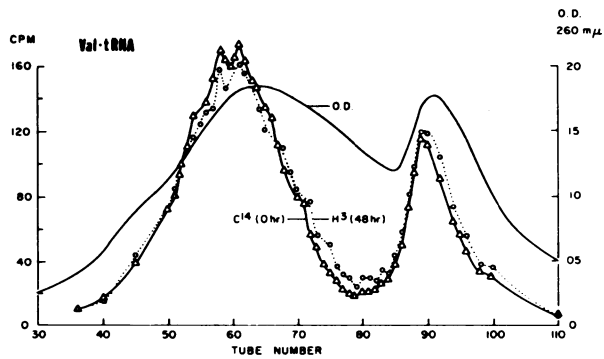

FIG. 2.—Coelution of 0-hr and 48-hr valyl-tRNA from MAK column. The RNA was prepared as in Fig. 1.

(2) three separate runs of 0-hour and 48-hour tRNA's charged with C^{14}-leucine and H^3-leucine, respectively, and cochromatographed (e.g., Fig. 1*E*). Valyl-tRNA columns A and B differed by 8.0 per cent (i.e., $f = 8.09$). Columns B and C had f values of 0.03 per cent. This comparison established the reproducibility of a single radioactivity profile. The leucyl-tRNA columns were compared to establish the reproducibility of f in the profiles for the cochromatography of differently labeled aminoacyl-tRNA's. The three leucyl-tRNA columns showed f values between the two isotopes of 14.2, 6.9, and 2.5. On the basis of these experiments, the f value above which differences are considered significant was arbitrarily set at 15 per cent. It must be pointed out, however, that we are basing this figure on the greatest variation experienced for a single amino acid, and not on the mean variation. If our choice of 15 per cent for

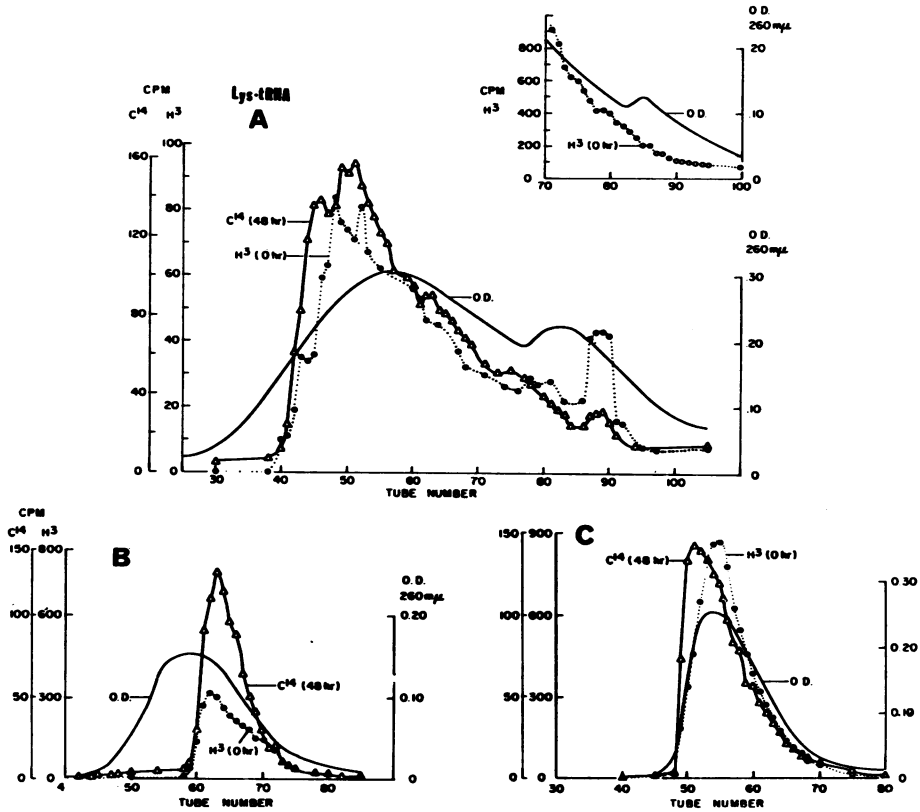

FIG 3.—(*A*) Coelution of 0-hr and 48-hr lysyl-tRNA from MAK. The inset shows the region from fraction 70 to 100 of a second elution of 0-hr tRNA. The regions corresponding to fractions 38–47 and 48–60 were rechromatographed in (*B*) and (*C*), respectively.

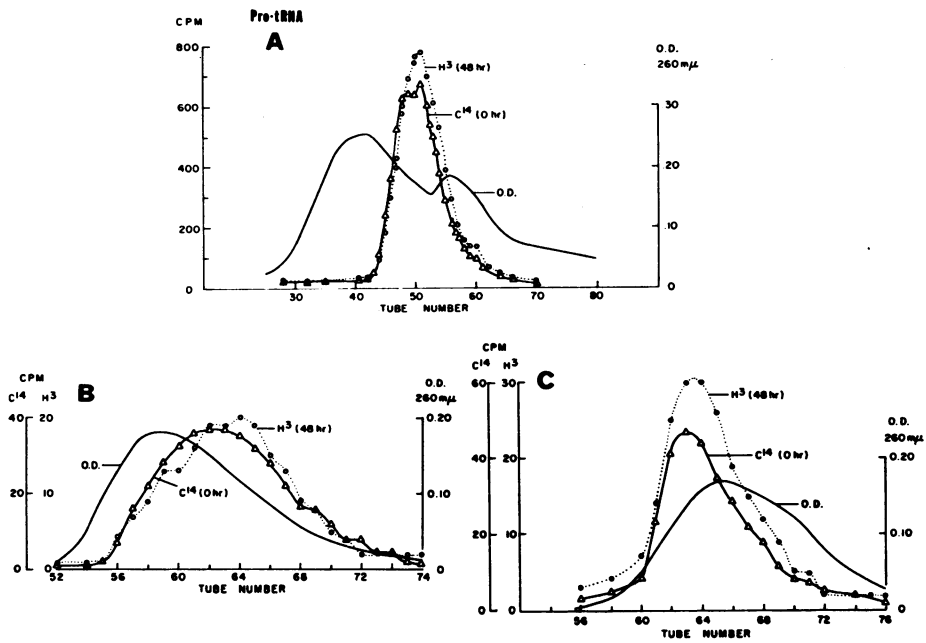

FIG. 4.—(*A*) Coelution of 0-hr and 48-hr prolyl-tRNA from MAK. The regions corresponding to fractions 43–49 and 50–58 were rechromatographed in (*B*) and (*C*), respectively.

TABLE 1. *f Values for various aminoacyl-tRNA's.*

Amino acid	Value	Amino acid	*f* Value*	Amino acid	*f* Value
Arginine	12.5	Leucine	8.4	Serine	*18.1*
Glutamic acid	3.6; 11.0†	Lysine	*28.4*	Threonine	13.7
Histidine	8.8	Proline	*22.5*	Valine	14.2

* Measure of the difference between peak ratios for embryo and seedling amino acyl-tRNA's, see text.

† Values for peak ratios 1:2 and 1:3, respectively.

significance is too high, we will overlook some significant difference in certain aminoacylated tRNA's.

(b) *Differences in isotopic peak ratios:* Table 1 gives the *f* values for nine aminoacyl-tRNA's. Phenylalanyl-tRNA and glycyl-tRNA each chromatograph in one symmetric peak (Figs. 1*C* and *D*) so that *f* values could not be calculated. The aminoacyl-tRNA's for serine, lysine, and proline are the only ones which show ratio differences above 15 per cent. The peak which occurs in the tail of the lysine profile (Fig. 3*A*) is not reproducible. The insert on the lysyl-tRNA graph represents this section from another column which does not show this variation.

Both the chromatographic profile for seryl-tRNA (Fig. 1*G*) and the ratio comparisons (*f* values) reveal a peak in the central region which is higher at 48 hours than at 0 hours of germination.

Even though the variations were reproducible, each peak was rechromatographed to make the variation for lysyl-tRNA and prolyl-tRNA more obvious. Peak fractions in Figures 3*A* and 4*A* were combined and then rechromatographed on separate MAK columns. The position of the radioactivity peaks from the original column was located by comparing the optical density profile with a previous column for which both the optical density and radioactivity had been measured.

The results for lysyl-tRNA are shown in Figures 3*A*, *B*, and *C*. It is evident from the profiles for the rechromatographed lysyl-tRNA peaks that there is much less tritium in peak one than in peak two. The difference in isotopic peak ratios obtained by comparing the two columns is 45 per cent. The tritium peak in part (*B*) is shifted to the left of the C^{14} peak, whereas in part (*C*) it is shifted to the right. It is possible that a lysyl-tRNA species in this region is not represented at all in nongerminated (0-hour) embryos, but appears only in the seedling before 48 hours of germination.

Figures 4*B* and *C* show the rechromatography of prolyl-tRNA. The amount of tritium and the position of the tritium peak is shifted to the right of the C^{14} profile in both cases. The difference in isotopic peak ratios obtained by comparing the two columns is 25.5 per cent.

Discussion.—The purpose of this study was to determine whether or not detectable changes could be observed in the distribution of the various aminoacyl-tRNA's during morphogenesis. It is obvious that we were limited to changes which could be resolved by the MAK chromatographic technique. The data presented in this report show that there are quantitative changes between the species of given aminoacyl-tRNA's. The changes, demonstrated by in-

creases or decreases in certain peaks eluted from MAK, may be accounted for in two ways. There could occur during differentiation an increase in the quantity of one degenerate species of lysyl-tRNA. Alternatively, a quantitative change could result from the formation of a completely new degenerate species of lysyl-tRNA, but a species which coelutes with one extant in both embryo and seedling. Neither alternative can be favored at this time. In either case, differences in tRNA's which can be resolved by MAK chromatography do not necessarily represent anticodon changes. Changes in tRNA molecules which are not associated with changes in the anticodon region result in altered chromatographic profiles.[12-14]

Whatever produces the alterations observed here, they occur when the seedling is undergoing rapid differentiation. In view of the role of tRNA's in the translation process, changes in the constitution of tRNA molecules may be necessary for the events of differentiation. Long-lived messenger RNA molecules which are necessary for the early stages of differentiation apparently exist in the embryos of seeds[15-17] and unfertilized eggs of the sea urchin.[18] These messages could be activated by the modification or synthesis of a tRNA with specificity for the initial sequences of the message.

Similarly, transfer RNA could implement the inactivation of "old" messages begun with a common codon sequence recognized by a regulatory tRNA anticodon. The presence or absence of this unique tRNA would then determine whether or not the messengers could be read. Modulating triplets could thus temporally regulate the expression of a group of genes, serving the same role in differentiation which has been suggested for histones.[19, 20]

The data presented here were taken from a thesis presented by Barbara S. Vold to the Graduate College, University of Illinois, in partial fulfillment of the degree of Doctor of Philosophy. One of us (B.S.V.) was the recipient of a predoctoral fellowship from the National Institutes of Health.

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[1] Ames, B. N., and P. E. Hartman, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 349.
[2] Stent, G., *Science*, **144**, 816 (1964).
[3] Kaneko, I., and R. Doi, these PROCEEDINGS, **55**, 564 (1966).
[4] Lazzarini, R. A., these PROCEEDINGS, **56**, 185 (1966).
[5] Kano-Sueoka, T., and N. Sueoka, *J. Mol. Biol.*, **20**, 183 (1966).
[6] Waters, L. C., and G. D. Novelli, these PROCEEDINGS, **57**, 979 (1967).
[7] Goehler, B., I. Kaneko, and R. Doi, *Biochem. Biophys. Res. Commun.*, **24**, 466 (1966).
[8] Johnston, F. B., and H. Stern, *Nature*, **179**, 160 (1957).
[9] Mandell, J. D., and A. D. Hershey, *Anal. Biochem.*, **1**, 66 (1960).
[10] Vold, B. S., and P. S. Sypherd, *J. Plant Physiol.* in press.
[11] Sueoka, N., and T. Yamane, these PROCEEDINGS, **48**, 1454 (1962).
[12] Lazzarini, R. A., and A. Peterkofsky, these PROCEEDINGS, **53**, 549 (1965).
[13] Lebowitz, P., P. L. Ipata, M. H. Makman, H. H. Richards, and G. L. Cantoni, *Biochemistry*, **5**, 3617 (1966).
[14] Zachau, H. G., D. Dütting, and H. Feldman, *Angew. Chem.*, **78**, 392 (1966).
[15] Marcus, A., and J. Feeley, these PROCEEDINGS, **51**, 1075 (1964).
[16] Waters, L. C., and L. S. Dure, III, *J. Mol. Biol.*, **19**, 1 (1966).
[17] Dure, L. S., III, and L. C. Waters, *Science*, **147**, 410 (1965).
[18] Slater, D. W., and S. Spiegelman, these PROCEEDINGS, **56**, 164 (1966).
[19] Huang, R. C., and J. Bonner, these PROCEEDINGS, **48**, 1216 (1962).
[20] Allfrey, V. G., V. C. Littau, and A. E. Mirsky, these PROCEEDINGS, **49**, 414 (1963).