Transfer RNAs and aminoacyl-tRNA synthetases were isolated from vegetative and developing cells of the cellular slime mold *Dictyostelium discoideum*. Using the homologous synthetases, the tRNAs were compared for their levels of acceptance of 17 amino acids. These levels were found to be the same. The tRNAs were further compared by double label chromatography on reversed phase columns. Some major quantitative differences in individual peaks were observed. In addition, for a number of aminoacyl-tRNAs, small but reproducible displacements of individual peaks were apparent. In many cases the displacement was in the major peak, and in all but one case the developmental peak eluted ahead of the vegetative peak. Taken together, these results suggest that there is no change from growth to development in the transcription of tRNA genes, but that there are important changes in post-transcriptional modification.

Differences in chromatographic mobilities have been demonstrated for tRNAs from a wide variety of tissues in different metabolic or developmental states (for detailed reviews see Refs. 1 and 2). Since the existence of such differences is quite common, an understanding of their nature and functional significance is of general biological interest. In some highly specialized differentiating systems, tRNAs have been shown to adapt to the amino acid composition of the few major proteins being synthesized (3-6), and therefore appear to be components of the differentiation process itself. In a few of these cases there is also some evidence that the availability of a few tRNAs may limit and thereby regulate the rate of synthesis of these proteins (5, 7). However, most changes in metabolic or developmental state do not involve gross changes in the amino acid composition of the proteins being made. In these more common instances it has proved difficult to proceed beyond observations of correlations between changes in tRNAs and changes in the biological state of cells to the goal of understanding cause and effect relationships. Only in a few studies with bacteria has it been possible to ascribe functions to the tRNA changes. In *Salmonella typhimurium* a tRNA modification has been found essential for proper transcriptional control of several amino acid biosynthetic operons (8, 9). For technical reasons, eukaryotes have not been as amenable to analysis as bacteria. Except for the highly specialized systems noted above, the significance of tRNA changes in eukaryotic systems remains unknown.

Among eukaryotic organisms, the cellular slime mold *Dictyostelium discoideum* appears to be especially promising for pursuing an analysis of developmental changes in tRNAs beyond the stage of simple correlation. In addition to its ease of manipulation and the fact that it differentiates in 24 h into only two cell types, the technology exists for assaying for the transcriptional and translational consequences of any detected alterations. Wheat germ and rabbit reticulocyte cell-free protein-synthesizing systems have been shown to be extremely efficient in translating stage-specific *Dictyostelium* mRNAs (10). Furthermore, an *in vitro* system for synthesizing mRNA precursor, using isolated nuclei, has been developed and has been shown to give excellent initiation (11).1 Finally, the haploid nature of the organism facilitates obtaining mutants (12, 19). The utilization of mutants to elucidate the role of tRNA in the repression of certain amino acid biosynthetic operons in bacteria (14) indicates the utility of this feature.

We are interested in the possibility that tRNAs might be involved in regulation of either translational or transcriptional events during development of *D. discoideum*. Because of the well known role of tRNA in decoding the base sequence of mRNA during protein synthesis, considerable attention has been given to the possibility that tRNAs may be involved in regulating the translation of messenger RNAs (1, 2). But in addition to their part in translation, tRNAs act also to donate amino acids to lipids, peptidoglycans, and proteins (15); and in bacterial systems at least, have been implicated in the transcriptional regulation of certain amino acid biosynthetic pathways (8, 9, 14). Hence tRNAs may assume multiple roles within an organism, including functioning as regulatory agents at several levels of control.

1 C. T. Mabie and A. Jacobson, personal communication.
We have become intrigued by the recent finding that starvation for certain amino acids is apparently required for initiation of development (16). Changes in in vivo levels of tRNA aminoacylation could conceivably alter the protein synthetic pattern in early development. In addition, by analogy with the role of tRNAs in expression of certain amino acid biosynthetic operons in bacteria (8, 9, 14), the signal for amino acid starvation and consequently the signal for initiation of development in Dictyostelium may well be communicated to the transcriptional control apparatus by tRNAs.

In order to provide a broad framework from which to initiate a functional analysis of tRNAs in Dictyostelium development, a general characterization of tRNAs present during growth and 18 h of development (initiation of culmination) was made. This developmental stage was chosen as it allowed a maximum of time for changes in tRNAs and aminoacyl-tRNA synthetases to accumulate. Still later times were not satisfactory because during culmination stalk cells extrude their contents, vacculate, and die. Following the isolation of tRNAs and aminoacyl-tRNA synthetases, we have determined the reaction conditions for optimum aminoacylation of the tRNAs with each of 17 amino acids, and this has in turn enabled us to assay for quantitative changes in acceptance for each of these amino acids during development. Finally, we have analyzed isoaccepting species of aminoacyl-tRNAs using reversed phase column chromatography. The combined results of these studies suggest that during Dictyostelium development tRNAs are regulated primarily at the level of post-transcriptional modification.

**Materials and Methods**

**Culturing and Harvesting of Cells—Dictyostelium discoideum** strain A3 (17) was grown at 22-23°C on a gyratory platform shaker rotating at 175 to 200 rpm. Cells were grown in HL-5 medium containing yeast extract (Difco), proteose peptone (Difco), glucose and phosphate buffer at pH 6.5 (18, 19). Cultures containing 2 liters of medium were routinely grown in 4-liter flasks and had a generation time of 9 to 10 h. Exponentially growing cells at concentrations of less than 6 \times 10^7 cells/ml were harvested in a Sorvall RC-2B centrifuge at 19,000 \times g for 30 s at 4°C or in a Sharples continuous flow centrifuge.

**Plating Cells for Development and Harvesting of Developing Cells**—Exponentially growing cells were harvested, washed once at a titer of 1 \times 10^8/ml with PDF buffer (40 mM KCl, 10 mM MgCl₂, 18.5 mM KH₂PO₄, 28.5 mM KHPO₄, and 500 µg/ml of streptomycin sulfate, pH 6.4 to 6.6), and resuspended in PDF buffer at a final concentration of 3 \times 10^7 cells/ml. Five milliliters of the cell suspension were evenly distributed on a 12.5-cm Whatman No. 50 filter, supported by eight Whatman No. 3 12.5-cm filters in a 15-cm Petri dish. The filters had been previously saturated with 40 ml of PDF buffer and freed of air bubbles with a glass spreader. Petri dishes were placed in polyethylene bags on flat trays containing moist paper towels. Bags were sealed with tape and incubated at 22°C. After 18 h of development, just before culmination commenced, cells were harvested, washed and resuspended at a final concentration of about 0.8 to 2 \times 10^9 cells/ml. Cells were homogenized with a tight fitting Dounce homogenizer until 90% breakage was achieved. Lower cell concentrations resulted in less efficient breakage. Alternate procedures for cell breakage (sonication, freeze-thawing, detergent lysis) gave much poorer activities.

**Preparation of Aminoacyl-tRNA Synthetases—Aminoacyl-tRNA synthetases from exponentially growing cells (vegetative synthetase) and from cells at 18 h of development (developmental synthetase) were prepared in identical fashion. Vegetatively growing cells were harvested, washed once at a titer of 1 \times 10^9/ml with cold synthetase buffer (10 mM Tris/HCl, pH 7.5, 10 mM MgCl₂, 10% glycerol, and 14 mM mercaptoethanol), resuspended at a final concentration of about 0.8 to 2 \times 10^9 cells/ml. Cells were homogenized with a tight fitting Dounce homogenizer until 90% breakage was achieved. Lower cell concentrations resulted in less efficient breakage. Alternate procedures for cell breakage (sonication, freeze-thawing, detergent lysis) gave much poorer activities.**

The \( A_{260} / A_{230} \) of tRNA prepared in this manner was 2.1 and the average yield was about 1 mg/g of cells. The \( A_{260} \) of tRNA in a buffer of 100 mM sodium cacodylate, pH 7.5, 10 mM MgCl₂, and 2 mM Na₃SO₄ was 15. The tRNA preparations contained about 5% protein as determined by the biuret method (22) and less than 0.1% primary amines (which include DNA) as measured by the diaminobenzoic acid assay (23). There were no apparent differences in these properties for vegetative and developmental tRNA preparations. Electrophoresis on polyacrylamide gels showed the tRNA preparation to be about 50% pure before the DEAE-cellulose chromatography and 60 to 65% pure at the final step, the primary contaminant being ribosomal RNA. This estimate of purity agrees well with the results from aminoacylation (Table II). Assuming that alanine, glutamine, and cysteine are accepted by the preparations to the same extent as the average of the other 17 amino acids, a total of about 1090 pmol of amino acids were accepted per A₅₅₀ unit of tRNA (average for the vegetative and developmental preparations shown in Table II). Since pure tRNAs accept about 1700 pmol of amino acids/A₅₅₀ unit (24), on the basis of amino acid acceptance our preparation was also about 60% pure.

**Preparation of Aminoacyl-tRNA Synthetases—** Aminoacyl-tRNA synthetases from exponentially growing cells (vegetative synthetase) and from cells at 18 h of development (developmental synthetase) were prepared in identical fashion. Vegetatively growing cells were harvested, washed once at a titer of 1 \times 10^9/ml with cold synthetase buffer (10 mM Tris/HCl, pH 7.5, 10 mM MgCl₂, 10% glycerol, and 14 mM mercaptoethanol), and resuspended at a final concentration of about 0.8 to 2 \times 10^9 cells/ml. Cells were homogenized with a tight fitting Dounce homogenizer until 90% breakage was achieved. Lower cell concentrations resulted in less efficient breakage. Alternate procedures for cell breakage (sonication, freeze-thawing, detergent lysis) gave much poorer activities.

The \( A_{260} \) of the homogenate was adjusted to about 80 and the homogenate centrifuged at 37,000 \times g for 30 min at 4°C. The clear supernatant was removed, transferred to a new centrifuge tube and centrifuged at 105,000 \times g for 1 h. Failure to dilute the cell extract occasionally yielded preparations of reduced activity, probably because particulate material remaining in suspension interfered with the DEAE-cellulose chromatography which followed.

The supernatant from the 105,000 \times g centrifugation step was withdrawn and 1/4 volume of 1 M KCl was added to give a final concentration of 0.2 M KCl. It was then passed onto a DEAE-cellulose column (Whatman DE52, 6-ml bed volume/g of cells), which had been previously equilibrated at 4°C with buffer containing 0.1 M KCl, 50 mM Tris/HCl, pH 7.5, 10 mM MgCl₂, 10% glycerol, and 14 mM mercaptoethanol. It was eluted directly with the same buffer. Fractions containing an \( A_{260} / A_{230} \) of less than 1.9 (greater than 95% protein) were pooled. The pooled fractions were concentrated and free amino acids were removed by vacuum dialysis against 2 changes of synthetase buffer, the second containing 50% glycerol.
tRNAs of Dictyostelium discoideum

The first dialysis was for a minimum of 2 h against at least 10 volumes of buffer and the second was for a minimum of 4 h against at least 200 volumes of buffer. Alternatively, the pooled column effluent was brought to 85% saturation with ammonium sulfate at pH 4.5, and 0.25 M NaCl; molarity refers to NaCl; and centrifuged at 27,000 x g for 10 min. The aqueous phase was withdrawn and extracted two or three times with ether saturated with 0.25 M RPC-5 buffer. The resulting aqueous phase was gently warmed and mixed on a Vortex mixer to remove the ether, and the solution was then either used immediately for chromatography on the RPC-5 column or frozen for later use. No significant loss of trichloroacetic acid-precipitable counts or changes in profile were observed after storage of aminoacyl-tRNA for as long as 2 weeks.

In some cases when the aminoacyl-tRNA was prepared in the above fashion, spurious peaks were detected which appeared to be associated with the particular isotope utilized rather than the stage-specific tRNA. In these cases (tyrosine, tryptophan, histidine, and methionine), the aminoacyl-tRNA was purified by passing the combined reaction mixtures (which had been adjusted to pH 4.5 with acetic acid and mixed with an excess of 0.25 M RPC-5 buffer) over a DEAE-cellulose column poured in a Pasteur pipette. The column had been previously equilibrated with 0.25 M RPC-5 buffer and, following application of the sample, was washed with 20 ml of the same buffer.

The RPC-5 column was then re-equilibrated with 50 ml of 0.25 M RPC-5 buffer. A mock run was made using unlabeled crude yeast tRNA (Sigma) with a 0.25 to 1.0 M RPC-5 buffer gradient (250 ml) (Pharmacia Fine Chemicals, Gradient Mixer GM-1). The column was washed with 50 ml of 1.5 M RPC-5 buffer and re-equilibrated with 0.25 M RPC-5 buffer. Salt concentrations were monitored with a Radiometer conductivity meter. The initial large wash volumes and the mock run were employed to avoid poor resolution in the first run, a phenomenon which occurs occasionally with the crude yeast amino acids.

Analysis and Plotting of Data—One-milliliter fractions were mixed with 3 ml of Aquasol (New England Nuclear) and counted in a liquid scintillation counter (Beckman or Searle). Under these conditions, the samples formed clear gels which gave constant quench and cross-over throughout the entire range of salt concentrations in which the tRNAs eluted. Corrections for background and cross-over were made by a computer program (30) which was modified to accept scintillation counter data on paper tape for analysis in a Data General Corp. computer. The modified program calculated the per cent of the total recovered counts per min for each label in each fraction (per cent total counts per min), calculated the ratio of the per cent total vegetative counts per min to the per cent total developmental counts per min for each fraction, and plotted this data with a Hewlett-Packard 7300A graphic plotter. The data for vegetative fractions was plotted as a dashed line connecting every other data point, and ratios were plotted as individual points. Salt gradients were drawn in by hand.

Sources of Radioactive Amino Acids—Radioactive amino acids were obtained as follows (specific activities in Ci/mmol): from New England Nuclear, L-[^14C]labeled amino acid mixture, L-[3,5-[^3H]arginine (24.2), L-[1-[^14C]asparagine (0.179), L-[2,3-[^3H]aspartic acid (23.66 and 26), L-[3-[^3H]glutamic acid (20.4), L-[2,3-[^3H]glycine (10.2), L-[3,5-[^3H]histidine hydrochloride (3.13), L-[3,5-[^3H]histidine (10.3), L-[4,6,8,11-[^14C]lysine (108), L-[4,5,10,12-[^3H]lysine (20), L-[methyl-[^3H]methionine (0.132), L-[[^7S]methionine (100), L-[[^14C]phenylalanine (12.8), L-[[^14C]serine (30 and 38), L-[[^14C]serine (1.23 and 3.38), L-[[^14C]threonine (2.68 and 2.38), L-[[^14C]trytophan (1.15 and 7.7), L-[[^14C]trytophan (0.9522), L-[3,5-[^3H]tyrosine (48.2).

Preparation of Aminoacyl-tRNA for Columns—Following aminoacylation of the tRNA from both stages under conditions which gave optimum aminoacylation, the reaction mixtures from each stage were made 0.01 M with acetic acid to reduce the pH to 4.5. They were then brought up to 1 ml with 0.25 M RPC-5 buffer (10 mM MgCl₂, 10 mM sodium acetate at pH 4.5, and 0.25 M NaCl; molarity refers to NaCl; MgCl₂, and sodium acetate remain constant at 10 mM). An equal volume of phenol saturated with 0.25 M RPC-5 buffer was added to each and they were blended on a Vortex mixer, combined, and centrifuged at 27,000 x g for 10 min. The aqueous phase was withdrawn and extracted two or three times with ether saturated with 0.25 M RPC-5 buffer. The resulting aqueous phase was gently warmed and mixed on a Vortex mixer to remove the ether, and the solution was then either used immediately for chromatography on the RPC-5 column or frozen for later use. No significant loss of trichloroacetic acid-p precipitable counts or changes in profile were observed after storage of aminoacyl-tRNA for as long as 2 weeks.
and 60.3), and L-[3H]valine (1.11); from Amersham/Searle, L-(methyl- 
3H)methionine (8.2); and from Schwarzi/Mann, L-(2,3-3H)asparagine 
(12) and all remaining 14C-amino acids (0.650). Unless otherwise 
indicated, these amino acids were either generally or uniformly 
labelled.

RESULTS

Optimal Conditions for Amino Acid Acceptance of Vegeta-
tive and Developmental tRNA – Optimal conditions for amino 
acid acceptance of vegetative tRNA are described in Table I. 
All the reaction conditions described gave amino acid incor-
poration directly proportional to tRNA concentration up to at 
least 3.82 A250 units/50 ml of reaction mixture, the highest used 
for any of the aminoaclylation reactions reported here. Also, all 
reached and maintained a reproducible plateau value for at 
least a 70-min reaction time (Fig. 1). The same conditions were 
used with developmental synthetase to determine whether it 
yielded linear incorporation with vegetative tRNA concentra-
tion. For all amino acids tested, this was the case.

We have not rigorously examined the vegetative and devel-
opmental aminoaclyl-tRNA synthetases for differences in their 
ability to aminoaclylate tRNA. However, in analyses of the 
chromatographic behavior of the aminoaclylated tRNAs we 
have found a given tRNA to give similar extents of amino-
acylation and an identical profile regardless of whether it is 
aminoaclylated with vegetative or developmental enzymes. 
Hence the enzymes from one stage are capable of aminoaclyl-
at the all the isoaccepting species of tRNA from the other stage.

Differences in rates of aminoaclylation, however, were not 
investigated.

Comparison of Vegetative and Developmental tRNA Prepa-
rations for Acceptance of Amino Acids – Using the conditions 
listed in Table I, samples of vegetative and developmental 
tRNA were aminoaclylated with each of the 17 14C-aminoacids 
(Table II). The overall acceptance of the amino acids by the 
vegetative preparation (1026 pmol/A250 unit) is greater than 
that for the developmental one (781 pmol/A250 unit), indicating 
that in this case the latter preparation is less pure (there was 
no systematic difference in purities between vegetative and 
developmental tRNA preparations). The third column of data, 
the adjusted ratio of acceptance by the two tRNA prepara-
tions, has been corrected for this difference in purity. This was

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optimal conditions for amino acid acceptance of tRNA</strong></td>
</tr>
<tr>
<td>Amino acid</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Asparagine</td>
</tr>
<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Tryptophan</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
</tbody>
</table>

* These concentrations vary with the activity of a given synthetase preparation and are listed only for purposes of general compari-

Table II

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Vegetative tRNA</th>
<th>Developmental tRNA</th>
<th>Adjusted ratio of acceptance</th>
</tr>
</thead>
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<tr>
<td>Arginine</td>
<td>60</td>
<td>43</td>
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</tr>
<tr>
<td>Asparagine</td>
<td>51</td>
<td>39</td>
<td>1.30</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>38</td>
<td>27</td>
<td>1.07</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>67</td>
<td>47</td>
<td>1.40</td>
</tr>
<tr>
<td>Glycine</td>
<td>72</td>
<td>57</td>
<td>1.27</td>
</tr>
<tr>
<td>Histidine</td>
<td>40</td>
<td>32</td>
<td>1.09</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>94</td>
<td>68</td>
<td>1.40</td>
</tr>
<tr>
<td>Leucine</td>
<td>90</td>
<td>78</td>
<td>1.17</td>
</tr>
<tr>
<td>Lysine</td>
<td>57</td>
<td>37</td>
<td>1.17</td>
</tr>
<tr>
<td>Methionine</td>
<td>10</td>
<td>9</td>
<td>0.85</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>47</td>
<td>37</td>
<td>0.97</td>
</tr>
<tr>
<td>Proline</td>
<td>43</td>
<td>32</td>
<td>1.02</td>
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<tr>
<td>Serine</td>
<td>127</td>
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<tr>
<td>Tryptophan</td>
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<td>37</td>
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</tr>
<tr>
<td>Valine</td>
<td>74</td>
<td>56</td>
<td>1.01</td>
</tr>
<tr>
<td>Total</td>
<td>1026</td>
<td>781</td>
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</table>

Fig. 1. Aminoacylation of tRNA""s. Vegetative tRNA was amino-
acylated with (14H)leucine and vegetative synthetase according to the 
assay conditions described in Table I. Four independent experiments 
were done, each on a different day. For three the specific activity of 
the (14H)leucine was 200 mCi/mmoll 2.82 A250 units of tRNA were 
used (A, A, O); for the other the specific activity of the (14H)leucine 
was 975 mCi/mmoll and 1.41 A250 units of tRNA were used (O).
done by multiplying the ratio of the raw data (first column value/second column value) by the ratio for total acceptance (781/1026). These adjusted ratios show that within an error of about 15% there are no differences in amino acid acceptance by these preparations.

**RPC-5 Profiles of Aminoacyl-tRNAs from Growth and Development**

Developmental tRNA, aminoacylated with "H-amino acid using developmental synthetase, and vegetative tRNA, aminoacylated with "C-amino acid using vegetative synthetase, were initially co-chromatographed with a steep gradient of 0.25 to 1.0 M RPC-5 buffer (100 ml). This initial run served two purposes: (a) it indicated the salt concentration elution range of the aminoacyl-tRNAs and (b) it increased the peak heights of small peaks, making them easier to detect. Independently isolated tRNA preparations were then aminoacylated, but this time the "C-amino acid and vegetative synthetase were used with developmental tRNA and the "H amino acid and developmental synthetase were used with the vegetative tRNA. These aminoacyl-tRNA preparations were combined and run with a very shallow gradient using the previously determined salt concentration elution range. If there were any inconsistencies between these two runs or if any aspects of the profiles remained to be clarified, further combinations of label and synthetase were used with different tRNA preparations. In all, up to five vegetative and three developmental tRNA preparations were used for a particular aminoacyl-tRNA. Recovery of labeled material from the columns always exceeded 90%.

Figs. 2 to 18 are representative profiles of aminoacyl-tRNAs from growth and development. Reproducible quantitative differences, marked with a K on the figures, are found for some amino acids. The most dramatic quantitative differences are found in the profiles for asparaginyl-tRNA (Fig. 3), aspartyl-tRNA (Fig. 4), and tyrosyl-tRNA (Fig. 17). The only other reproducible quantitative differences are in minor peaks: a minor peak for lysine (Fig. 10), the final small peak for methionine (Fig. 11), the fourth proline peak (Fig. 13), three minor peaks for serine (Fig. 14), the minor peak for threonine (Fig. 15), the third peak for tryptophan (Fig. 16), and a minor peak for valine (Fig. 18).

More common than major quantitative differences is the displacement of peaks from one stage with respect to the other. In some cases, there is an actual change in the position of the peak fraction, while in others the displacement is indicated only by a change in the ratio of vegetative to developmental aminoacyl-tRNA indicating changes in the relative amounts of vegetative and developmental tRNA across the peak. Examples of this kind of change in which the developmental peak elutes early (marked with a D on the figures) include the major arginine peak (Fig. 2), the second aspartic acid peak (Fig. 4), the two major glycine peaks (Fig. 6), the histidine peak (Fig. 7), the major and two minor isoleucine peaks (Fig. 8), the major leucine peak (Fig. 9), the second methionine peak (Fig. 11), the minor phenylalanine peak (Fig. 12), the two major threonine peaks (Fig. 15), and the major valine peak (Fig. 18). The only example of a displacement in which the vegetative peak elutes early (marked with a V on the figure) is the second lysine peak (Fig. 10). The displacement in the second tyrosine peak (Fig. 17) was not reproduced in all runs and is not marked on the figure.

**Reproducibility of RPC-5 Chromatographic Profiles**

The performance of the column was periodically monitored by passing Escherichia coli K-12 tRNA (Schwarz/Mann), aminoacylated with ["H]leucine, through the column, and comparing the elution profile with published results (28). A typical profile for E. coli leucyl-tRNA is shown in Fig. 19. Columns were repacked when resolution began to deteriorate (usually after about 25 column runs).

RPC-5 chromatographic profiles of Dictyostelium tRNA are summarized in Table III. Only those profiles which showed sufficient resolution are included in the table. As indicated in the table, profiles were generally highly reproducible. Although individual tRNA preparations gave consistent profiles, however, some differences between tRNA preparations from the same stage were occasionally observed. For example, the quantitative differences in the two major glycine peaks (Fig.
tRNAs of Dictyostelium discoideum

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Fig. 5 (left). RPC-5 chromatographic profile of glutamyl-tRNA from vegetative (veg) and developing (dev) cells. Recovered from the column were 228,000 cpm of developmental [3H]tRNA and 59,000 cpm of vegetative [14C]tRNA.

Fig. 6 (center). RPC-5 chromatographic profile of glycyl-tRNA from vegetative (veg) and developing (dev) cells. Recovered from the column were 302,000 cpm of developmental [3H]tRNA and 22,000 cpm of vegetative [14C]tRNA.

Fig. 7 (right). RPC-5 chromatographic profile of histidyl-tRNA from vegetative (veg) and developing (dev) cells. Recovered from the column were 221,000 cpm of developmental [3H]tRNA and 49,000 cpm of vegetative [14C]tRNA.

Fig. 8 (left). RPC-5 chromatographic profile of isoleucyl-tRNA from vegetative (veg) and developing (dev) cells. Recovered from the column were 80,000 cpm of developmental [3H]tRNA and 56,000 cpm of vegetative [14C]tRNA.

Fig. 9 (center). RPC-5 chromatographic profile of leucyl-tRNA from vegetative (veg) and developing (dev) cells. Recovered from the column were 427,000 cpm of developmental [3H]tRNA and 76,000 cpm of vegetative [14C]tRNA.

Fig. 10 (right). RPC-5 chromatographic profile of lysyl-tRNA from vegetative (veg) and developing (dev) cells. Recovered from the column were 34,000 cpm of vegetative [3H]tRNA and 33,000 cpm of developmental [14C]tRNA.

6) and the second methionine peak (Fig. 11) were not found in other runs. Also, although quantitative differences in the first methionine peak (Fig. 11) were reproduced in all runs, when two vegetative methionyl-tRNA preparations were co-chromatographed, they showed quantitative differences of similar magnitude. Because of results such as these, quantitative differences of less than 20%, especially in minor peaks, were extremely difficult to substantiate. Although such differences might exist in vivo, they were beyond the resolution of this analysis. In addition, some minor peaks were encountered in which the peak tube represented less than 0.1% of the total recovered counts. Peaks in this size range could not be accurately analyzed.

It is impossible for us to equate the number of peaks found for a given aminocyl-tRNA with its number of isoaccepting species. Besides the possibility that some peaks are different modifications of the same gene product, some may be due to the presence of aggregates, tRNA fragments, or amino acid acceptors other than tRNAs (15). Since the purpose of this analysis was only to screen for differences between vegetative and developmental tRNA, these other possibilities have not as yet been pursued.
DISCUSSION

Comparison of Vegetative and Developmental tRNA for Acceptance of Amino Acids—We have found no major difference between vegetative and developmental tRNAs in their level of aminoacylation with each of the 17 amino acids investigated (Table II). In contrast, significant changes in tRNA populations do occur in several other developing systems, including the silk gland, lens, and reticulocyte (4, 5). All of these systems, however, are characterized by differentiation to produce large quantities of a few proteins having an unusual amino acid content, and in each case the change in tRNA populations parallels the change in amino acid composition of the proteins being made. Hence the concentrations of the tRNAs are believed to adjust to permit efficient synthesis of the specialized proteins (4-6, 31). Since D. discoideum synthesizes many different proteins throughout development, it is not surprising that no major readjustment in levels of amino acid acceptance occur. However, these results do not preclude there being important developmental changes in the tRNAs; there could be qualitative changes in the tRNA molecules, or there could be compensating quantitative changes within the subpopulation of isoaccepting species for a given amino acid.
tRNAs of Dictyostelium discoideum

**Fig. 17 (left).** RPC-5 chromatographic profile of tyrosyl-tRNA from vegetative (ueg) and developing (deu) cells. Recovered from the column were 218,000 cpm of developmental [\(^{3}P\)]tRNA and 26,000 cpm of vegetative [\(^{14}C\)]tRNA.

**Fig. 18 (right).** RPC-5 chromatographic profile of valyl-tRNA from vegetative (ueg) and developing (deu) cells. Recovered from the column were 317,000 cpm of developmental [\(^{3}P\)]tRNA and 19,000 cpm of vegetative [\(^{14}C\)]tRNA.

The quantitative differences between aminoacyl-tRNAs from growth and 18 H of development—Quantitative differences between aminoacyl-tRNAs from growth and development are found in both major and minor peaks. The most dramatic quantitative differences are found in the profiles for only three aminoacyl-tRNAs: asparaginyl-tRNA (Fig. 3), aspartyl-tRNA (Fig. 4), and tyrosyl tRNA (Fig. 17). Curiously, those tRNAs, along with histidyl-tRNAs, are the only ones for which major quantitative differences were observed during Drosophila development (32). Since it was shown in that system that differences in a Q base modification were responsible for the shifting of the aminoacyl-tRNAs from one peak to another, it is possible that a similar post-transcriptional change, rather than quantitative differences in gene products, may be responsible for the analogous differences which we observe. Some of the differences in minor peaks may be due to the presence of mitochondrial tRNAs. In general, however, differences in minor peaks accounted for a very small number of the actual changes observed.

The quantitative difference in the final small peak of methionine (Fig. 11) is interesting in light of the restriction in protein synthesis which occurs early in Dictyostelium development (33, 34). We do not as yet know when this quantitative change takes place or if the peak represents a minor cytoplasmic initiator tRNA.

**Displacement of Peaks of Aminoacyl-tRNAs—**The most common class of differences between aminoacyl-tRNAs from growth and development is the displacement of peaks from one stage with respect to the other. This class of differences is highly reproducible for all independent tRNA preparations studied and does not depend on either the label utilized or the source of the synthetase in the aminoacylation reaction. This type of change is apparent for peaks corresponding to 11 out of the 17 amino acids studied. In many cases, the displacement is in the major peak, and in all but one case, the developmental tRNA elutes ahead of the vegetative tRNA. In most instances, a single peak for each amino acid is displaced. Independent confirmation of peak displacements for three amino acid peaks has been obtained on RPC-3 columns.

Preferential degradation is not a likely cause for the peak displacements observed. If there were degradation, it would have to reside in the tRNA preparations themselves since displacements did not change when stage-specific synthetases were interchanged in the aminoacylation reactions for the RPC-5 column runs. Such preferential degradation of the

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*Fig. 19. RPC-5 chromatographic profile of \[^{3}P\]leucyl-tRNA from Escherichia coli K-12. A gradient of 0.45 to 1.0 M RPC-5 buffer (200 ml) was used. Recovered from the column were 186,000 cpm.*

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*Fig. 20. RPC-5 chromatographic profile of \[^{3}P\]leucyl-tRNA from vegetative (ueg) and developing (deu) cells. Recovered from the column were 186,000 cpm of developmental \[^{3}P\]tRNA and 26,000 cpm of vegetative \[^{14}C\]tRNA.*

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*M. Brenner, unpublished observations.*
tRNA preparations is unlikely because (a) vegetative and
developmental tRNAs were prepared in identical fashion, (b)
displacements were consistent for five different vegetative and
three different developmental tRNA preparations, (c) gel
electrophoretic analyses of RNA preparations showed little evi-
dence of degradation, (d) rates and extents of aminoacylation
were identical for tRNAs from both stages, even when stage-
specific synthetases were switched, suggesting (but not prov-
ing) that synthetase recognition was identical for both, (e)
only certain peaks were affected, and (f) heating of vegetative

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>No. of tRNA preparations analyzed</th>
<th>No. of columns</th>
<th>% Total in Given Peak ± S.D.¹</th>
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<tr>
<td>Arg</td>
<td>4</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>V 68.3 ± 4.4</td>
<td>31.7 ± 4.4</td>
<td>3.2 ± 0.4</td>
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<tr>
<td></td>
<td>D 71.1 ± 1.7</td>
<td>28.4 ± 1.7</td>
<td>5.3 ± 0.3</td>
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<tr>
<td></td>
<td>R 0.96 ± 0.06</td>
<td>1.11 ± 0.15</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Asn</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>V 2.4 ± 0.4</td>
<td>30.6 ± 5.1</td>
<td>79.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>D 1.9 ± 1.1</td>
<td>48.9 ± 4.1</td>
<td>49.7 ± 0.4</td>
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<tr>
<td></td>
<td>R 0.35 ± 0.13</td>
<td>1.63 ± 0.15</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Asp</td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>V 65.0 ± 3.0</td>
<td>35.0 ± 3.0</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>D 93.0 ± 3.8</td>
<td>7.0 ± 3.8</td>
<td>28.6 ± 1.7</td>
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<tr>
<td></td>
<td>R 0.70 ± 0.03</td>
<td>10.36 ± 6.55</td>
<td>1.1 ± 0.3</td>
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<td>Glu</td>
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<td>2</td>
</tr>
<tr>
<td></td>
<td>V 9.1 ± 4.2</td>
<td>3.1 ± 0.4</td>
<td>87.9 ± 4.6</td>
</tr>
<tr>
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<td>D 12.2 ± 1.2</td>
<td>3.5 ± 1.4</td>
<td>84.4 ± 2.5</td>
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<tr>
<td></td>
<td>R 0.73 ± 0.28</td>
<td>0.99 ± 0.30</td>
<td>1.04 ± 0.02</td>
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<tr>
<td>Gly</td>
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<td>V 29.7 ± 0.5</td>
<td>70.3 ± 0.5</td>
<td>6.7 ± 1.0</td>
</tr>
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<td>D 26.6 ± 9.0</td>
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<tr>
<td></td>
<td>R 1.35 ± 0.35</td>
<td>1.00 ± 0.12</td>
<td>28.6 ± 1.7</td>
</tr>
<tr>
<td>His</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(one peak only)</td>
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<td></td>
</tr>
<tr>
<td>Ile</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>V 3.0 ± 0.6</td>
<td>85.0 ± 0.5</td>
<td>3.2 ± 0.4</td>
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<td>D 78.0 ± 0.7</td>
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<td>75.5 ± 1.4</td>
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<td>R 1.25 ± 0.03</td>
<td>1.03 ± 0.12</td>
<td>1.12 ± 0.15</td>
</tr>
</tbody>
</table>

Table III

Summary of RPC-5 chromatographic profiles

8The first line for each amino acid (V) gives the percentages of the total cpm of vegetative aminoacyl-tRNA found in each peak,
and the second line (D) gives the corresponding values for developmental tRNA. The third line (R) gives the ratio for each peak
of the percentage vegetative tRNA to the percent developmental tRNA. All data are the averages for the number of columns indicated,
plus or minus the standard deviation of the mean. (Note that the third line is not the quotient of the first line divided by
the second, but that the ratio of the averages rather than the average of the ratios.)
tRNAs of Dictyostelium discoideum

Peaks in the peak displacements observed here to those resulting from the; the observed profile and the heights of the specific peaks in question are also strikingly similar. Even the differences observed in the aspartic acid, asparagine, and tyrosine prototrophs may represent differences in modification, as was demonstrated for changes in the same tRNAs during Drosophila development (32). The fact that displaced peaks almost always have the developmental tRNA eluting ahead of the vegetative tRNA further suggests that a common change or a very small number of changes in modification of these tRNAs are occurring during Dictyostelium development. In the his T mutants of Salmonella, for example, it was shown that a lack of pseudouridine in the anticodon loop, caused by the absence of a single modifying enzyme activity, resulted in at least 10 peak displacements, corresponding to several different amino acids. All peak displacements were in the same direction (8, 9, 36).

Peak Displacements Are Not Unique to Dictyostelium Development – The observation that there were peaks for most of the amino acids tested prompted us to re-examine published profiles of aminoacyl-tRNAs in other developing systems to see if peak displacements could also be detected. Such examination suggests that displacements are present. Some examples include the tryptophan and methionyl-tRNAs of developing brine shrimp (Ref. 37, p. 30), the scyllo-tRNA of milkweed bugs undergoing embryogenesis (Ref. 38, p. 127), and the histidyl-tRNA from different tissues during mouse development (Ref. 39, pp. 68–69). Peak displacements are therefore probably not unique to Dictyostelium development and should be considered as examples of unresolved heterogeneity, possibly due to differences in post-transcriptional modification.

Relation of Differences to Translational Control – Because of the central position occupied by tRNAs in protein synthesis, any analysis of tRNA changes during development must consider the postulated role of tRNA in translational control (1). The possibility that some of the differences we observe may represent differences in Q base modification in the anticodon loop is of interest in this regard. In preliminary work with an in vitro protein synthesizing system from wheat germ programmed with Dictyostelium vegetative mRNA, however, tRNAs from both stages appeared to function equally well in their ability to stimulate incorporation of radioactive amino acids into hot trichloroacetic-acid-precipitable material. This approach is presently being extended by comparing on gels products synthesized in vitro with stage-specific tRNAs.

The possibility that some of the tRNA changes affect functions other than translation should also be considered. The analogy of the peak displacements observed here to those resulting from the his T mutation in Salmonella, which is known to affect gene transcription (8, 9, 14), has already been pointed out.

Acknowledgments – We are extremely grateful to Paula A. Palatnik for help with the computer analysis of the data and to Larry E. Fields and Macy Koehler for help with the experimental work.

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C M Palatnik and E R Katz


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