Ribonucleic Acid Code Words in Wheat Germ*

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SUMMARY

An amino acid-incorporating system has been obtained from ungerminated wheat germ, which can be stimulated by the addition of uridylic-rich polynucleotides. Pol adenyllic and polycytidylic acids fail to stimulate the incorporation of lysine and proline, respectively.

The amino acid codons determined by the use of the uridylic-rich polynucleotides coincide almost perfectly with those reported for Escherichia coli.

Binding of aminoacyl-sRNA to wheat germ ribosomes can be directed specifically by polynucleotides and does not require the presence of supernatant fraction or guanosine triphosphate. Polycytidylic acid failed to promote prolyl-sRNA binding by ribosomes.

Synthetic polynucleotides stimulate specifically the incorporation of amino acids into polypeptides in cell-free systems. This phenomenon has been used to elucidate the amino acid code in Escherichia coli (1, 2). The effect of a few polynucleotides has also been tested in systems derived from other species (3-7). In these studies the RNA code words (codons) have been found to be the same for all the organisms investigated. On these grounds the important principle of the universality of the genetic code has been postulated. However, no detailed evidence has been reported for systems obtained from higher plants.

A cell-free system from ungerminated wheat that incorporates amino acids into polypeptides in cell-free systems has been developed and will be described later. In this communication, some RNA code words found in this system are presented.

A genetically pure strain of wheat (Triticum durum CP x St 464 PM 60 Cp 64N) was kindly supplied by the Instituto de Desarrollo Agropecuario of the Chilean Department of Agriculture. 4C-Amino acids were obtained from New England Nuclear. ATP, creatine phosphate, creatine phosphate kinase, and glutathione were purchased from Sigma. GTP and nucleoside diphosphates were obtained from Schwarz BioResearch. Poluridylic acid was purchased from Miles Laboratories, and yeast sRNA1 was obtained from General Biochemicals.

Wheat germ was isolated according to the method of Johnston and Stern (8) and was stored frozen at -20°C. A ribosomal fraction was prepared according to the method of Marcus and Feeley (9), except that the pH was maintained at 7.5. A fresh preparation was used for every experiment.

Washed ribosomes were prepared by resedimenting the particulates at 105,000 × g for 1 hour. The yield of particles obtained from 8 g of wheat germ was approximately 8 mg of ribosomal RNA.

The protein fraction of the high speed supernatant was freed of contaminating amino acids by passage through a Sephadex G-25 column.

Polynucleotides were synthesized with polynucleotide phosphorylase from Azotobacter vinelandii as reported by Basilio and Ochoa (10).

sRNA from wheat germ and rat liver was prepared by the method of Brumgraber (11), except that, previous to the last ethanol precipitation, the nucleic acid preparation was treated with DNase (1 µg per ml) in the presence of 0.001 M MgCl2.

14C-Aminoacyl-sRNAs were prepared with rat liver sRNA and a rat liver supernatant fraction by the method of von Ehrenstein and Lipmann (12).

The amino acid-incorporating system contained, in a total volume of 1 ml, Tris-HCl (pH 7.5), 50 mM; MgCl2, 5.7 mM; KCl, 25 mM; creatine phosphate, 5 mM; creatine phosphate kinase, 50 µM; ATP, 1 mM; GTP, 0.5 mM; ribosomes, 150 µg of ribosomal RNA; wheat germ sRNA, 150 µg; 105,000 × g supernatant fraction, 0.2 mg of protein; 14C-amino acid (specific activity, 25 µC per µmole), 8 µM; polynucleotides, 100 µg, or as specified.

This system was incubated for 45 min at 30°C, and the reaction was stopped by addition of 4% trichloroacetic acid. After addition of 4% of casein as carrier, the precipitated protein was treated as described by Zamecnik et al. (13) and counted in a Nuclear-Chicago gas flow counter with an efficiency of 39%.

When C-rich polymers were used, the precipitation agent was 20% TCA; for the A-rich polymers, a mixture of 5% TCA and 0.25% sodium tungstate adjusted to pH 2 was utilized. Binding of aminoacyl-sRNA to washed ribosomes in the presence of polynucleotides was assayed as described by Nirenberg and Leder (14).

As reported by Marcus and Feeley (9), poly U greatly enhances the incorporation of phenylalanine in the wheat germ system. In the absence of the polynucleotide, phenylalanine incorporation is approximately 16 µmoles of amino acid per mg of ribosomal RNA; in the presence of 40 µg of poly U, this value reaches levels of 1600 µmoles or more. However, the other homopolymers, namely, poly A, poly C, and poly I, did not stimulate the incorporation of any of the amino acids tested. Several preparations of these polymers remained inactive in a wide range of concentrations of both polynucleotide and magnesium ion.

The specific incorporation of amino acids directed by U-rich copolymers is shown in Table I. Glutamine, asparagine, and cysteine were not tested. The codons were assigned by comparing the frequencies of the different triplets present in the copolymers with the relative incorporation of the various amino acids.

Poly UC (3:1) stimulated the incorporation of phenylalanine, leucine, proline, and serine, while poly UG (5:1) directed the incorporation of phenylalanine, leucine, valine, glycine, and tryptophan. The incorporation of valine with this last copolymer was unexpectedly high. Poly UA (5:1) significantly promoted the incorporation of phenylalanine, leucine, isoleucine, tyrosine, glutamic acid, aspartic acid, and methionine. The values for leucine with all four polynucleotides were higher than one would infer from theoretical grounds.

The codon assignment deduced from the stimulation of amino acid incorporation with the U-rich polynucleotides coincides with the numbers in parentheses.
### Table I

**Amino acid incorporation in wheat germ system with various polynucleotides**

Values without polynucleotide are subtracted from those with polynucleotide.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Polynucleotides and experiment No.</th>
<th>Codons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>UC (5:1)</td>
<td>UGG (5:1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>10.0</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>15.4</td>
<td>20.5</td>
</tr>
<tr>
<td>Proline</td>
<td>8.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Serine</td>
<td>7.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>9.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Histidine</td>
<td>10.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>8.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>7.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>5.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Valine</td>
<td>6.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>9.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

**Table II**

**Binding of aminoacyl-sRNAs to ribosomes in presence of polynucleotides**

The incubation mixtures contained, in a total volume of 0.2 ml, washed ribosomes containing 120 μg of RNA; Tris-HCl, pH 7.6, 100 mM; MgCl₂, 20 mM; KCl, 50 mM; mercaptoethanol, 0.5 mM; and the following components, when indicated: poly U, 40 pg; poly C, 200 ng; poly UC (3:1), 100 μg; GTP, 2.5 mM; 105,000 × g supernatant, 0.2 mg of protein; [3C]-phenylalanyl-sRNA, 23.3 μmoles; [3C]-prolyl-sRNA, 45.2 μmoles; and [3C]-histidyl-sRNA, 33.4 μmoles. After 10 min of incubation, the tubes were treated as described by Nirenberg and Leder (14). Duplicate treated according to Zamecnik et al. (13) showed no incorporation into protein.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Phenylalanyl-sRNA</th>
<th>Prolyl-sRNA</th>
<th>Histidyl-sRNA</th>
<th>Aminoacyl-sRNA bound</th>
<th>μmoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Poly U</td>
<td>10.7</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Poly U, GTP</td>
<td>6.7</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Poly U, 105,000 × g supernatant</td>
<td>6.2</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Poly U, GTP, without Mg++</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Poly UC (3:1)</td>
<td>0.4</td>
<td>1.1</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

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In *E. coli*, aminoacyl-sRNA binding to the ribosome-messenger complex likewise does not require GTP or transfer enzymes, as shown by several investigators (14, 17), and more conclusively by Kurland.2 In reticulocytes, however, a requirement for one of the two transfer enzymes and GTP to carry out the polymerization of phenylalanyl-sRNA from phenylalanyl-sRNA.

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The failure of the homopolymers, with the exception of poly U, to stimulate significantly amino acid incorporation warrants further investigation. This failure is probably not due to the absence of sRNAs containing the appropriate anticodons in wheat germ, since the addition of rat liver sRNA and supernatant fraction from the same source did not activate this system in the presence of poly U and poly UC. Prolyl-sRNA binding was stimulated by poly UC but not by poly C. Supernatant fraction and GTP do not seem to be required for aminoacyl-sRNA binding to washed ribosomes. However, these ribosomes showed complete dependence on both supernatant fraction and GTP to carry out the polymerization of phenylalanyl-sRNA from phenylalanyl-sRNA.

Poly A has been shown to stimulate the polymerization of lysine in a rat liver system (7). Prolyl-sRNA obtained from rat liver also did not bind wheat germ ribosomes in the presence of poly C. The poly C preparation used in these experiments was tested and found active for a 7-fold stimulation of proline incorporation in a chick embryo system.

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possibility, which would imply specificity of binding between messenger RNA and ribosomes, is presently being explored.

A somewhat analogous situation is found in a system derived from unfertilized sea urchin eggs. Monroy, Maggio, and Rinaldi (20) have reported that this system responds to poly U but not to RNA obtained from the same source or from sea urchin embryos. They present evidence that indicates the existence of a ribosome-bound protein that would interfere with the binding of the native messenger RNA to the particles and that can be destroyed by trypsin digestion.

Treatment of ungerminated wheat germ ribosomes with different amounts of trypsin did not significantly activate either the endogenous amino acid incorporation or the polymerization of proline in the presence of poly C. Ribosomes obtained from wheat germ imbibed during 24 hours at room temperature did not bind prolyl-sRNA when incubated with poly C.

The evidence accumulated from different species leaves little doubt that the genetic code is essentially universal, despite the fact that the components involved in protein synthesis have mutated throughout evolution. As discussed by Crick (21), a change in the code would involve a mutation affecting the translation mechanism which rests upon the specificity of the aminoacyl-RNA synthetases. Unless this mutation would affect a very infrequently used triplet, it would be lethal since it would alter the primary structure of most cellular proteins. These considerations imply that a.11 presently living species arose from a common ancestor which had the same degenerate code that is presently being unraveled.

Acknowledgment—It is a pleasure to acknowledge the skillful technical assistance of Miss Lucinda Nunez.

REFERENCES


Separation of H+ and OH- in the Extramitochondrial and Mitochondrial Phases during Ca++-activated Electron Transport*

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When electron transport in isolated mitochondria is stimulated by divalent ions such as Ca++ (1-5) or monovalent ions such as K+ in the presence of valinomycin or gramicidin (6, 7), H+ ions are ejected into the medium. During Ca++-stimulated respiration, about 2.0 H+ ions appear and 2 Ca++ ions are accumulated as each pair of electrons passes each energy-conserving "site" in systems at pH near 7.4 and salt concentrations near 80 mm (1-5, 8-12). These findings have been taken as support for the view that electron transport can produce a pH gradient across the mitochondrial membrane (7, 13-15). However, little quantitative information is available on acid-base changes occurring within intact mitochondria during electron transport.

This communication reports experiments on the acid-base changes in the mitochondrial phase that accompany the appearance of H+ ions in the medium during Ca++-stimulated oxygen uptake. From pH measurements and acid-base titrations on rat liver mitochondria "solvulized" in a nionic detergent before and after Ca++-induced respiratory jumps, it has been found that the mitochondrial phase loses H+ ions in an amount that is nearly exactly equal to the H+ appearing in the suspending medium. This finding thus provides more complete evidence that the respiratory chain may act as a directional "H+ pump."

Freshly prepared rat liver mitochondria were added to a medium of 80 mm NaCl and 5 mm sodium succinate, lightly buffered with either Tris-chloride or glycylglycine; the respiratory jump was initiated by addition of 45CaCl2. Aliquots of the mitochondria were taken before and after Ca++ addition, rapidly chilled, and centrifuged at 10,000 x g. Uptake of 45Ca++ from the medium was measured as described before (8). H+ ejection was measured with a glass electrode and a Beckman Expandmatic pH meter coupled to a Sargent SR recorder. The absolute amounts of H+ ejected were determined by comparison with pH changes yielded on addition of internal standards of HCl in each experiment. Oxygen uptake was measured with the Clark oxygen electrode.

The measurement of acid-base changes occurring in the mitochondrial phase during Ca++-induced respiratory jumps was developed from Mitchell's finding that nonionic detergents abolish the impermeability of the mitochondrial membrane to H+ (cf. References 13 and 14). The mitochondrial pellets obtained from 2.0 ml of the suspension before Ca++ addition and 30 sec after completion of the Ca++-induced respiratory jump were carefully drained of adhering medium and taken up in 2.0 ml of a neutral solution of Lubrol (a nonionic detergent; ICL Organics, Inc., Providence, Rhode Island) in water (40 µg per ml). All of the mitochondrial pellet "dissolved" to yield a
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