Partition Chromatography of Yeast and *Escherichia coli* Soluble Ribonucleic Acid. Relation of Coding Properties to Fractionation*

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ABSTRACT: *Escherichia coli* and yeast soluble ribonucleic acid (s-RNA) were chromatographed on Sephadex G-25 using the Zachau solvent system. The distribution patterns of amino acid specific s-RNA's from the two sources were similar in general but did show some differences, the most pronounced of which were in the behavior of s-RNA's specific for histidine, tryptophan, proline, and aspartic acid s-RNA's. *E. coli* amino acid specific s-RNA's show a tendency to fractionate according to the amino acid codewords. Yeast s-RNA's demonstrate this tendency also but to a lesser extent.

Base analyses of various chromatographic fractions of s-RNA together with partition coefficient determinations on various nucleosides and polynucleotides suggest that the chromatographic separations observed are not explainable in terms of differences in base composition alone. To explain the fractionation of the *E. coli* s-RNA according to codeword, the hypothesis is proposed that a complementary coding area exists in each s-RNA molecule which, perhaps by virtue of its structure or position, exerts a disproportionately great influence on the partition coefficient of the s-RNA molecule.

Comparative studies of soluble ribonucleic acid (s-RNA) preparations of different biological origin (guinea pig, rat, rabbit, yeast, *Neurospora*, and *Escherichia coli*) have documented complex similarities and differences. The base composition of average s-RNA from various organisms has been shown to be remarkably similar in the near equivalence of guanine monophosphate (GMP)[1] and cytidine monophosphate (CMP) as well as of adenine monophosphate (AMP) and uridine monophosphate (UMP), a high GMP and CMP content, and the presence of pseudo-UMP (Dunn *et al.*, 1960; Singer and Cantoni, 1960; Monier *et al.*, 1960; Ofengand *et al.*, 1961; Brown *et al.*, 1962). Average s-RNA preparations from various organisms are also alike in the presence of methylated bases (Dunn, 1959; Cantoni *et al.*, 1962) and in the presence of identical terminal sequences at both ends of the molecule (Hecht *et al.*, 1959; Berg *et al.*, 1961; Singer and Cantoni, 1960; Zillig *et al.*, 1960). In addition to these compositional similarities, average s-RNA from various species also demonstrates relative uniformity in molecular weight with values around 25,000 ± 1000 having been reported by several investigators (Berg *et al.*, 1961; Tissières, 1959; Osawa, 1960; Luborsky and Cantoni, 1962).

While these interspecies compositional and structural similarities were revealed by comparison of average s-RNA preparations, comparisons between s-RNA's specific for a single amino acid have been much more limited due to the difficulties inherent in obtaining pure preparations. However, Nathenson and Cantoni have demonstrated the absence of dimethyl-GMP in serine s-RNA from *E. coli* (Nathenson *et al.*, 1964; Cantoni *et al.*, 1965), although, as first shown by Cantoni *et al.* (1963), one residue of dimethyl-GMP per chain is present in yeast serine s-RNA. Other differences in nucleotide composition and sequence were also demonstrated between serine s-RNA derived from yeast and *E. coli* [S. G. Nathenson and G. L. Cantoni (1964), unpublished observations]. Differences between *E. coli* and yeast s-RNA's specific for phenylalanine, tyrosine, and valine can also be demonstrated by differences in sensitivity to chemical bromination (Yu and Zamecnik, 1963).

Species specificity in the interaction between aminoacyl s-RNA synthetase and s-RNA is another phenomenon which has been used in the comparison of amino acid specific s-RNA's from different species. As first demonstrated by Berg and Ofengand (1958) and later studied in detail by Allen *et al.* (1960), Berg *et al.* (1961), Rendi and Ochoa (1961, 1962), and Bennett *et al.* (1963), instances of species specificity (total or partial inability of a synthetase from one organism to charge the s-RNA from another) are quite frequent although in many cases no difference in the interaction

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[1] Abbreviations used are: GMP, CMP, AMP, and UMP = the monophosphates of guanosine, cytidine, adenosine, and uridine, respectively; ATP = adenosine triphosphate; GpC = guanylyl-3′,5′-cytidine.

with an homologous or heterologous amino acid s-RNA synthetase is observed.

Another method of comparing specific s-RNA's of different biological origin involves comparison of their distribution patterns after chromatography or countercurrent distribution. Apgar and Holley (1962) utilized countercurrent distribution to examine yeast and rat liver s-RNA's and found that several of the amino acid specific s-RNA's are fractionated differently. They also demonstrated that differences in distribution did not always correlate with differences detectable by aminoacyl synthetases and, conversely, that similarity in distribution does not necessarily imply cross reactivity between the s-RNA's and their synthetases. Yamane *et al.* (1963), using methylated albumin columns, chromatographed charged leucine, methionine, and phenylalanine s-RNA's from yeast, *E. coli*, *M. lysodeikticus*, and *Bacillus subtilis*. They demonstrated that when different organisms are used as the source of any one of these three aminoacyl s-RNA's, the chromatographic patterns obtained showed a definite tendency to resemble one another, although no two patterns were identical. In the case of charged tryptophan s-RNA's from yeast and *E. coli*, a complete lack of similarity in their elution profiles was found.

In the course of purification of various s-RNA's by partition chromatography on Sephadex G-25, we have had the opportunity to survey for species differences between various *E. coli* and yeast s-RNA's which might be demonstrable by this method. This article will report these observations and will also report an unexpected result of this work, namely, the discovery of a definite tendency of s-RNA's to fractionate in this system according to their coding properties.

Methods

Yeast s-RNA was prepared by the method of Monier *et al.* (1960), as modified by Apgar *et al.* (1962). *E. coli* s-RNA was prepared by the method of Zubay (1962) from *E. coli* B which had been harvested in the logarithmic phase of growth.

Chromatography of *E. coli* and yeast s-RNA on Sephadex G-25 was carried out as described previously (Tanaka *et al.*, 1962). s-RNA (600 mg) as the tributylamine salt was chromatographed on a 2.5 × 200 cm column of Sephadex G-25. The solvent system used was system "29" of Zachau *et al.* (1961), which contains 1-butanol–water–tri-*n*-butylamine–acetic acid–di-*n*-butyl ether (100:130:10:2.5:29). The column speed was 38 ml/hr and 8-ml fractions were recovered. A constant temperature of 22–24° was assured by use of a water-jacketed column.

As a source of *E. coli* aminoacyl synthetases, we used a crude extract prepared as follows. *E. coli* B cell paste (5 g) was suspended in 20 ml of 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer at pH 7.45 and treated by sonication at full power for 20 min at 4° in a Ratheon 10 KC sonic oscillator. The resultant material was centrifuged for 1 hr at 25,000*g*. The clear yellow-green supernatant (approximately 30 mg/ml of protein) was dialyzed overnight against 2 l. of 0.05 M Tris buffer at pH 7.45, and then frozen in 0.5-ml aliquots. The yeast aminoacyl synthetases were prepared according to Makman and Cantoni (1965). The purification was carried through the 9000*g* centrifugation step after which the supernatant fluid was dialyzed and frozen.

The assay for amino acid acceptor activity of the *E. coli* s-RNA was done in a 200-μl reaction mixture containing potassium cacodylate buffer (pH 7.0, 20 μmoles), adenosine triphosphate (ATP, 200 mμmoles), $MgCl_2$ (400 mμmoles), mercaptoethanol (80 mμmoles), [^{14}C] amino acid (16 mμmoles), and *E. coli* extract (0.45 mg). Incubation was carried out at 37° for 20 min after which time 25 μl of iced [^{12}C] amino acid solution (0.02 M) was added to each tube. The reaction mixture (100 μl) was then pipetted onto Whatman No. 3 MM filter disks and treated according to the method of Mans and Novelli (1961). Counting was done in a Packard scintillation counter. The assay for amino acid acceptor activity of the yeast s-RNA was essentially as described by Makman and Cantoni (1965).

Partition coefficient measurements with yeast s-RNA were done in solvent system "29" of Zachau *et al.* (1961). Average yeast s-RNA (30 mg), as the tributylamine salt, was dissolved in 10 ml of lower phase which was then thoroughly equilibrated with 10 ml of upper phase in a Gyrotory Shaker (New Brunswick Scientific Co.) for 2 hr at room temperature. The s-RNA was recovered from each of the separated phases by adding 1 ml of 20% potassium acetate (pH 5.1) and precipitating with 22 ml of ethanol at 4°. The precipitate was then dissolved in potassium phosphate–sodium chloride buffer, pH 7.6 (0.025 mμ each). Amino acid acceptor activity of the s-RNA from each phase was then determined as described above.

The partition coefficients of adenosine, guanosine, uridine, and cytidine were determined by dissolving 0.5 mg of nucleoside in 1 ml of lower phase of solvent "29" and equilibrating with 1 ml of upper phase as above. After separation of the two phases and suitable dilution with potassium phosphate buffer, pH 7.0 (0.1 M), the optical density at the maximum wavelength of the nucleoside was determined for each phase.

Since polyuridylic acid is soluble in 0.1 N HCl, it was not feasible to prepare its tributylamine salt by the usual method. Therefore, for partition coefficient measurements of polyadenylic acid, polyuridylic acid, and polycytidylic acid, 100 mg of polymer was converted to the tributylamine salt by passing through a 1.8 × 44 cm Dowex-50 tributylammonium column. The ultraviolet absorbing material was pooled and lyophilized to dryness. Lower phase (1 ml) containing 5 optical density units (determined at wavelength of maximum absorbancy) of polymer was equilibrated with 1 ml of upper phase as described previously, after which the optical density in each phase was determined. Base analyses on s-RNA were carried out as described previously (Cantoni *et al.*, 1962).

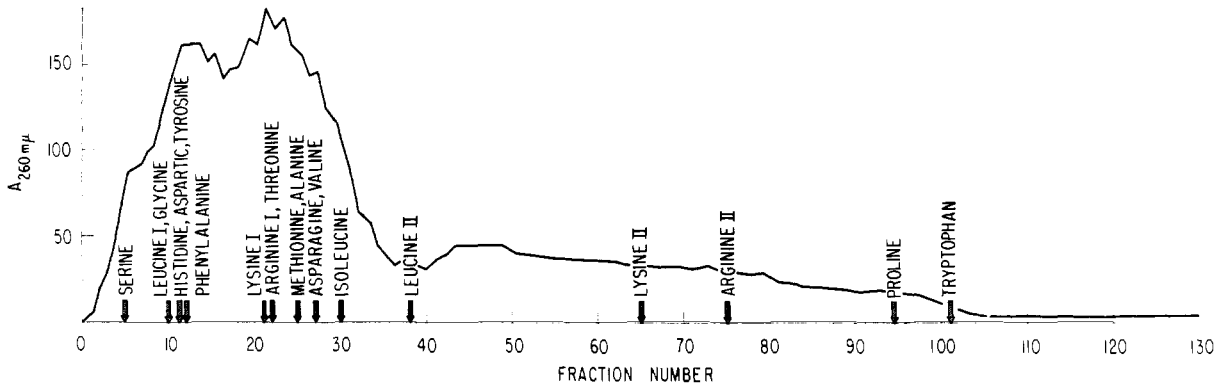

FIGURE 1: Partition chromatography of yeast s-RNA as described in text. A_{260} is plotted as total absorbancy recovered per fraction.

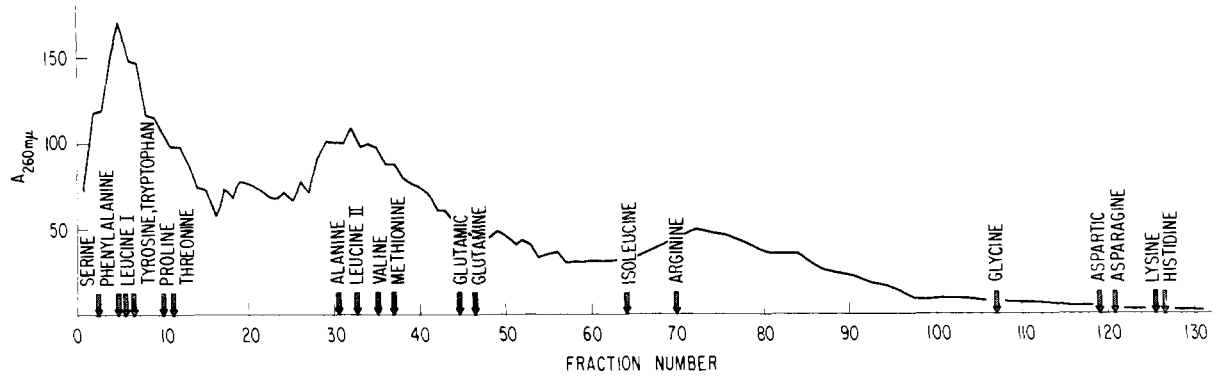

FIGURE 2: Partition chromatography of *E. coli* s-RNA as described in text. A_{260} is plotted as total absorbancy recovered per fraction.

Results and Discussion

Comparison of Yeast and E. coli s-RNA. s-RNA preparations from yeast and *E. coli* were subjected to partition chromatography on Sephadex G-25 under identical conditions and the position of the peak of acceptor activity for the various amino acid specific s-RNA's was determined (Figures 1 and 2). For a more quantitative comparison of the chromatographic properties of the various amino acid specific s-RNA's from yeast and *E. coli*, the R_F of the different amino acid specific s-RNA's was calculated by the equation

$$R_F = \frac{\text{bed volume}}{\text{bed volume} + \text{volume of upper phase eluted to peak of acceptor activity}}$$

The data are presented in this manner in Table I. From these data it can be seen that the patterns of distribution of the amino acid specific s-RNA's from yeast and *E. coli* exhibit remarkable similarities as well as significant differences. For example, in both yeast and *E. coli*, serine-specific s-RNA elutes first from the column. In addition, the phenylalanine, tyrosine, and leucine s-RNA's from yeast also have elution patterns similar to the corresponding s-RNA's from *E. coli*. The elution patterns of most of the other amino acid specific s-RNA's from the two sources are also similar, although to a less marked degree. A few of the s-RNA's, most notably histidine-, tryptophan-, proline-, and glycine-specific s-RNA's, show marked differences in elution pattern.

Thus, these results with the Zachau "29" solvent system on Sephadex G-25, demonstrating both similarities and differences in the elution patterns of the various amino acid specific s-RNA's from different biological origin, are analogous to those obtained with the Holley solvent system in countercurrent distribution with yeast and rat liver s-RNA's (Apgar and Holley, 1962) and those obtained with methylated albumin columns using charged s-RNA's from different organisms (Yamane *et al.*, 1963). It is of interest to note that one of the most marked differences in elution pattern between yeast and *E. coli* s-RNA's specific for the same amino acid is shown by the tryptophan-specific s-RNA's which, in the form of tryptophanyl s-RNA's, have marked differences in elution profile on methylated albumin columns. These findings suggest that yeast and *E. coli*

TABLE I: Relative Mobilities of s-RNA from Yeast and *E. coli*.

Yeast s-RNA	R_F	*E. coli* s-RNA	R_F
Serine	0.84	Serine	0.91
Leucine I	0.72	Phenylalanine	0.83
Glycine	0.71	Leucine I	0.81
Histidine	0.70	Tyrosine	0.79
Aspartic	0.70	Tryptophan	0.78
Tyrosine	0.69	Proline	0.71
Phenylalanine	0.68	Threonine	0.70
Lysine I	0.55	Alanine	0.44
Arginine I	0.54	Leucine II	0.43
Threonine	0.53	Valine	0.41
Methionine	0.50	Methionine	0.40
Alanine	0.49	Glutamic	0.35
Asparagine	0.48	Glutamine	0.34
Valine	0.47	Isoleucine	0.27
Isoleucine	0.46	Arginine	0.26
Leucine II	0.40	Glycine	0.22
Lysine II	0.28	Aspartic	0.20
Arginine II	0.26	Asparagine	0.19
Proline	0.21	Lysine	0.16
Tryptophan	0.20	Histidine	0.16

TABLE II: Partition Coefficients[a] in the Zachau System.

Compound	K
Yeast S-RNA	
Serine	2.30
Tyrosine	1.11
Phenylalanine	0.64
Threonine	0.25
Proline	0.02
Nucleosides	
Adenosine	0.246
Guanosine	0.108
Uridine	0.081
Cytidine	0.036
Polynucleotides	
Poly-A[b]	96.0
Poly-U[c]	0.08
Poly-C[d]	0.03

[a] Partition coefficients were calculated from data obtained in experiments described under procedures. Partition coefficient (K) is defined as: K = (mg/ml in upper phase)/(mg/ml in lower phase). [b] Polyriboadenylic acid. [c] Polyuridylic acid. [d] Polycytidylic acid.

TABLE III: Base Composition of Yeast s-RNA Fractions.[a]

	Serine	Tyrosine Rich	Lysine II Rich	Average
Adenosine	0.3	0.3	0.2	0.3
Cytidine	0.4	0.4	0.8	0.5
AMP	14.1	14.6	13.3	12.8
CMP	16.1	17.9	20.1	19.5
GMP	19.8	20.5	20.3	19.4
UMP	14.2	12.0	10.2	12.5
Ψ-UMP	3.0	2.5	1.9	2.7
Me-GMP[b]	1.0	1.7	2.1	1.4
Me-AMP	0.0	0.2	0.9	0.5
Me-CMP	0.7	0.4	0.5	0.6
Tr-P[c]	1.0	1.0	1.0	0.7

[a] Reported as μmoles per μmole of s-RNA (nucleotide content = 70). These data are reproducible to within 5% for a major compound and 15% for a minor compound. [b] A mixture of dimethyl-GMP and methyl-GMP except for serine s-RNA from yeast in which no Me-GMP can be detected. [c] Thymine riboside.

tryptophan s-RNA's may possess a species difference greater than most of the other yeast and *E. coli* amino acid specific s-RNA's.

Factors Affecting Chromatographic Separation. To determine some of the factors involved in the chromatographic separations, partition coefficients in the Zachau system were determined as described in Methods for several amino acid specific yeast s-RNA's as well as for several polynucleotides and nucleosides. When the partition coefficients for several yeast s-RNA's (Table II) are compared with the relative chromatographic mobility of these s-RNA's (Table I), the correlation is, as expected, excellent, and confirms the fact that differences in partition coefficients between the various s-RNA's are the basis for their chromatographic separation in this system.

The results of the experiments with the polynucleotides and nucleosides (Table II) show high partition coefficients for adenosine, guanosine, and polyadenylic acid (polyguanylic acid was unavailable) and low partition coefficients for uridine, cytidine, polycytidylic acid, and polyuridylic acid. From these data, one might anticipate that the fractions of *E. coli* and yeast s-RNA eluting early (high partition coefficients) should be relatively rich in AMP and GMP residues and the fractions eluting late (low partition coefficient) should be relatively rich in CMP and UMP residues.

In order to determine if the base composition of the s-RNA's in different chromatographic fractions could actually be related to the partition coefficient data in Table II, the base composition was determined for yeast and *E. coli* s-RNA fractions eluting early and late in chromatography. Base analyses (Table III) of a fraction of yeast s-RNA rich in tyrosine acceptor activity, rich in lysine acceptor activity, and a purified preparation of serine-specific s-RNA showed only slight differences in base composition. AMP residues were

present in a molar proportion of 14.1 for serine s-RNA and fell to 13.3 in the lysine-rich s-RNA fraction. CMP residues were present in a molar proportion of 16.1 in serine s-RNA, 17.9 in tyrosine-rich s-RNA, and 20.1 in the lysine-rich preparation. UMP is present in a molar proportion of 14.2 for serine s-RNA and fell to 10.2 in the lysine-rich fraction.

Examination of the base composition of *E. coli* s-RNA fractions (Table IV) eluting early (serine rich), moderately late (arginine rich), and very late (histidine rich) showed a greater difference in composition of AMP residues and a general trend from early to late fractions. CMP residues showed a slight rise from 19.6 in the serine-rich fraction to 21.0 in the histidine-rich fraction. In other nucleotide components there were no consistent differences.

TABLE IV: Base Composition of *E. coli* s-RNA Fractions.[a]

	Serine Rich	Arginine Rich	Histidine Rich	Average
Adenosine	0.68	0.75	0.82	0.72
Cytidine	<0.05	<0.05	<0.05	<0.05
CMP	19.6	20.1	21.0	20.4
AMP	14.5	12.0	10.8	11.75
GMP	19.8	22.6	20.2	20.1
UMP	10.7	8.2	10.8	10.7
Ψ-UMP	1.9	2.2	2.1	2.4
Me-GMP[b]	<0.2	1.05	1.0	1.0
Tr-P	0.93	1.05	0.87	0.8
Me-AMP	0.4	0.24	0.78	0.6
Me-CMP	[c]	0.62	0.78	[c]
X = unknown	2.00	1.1	1.0	1.2

[a] Reported as μmoles per μmole of s-RNA (nucleotide content = 70). These data are reproducible to within 5% for a major compound and 15% for a minor compound. [b] A mixture of dimethyl-GMP and Me-GMP except for serine s-RNA from yeast in which no Me-GMP can be detected. [c] Not analyzed.

When the experimentally determined base composition of the various chromatographic fractions of yeast and *E. coli* s-RNA are compared with the predictions based on the nucleoside and polynucleotide partition data, no consistent agreement is observed. Thus, while the changes in AMP are as predicted in the *E. coli* s-RNA fractions, no significant trend can be seen in the yeast fractions and, in both *E. coli* and yeast, significant changes in GMP are not found. UMP shows no significant trend in *E. coli* fractions and shows a trend in the direction opposite to that predicted in the yeast fractions. CMP changes as predicted in the yeast fractions, but shows only a slight change in the *E. coli* fractions. In light of these results, it would seem difficult, if not impossible, to account for the observed chromatographic separations solely on the basis of differences in average base composition of the various fractions.

Fractionation of E. coli s-RNA According to Coding Properties. Examination of the results of the chromatography of *E. coli* s-RNA (Table I) reveals an interesting fact, namely, that the order in which the various amino acid specific s-RNA's elute from the column is not random, but appears to be related to their coding properties. This can be seen more clearly in Table V in which the s-RNA's are listed in order of elution together with the RNA codewords for the corresponding amino acids as experimentally determined (Nirenberg *et al.*, 1965). s-RNA's specific for amino acids whose codewords are rich in pyrimidines show a striking tendency to elute early, while those specific for amino acids with codewords rich in purines tend to elute late. According to current concepts, where multiple codewords for a single amino acid are known, there are presumably multiple s-RNA's specific for the amino acid and responding to the different codewords (Weisblum *et al.*, 1962). As the chromatographic data shown above do not indicate how many or which of the multiple s-RNA's specific for a given amino acid are present at the peak of acceptor activity, in Table V all corresponding codewords are listed beside each peak.

That the correlation observed in Table V is not an accidental one is suggested by the fact that when the per cent of pyrimidine in the codewords for each s-RNA peak is compared with the R_F of the s-RNA by product–moment and rank correlation,[2] P is less than 0.02 by the former method and less than 0.01 by the latter method. The agreement between mobility and codewords is not perfect, however, and discrepancies are noted, the most striking of which are histidine and tryptophan s-RNA's.

Yeast s-RNA's (Table VI) also show a similar tendency to fractionate according to the amino acid codewords, but the phenomenon is considerably less marked than with *E. coli* s-RNA (Table V). The reason for this difference between *E. coli* and yeast s-RNA's is obscure.

We are unable to give a complete explanation for the correlation between coding properties and chromatographic properties in *E. coli* s-RNA. However, if we assume the presence of a complementary coding triplet in each s-RNA molecule, the chromatographic elution

[2] The correlation coefficients were calculated in the usual manner. The rank correlation coefficient, r_s, was calculated from the formula

$$r_s = -6\Sigma d^2/(n^2 - 1)$$

where n = number of s-RNA peaks, d = difference in rank according to R_F and according to per cent pyrimidine in codewords. The product–moment correlation coefficient, r, was obtained by the formula

$$r = \Sigma x_n y_n/(\Sigma x_n{}^2)(\Sigma y_n{}^2)$$

where $x_n = X_n - \bar{x}$, $y_n = Y_n - \bar{y}$, $X_n = R_F$ for s-RNA, Y_n = per cent pyrimidine in codewords for s-RNA$_n$, $\bar{x} = \Sigma X_n/n$, and $\bar{y} = \Sigma Y_n/n$. P was obtained from the correlation coefficients using the standard table for the distribution of correlation coefficients.

TABLE V: Relative Mobility of *E. coli* s-RNA and Amino Acid Codewords.

Order of Elution	Codewords	% Pyrimidine[a] in Codewords	Theoretical Complementary Coding Triplet
Serine	UCC, UCU, UCG, AGU, AGC	67	AGG, AGA, AGC, UCA, UCG
Phenylalanine	UUU, UUC	100	AAA, AAG
Leucine I	UUG, CUU, CUC, CUG	83	AAC, GAA, GAG, GAC
Tyrosine	UAU, UAC	67	AUA, AUG
Tryptophan	UGA	33	ACU
Proline	CCU, CCC, CCA	89	GGA, GGG, GGU
Threonine	ACU, ACC, ACA, ACG	50	UGA, UGG, UGU, UGC
Alanine	GCU, GCC	67	CGA, CGG
Leucine II	UUG, CUU, CUC, CUG	83	AAC, GAA, GAG, GAC
Valine	GUU	67	CAA
Methionine	AUG	33	UAC
Glutamic	GAA	0	CUU
Glutamine	CAA, CAG, UAA, UAG	33	GUU, GUC, AUU, AUC
Isoleucine	AUU, AUC	67	UAA, UAG
Arginine	CGC, CGA	50	GCG, GCU
Glycine	GGU	33	CCA
Aspartic	GAU, GAC	33	CUA, CUG
Asparagine	AAU, AAC	33	UUA, UUG
Lysine	AAA, AAG	0	UUU, UUC
Histidine	CAU, CAC	67	GUA, GUG

[a] Per cent pyrimidine = (total pyrimidines in all codewords)/(total nucleotides in all codewords) × 100.

TABLE VI: Relative Mobility of Yeast s-RNA and Amino Acid Codewords.

Order of Elution	Codewords	Pyrimidine[a] (%)
Serine	UCC, UCU, UCG, AGU, AGC	67
Leucine I	UUG, CUU, CUC, CUG	83
Glycine	GGU	33
Histidine	CAU, CAC	67
Aspartic	GAU, GAC	33
Tyrosine	UAU, UAC	67
Phenylalanine	UUU, UUC	100
Lysine I	AAA, AAG	0
Arginine I	CGC, CGA	50
Threonine	ACU, ACC, ACA, ACG	50
Methionine	AUG	33
Alanine	GCU, GCC	67
Asparagine	AAU, AAC	33
Valine	GUU	67
Isoleucine	AUU, AUC	67
Leucine II	UUG, CUU, CUC, CUG	83
Lysine II	AAA, AAG	0
Arginine II	CGC, CGA	50
Proline	CCU, CCC, CCA	89
Tryptophan	UGA	33

[a] See footnote *a*, Table V.

pattern can be explained by making the further assumption that the complementary coding triplets influence the partition coefficients in a manner predicted from their base compositions (Table III), but that for some structural or other reason (perhaps single-strandedness of the coding area is a requirement for proper base pairing) the influence on the partition coefficient is relatively much greater than that of the remainder of the molecule. Thus, from Table V it can be seen that s-RNA molecules which might be thought to have complementary coding triplets rich in adenosine and guanosine tend to elute early while those with triplets rich in uridine and cytidine tend to elute late. In conjunction with the hypothesis that a complementary coding triplet inordinately influences chromatographic mobility for a structural reason, it may be noted that in yeast alanine s-RNA the phosphodiester bond most sensitive to T_1 ribonuclease is the guanylyl-3′,5′-cytidine (GpC) bond located in the middle of the chain as part of the sequence IpGpC, one of the few possible alanine complementary coding triplets in the entire sequence (Holley *et al.*, 1965).

As present evidence suggests that reading of the messenger ribonucleic acid (m-RNA) codewords is not influenced by the biological origin of the s-RNA used in the decoding system, the fact that yeast s-RNA does not chromatograph according to codeword as well as *E. coli* s-RNA may be taken as an indication that, even

if the coding area does prove to be inordinately influential in fractionation in this system, it cannot be the only factor involved. Other differences in base sequence and secondary structure, as well as small differences in molecular weight, might be considered.

The concept that the mechanism of translation of the m-RNA code into a linear sequence of amino acids in polypeptides involves complementary base pairing is deeply rooted in present thinking, although not yet firmly established experimentally. The interpretation suggested above to explain the fractionation of s-RNA according to codeword in this partition system may be considered additional, although indirect, evidence for a direct interaction between m-RNA and s-RNA in the process of code reading.

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