# THE NUCLEOTIDE COMPOSITION OF RIBONUCLEIC ACIDS FROM SUBCELLULAR COMPONENTS OF YEAST, *ESCHERICHIA COLI* AND RAT LIVER, WITH SPECIAL REFERENCE TO THE OCCURRENCE OF PSEUDOURIDYLIC ACID IN SOLUBLE RIBONUCLEIC ACID

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## SUMMARY

The analyses of the nucleotide composition of RNA's derived from various subcellular fractions of yeast and *Escherichia coli* B, revealed that the RNA found in the supernatant fraction has a unique nucleotide composition as compared with the RNA from the particle fractions. The supernatant RNA is characterized by the presence of a considerable amount of pseudouridylic acid and by higher cytidylic acid and lower adenylic and uridylic acid contents. Predominant localization of pseudouridylic acid in the RNA of the "pH 5 enzyme fraction" of rat liver was also observed.

The relation between the ability to bind [$^{14}$C]leucine and the content of pseudouridylic acid in RNA's prepared from large granules, small granules, and some subfractions of the 105,000 × *g* supernatant of yeast cells has also been investigated. The RNA prepared from the supernatant fraction is the only active RNA, those prepared from particle fractions failing to incorporate [$^{14}$C]leucine. In five preparations of RNA derived from the acid-fractionated supernatant subfractions and the unfractionated supernatant, it was found that the ability to incorporate [$^{14}$C]leucine into different RNA's is directly proportional to their pseudouridylic acid content. These data suggest that this nucleotide is characteristic of the RNA which incorporates amino acids (at least leucine).

## INTRODUCTION

It is well known that the RNA in the cells is not homogeneous and shows variations in the nucleotide composition[1-4] and the turnover rate[4-7], which depend on the intracellular location. Such structural and metabolic heterogeneity of cellular RNA's would underlie the heterogeneity of their function. There has been much speculation about the involvement of microsomal RNA in the determination of the specificity of proteins. However, one of the most remarkable and also definite functions is that of the RNA found in the supernatant fraction. It has been reported[8] that RNA

Abbreviations used: RNA, ribonucleic acid; ATP, adenosine triphosphate; Tris, tris (hydroxymethyl) aminomethane.

in the supernatant fraction (soluble RNA) binds amino acids in the presence of ATP and amino acid-activating enzymes, and that amino acids so bound are then transferred to the microsomal ribonucleoprotein particles. The amino acid–RNA complex formed is thus considered to be an intermediate of protein synthesis[8]. As a basis for this unique function of the active RNA there should be a specific molecular structure. It is now generally recognized from the work of HECHT *et al.*[9] and others[10,11] that the active RNA possesses the specific terminal group (–pCpCpA), the existence of which is required for the attachment of amino acids. It is not, however, likely that the difference between active and inactive RNA's resides only in the presence or absence of the specific terminal group. For a full understanding of the specific function of the active RNA we require a complete knowledge, not only at the terminal group, but also of the primary and secondary structure of the molecule.

The present paper reports that RNA, in the 105,000 × *g* supernatant of yeast and *E. coli* homogenates, as well as in the "pH 5 enzyme fraction" of the rat liver supernatant, contains a considerable amount of pseudouridylic acid. Because this special nucleotide was not found to any significant extent in RNA's of particle fractions, its presence may be, in addition to the specific terminal group, a feature of the active molecule. In support of this idea it has been demonstrated that the ability to incorporate [$^{14}$C]leucine into different RNA's prepared from the particle fractions, as well as from some subfractions of the 105,000 × *g* supernatant, is directly proportional to their content of pseudouridylic acid.

Preliminary accounts of this work have already been published[12,13].

## EXPERIMENTAL

### *Organisms*

Yeast cells (*Saccharomyces cerevisiae*, Strain Kaneka) were obtained from Drs. S. SIRAI and M. ASAI of the Kanekafuchi Kagaku Co. Ltd., Takasago. *E. coli* B(H) was provided by Dr. R. OKAZAKI of our institute. Rats of an unspecified strain, weighing about 200 g, were used for the preparation of liver fractions.

### *Culture conditions*

For the nucleotide composition analyses of RNA contained in the whole cells as well as in various subcellular fractions, yeast cells were inoculated in the synthetic medium of HALVORSON AND SPIEGELMAN[14] in a 5-l Erlenmeyer flask. Anaerobic conditions were obtained by filling the flask with the medium and sealing it with a rubber stopper connected to a 50 ml-flask containing 5 % pyrogallol in 4 *N* KOH. The cultivation was carried out at 30° under continuous magnetic stirring. After about 14 h (late log phase) cells were collected and washed twice with the medium of CHAO AND SCHACHMAN[15] (CS-medium). For the preparation of RNA or enzymes for the amino acid binding test *in vitro*, late log phase cells grown under simple standing culture at 30° were used.

*E. coli* was grown in 6 l Tris–glucose synthetic medium[16] in three 5-l Erlenmeyer flasks at 37° in a mechanical shaker. In some experiments, glucose was substituted by lactose. The cells of late log phase were collected, and washed with a cold 0.001 *M* Tris buffer (pH 7.0) containing 0.001 *M* $MgCl_2$ (see ref. 17).

*Fractionation of subcellular components*

For the RNA nucleotide composition analyses, 5 g wet yeast cells were ground in a hand mortar for 20 min with the occasional addition of quartz sand. About 15 g of sand were required for the complete disruption of the cells during the period indicated. Homogenate was then suspended with a mechanical stirrer in 30 ml of CS-medium. The suspension was centrifuged at 2,240 × *g* for 5 min. The sediment was washed 3 times with CS-medium and designated as the cell wall fraction. The washings were combined with the first supernatant and centrifuged at 10,000 × g for 20 min in a Servall SS-1 centrifuge. The large granule fraction (LG) was sedimented. The volume of the 10,000 × *g* supernatant was adjusted to 150 ml with CS-medium and centrifuged in Spinco L ultracentrifuge (Rotor No. 40) at 105,000 × *g* for 90 min. The clear supernatant was carefully pipetted out, leaving about 1 cm volume from the bottom of the tube (supernatant fraction (Sp)). The yellowish, transparent pellet firmly packed at the bottom was designated as the small granule fraction (SG). In some experiments the Sp fraction was obtained directly by centrifuging the first 2,240 × *g* supernatant at 105,000 × *g* for 90 min.

For the preparation of RNA the subcellular components were obtained from about 50 g wet cells by essentially the same cell fractionation technique as that described above. When the subfraction of the 105,000 × *g* supernatant was prepared, the pH of the supernatant fraction was adjusted to 5.0 or 5.3 with 1 *N* acetic acid in the cold. The turbid solution was immediately centrifuged at 15,000 × *g* for 10 min in a Servall SS-1 centrifuge, yielding the supernatant and the precipitate at each pH. The precipitate was suspended in 50 ml CS-medium and the pH was adjusted to 6.8 with 1 *N* KOH. The pH of the acid-supernatant was also brought to 6.8 with KOH.

The general procedure of fractionating *E. coli* cells was the same as in the case of yeast cells, except the use of Tris–$Mg^{++}$ suspending medium[7]. The first low speed sediment was discarded, because of its negligible content in RNA.

Fractionation of rat liver components was conducted by the method of KELLER AND ZAMECNIK[18].

All operations were done at 0–2°.

*Nucleotide composition analyses of RNA*

The RNA nucleotide composition of each subcellular fraction was determined by Dowex-1-formate ion exchange chromatography after 0.5 *N* KOH hydrolysis (18 h at 38°) as described previously[4]. For the analyse of the composition of purified RNA, RNA was treated with 1 *N* KOH at 37° for 24 h, to insure more complete hydrolysis. The individual mononucleotides were separated on a 0.6 cm × 28 cm Dowex-1-formate column[4].

For the millimolar extinction coefficient of pseudouridylic acid, 8.3 at 260 mμ at acidic condition[19] was adopted.

*RNA* preparations*

An equal volume of 90% commercial phenol[20] was added to each subcellular

* The RNA preparations, especially those of the supernatant fraction, are contaminated with polysaccharides. We have some evidence which shows that these polysaccharides do not alter the conclusions presented in this paper. Isolated polysaccharides from this RNA preparation have no effect on the incorporation of [$^{14}$C]leucine into the RNA contained in the enzyme preparation. The purified RNA free from polysaccharides has a specific activity similar to that of the crude RNA.

fraction described above and stirred for 1 h at room temperature (about 23°) with a mechanical stirrer. The resulting emulsion was centrifuged in the cold at 16,300 × *g* (Lourdes Centrifuge, Volume rotor) for 30 min. The aqueous layer was pipetted out. The remainder was mixed with an appropriate volume of cold water and centrifuged again. The combined aqueous layer was made up to 2 % with respect to potassium acetate. 2 volumes of cold ethanol were then added and the content was kept at −5° for 18 h. The precipitate was collected by centrifugation and was dissolved in 20 to 50 ml of water. Insoluble matter was centrifuged down at 105,000 × *g* for 30 min. The RNA was precipitated again by adding 2 volumes of ethanol and 2 % potassium acetate. It was dissolved in water and dialysed for 4 h against cold water. After dialysis, it was again centrifuged at 20,000 × *g* for 20 min. The clear solution was then frozen and lyophilized. Dried samples were kept in a desiccator in a cold room before use.

*Preparation of cell extract as the enzymes for the activity test of RNA*

5 g of wet yeast cells, washed with CS-medium, were ground with quartz sand for 20 min. The homogenate was suspended in 15 ml of CS-medium and centrifuged at 2,000 rev./min for 3 min to remove sand. The supernatant was again centrifuged at 105,000 × *g* for 90 min. The fat layer at the top of the tube was removed and the upper 3/4 of the supernatant was pipetted out and dialysed against 0.003 *M* Tris buffer at pH 6.8. After dialysis for 18 h, the pH of the content was brought to 7.6 by careful addition of saturated potassium bicarbonate. All operations were done at 0–2°. The preparation usually contained about 6 mg proteins and 0.35 mg RNA/ml.

*Test of the ability of RNA to combine* L-*leucine*

0.2 ml of RNA dissolved in 0.2 *M* Tris buffer (pH 7.6) was shaken at 30° for 5 to 15 min with 0.3 ml of the dialyzed 105,000 × *g* supernatant, 1 μmole ATP, 1 μmole $MgCl_2$, 13.3 μmoles Tris buffer, pH 7.6, and 0.41 μmole (0.25 μC) L-[$^{14}$C]-leucine in a total volume of 1 ml. Duplicate or triplicate flasks were usually run. In some experiments, 0.05 μmole cytidine triphosphate was added without effect. Variation in the amount of ATP from 0.5 μmole to 10 μmoles, of the enzymes from 0.2 ml to 0.5 ml, of $MgCl_2$ from 1 μmole to 3.3 μmoles and of Tris buffer from 13.3 μmoles to 50 μmoles, did not change the incorporation of [$^{14}$C]leucine into RNA. After incubation, the flasks were chilled and 8 ml of 0.4 *N* $HClO_4$, plus 2.0 ml of carrier proteins, were added. The precipitate was washed 4 times with cold 0.2 *N* $HClO_4$, twice with cold ethanol, once with acetone, twice with ether, and dried. The powder was suspended in acetone and deposited quantitatively on Whatman No. 1 filter paper with the aid of a Tracerlab E8A filtration apparatus. The activity of samples was counted in a $2\pi$ gasflow counter, and corrected for self-absorption. Duplicate blanks, without added RNA, were always run in parallel. The total activity of added RNA was estimated as the difference between the total activity of experimental series and the total activity of the blank. Similarly the amount of added RNA is known by the difference between the RNA contained in the sample of the experimental series and that contained in the blank. For the determination of RNA, u.v. absorption at 260 mμ was estimated on a hot 10 % $HClO_4$ extract (70°, 20 min) of the sample which had been used for counting.

The L-[$^{14}$C]leucine used in this work was part of an allocation of it made by The Radiochemical Centre, Amersham, England to The Japan Isotope Association.

## RESULTS AND DISCUSSION

*Occurrence of pseudouridylic acid in RNA of the supernatant fraction of yeast, E. coli, and rat liver cells*

In Table I, column 1, are shown the nucleotide compositions of RNA's of different subcellular components of yeast cells. Because no special effort was made in the present study to identify the cells in the fractions used, the fractions collected at the centrifugal field of 10,000 × *g* (20 min), and that at the field of 105,000 × *g* (90 min) were provisionally named the "large granule (LG) fraction", the "small granule (SG) fraction", and the "supernatant (Sp) fraction", respectively. The SG fraction includes the bulk of ribonucleoprotein particles (80 S particles of CHAO AND SCHACHMAN[15]) of the yeast cells. In any event, it is clear from the table that the RNA's from the LG and SG fractions were very similar, whereas that of the RNA of the supernatant fraction was significantly different. The supernatant RNA, as compared with the RNA of the particle fractions, is characterized by a much higher content of cytidylic acid and a lower content of adenylic and uridylic acid.

In addition to the distinct composition of the four nucleotides which commonly occur in SpRNA, the chromatogram of these RNA nucleotides indicated the presence

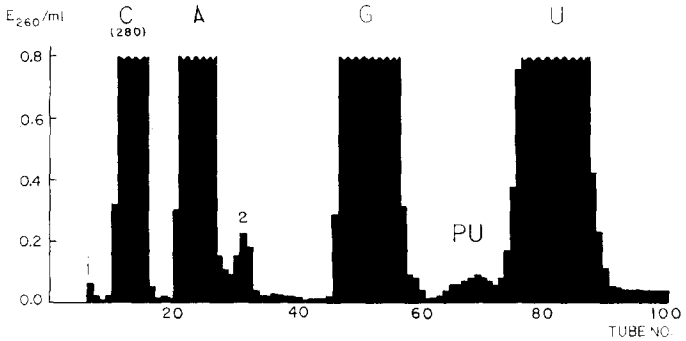


Fig. 1a.

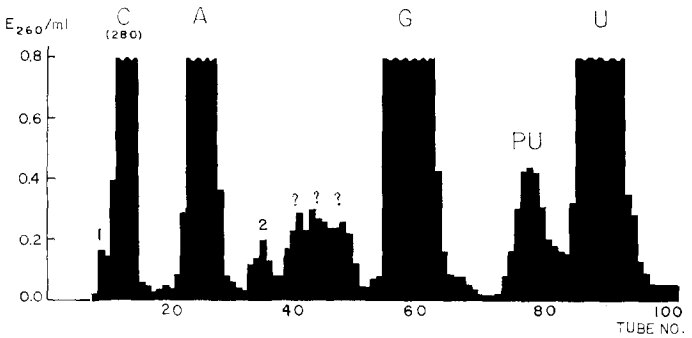


Fig. 1b.

Fig. 1. Chromatographic separation of individual mononucleotides of RNA in the small granule fraction (a) and in the supernatant fraction (b) of *Saccharomyces cerevisiae*. C, cytidylic acid; A, adenylic acid; G, guanylic acid; U, uridylic acid; PU, pseudouridylic acid; 1,2, probably not nucleotide; ? unidentified.

of a small but definite peak just before that of uridylic acid*. The contents of the tubes of the main part of this peak were pooled and lyophilized to remove formic acid. The u.v. absorption spectra taken at different pH's agreed closely with those reported by DAVIS AND ALLEN[19] for 5-ribosyluracil nucleotide[26,27]. We therefore tentatively assumed this to be the same nucleotide and, the name "pseudouridylic acid" proposed by COHN[27] for this substance has been adopted throughout the present paper. Our sample showed $\lambda_{max}$ 262 m$\mu$, $\lambda_{min}$ 233 m$\mu$, 260/280 ratio = 0.43 in 0.1 *N* HCl, and $\lambda_{max}$ 285 m$\mu$, $\lambda_{min}$ 245 m$\mu$, 260/280 ratio = 2.0 in 0.1 *N* KOH.

Fig. 1b shows a chromatographic pattern of RNA mononucleotides from the Sp fraction, indicating the presence of a considerable amount of pseudouridylic acid. The peak corresponding to this nucleotide is either absent or insignificantly small in the chromatograms of the other cellular fractions (*cf.* Fig. 1a). A small amount of this nucleotide detectable in the RNA's of particle fractions would possibly be due to the contamination of the supernatant fraction, because none of the fractions obtained were thoroughly washed to remove traces of the supernatant fluid. However, the possibility of the presence of a very small amount of this substance in RNA's of the particle fractions remains to be examined.

The essential results obtained in the yeast cell fractions were also confirmed in the nucleotide composition analyses of RNA's of *E. coli* subcellular fractions**. The data shown in Table I, column 2, indicated the following characteristics of the SpRNA compared with RNA's from the particle fractions: (a) a higher cytidylic acid content, (b) lower adenylic acid and uridylic acid contents, and (c) a predominant localization of pseudouridylic acid.

Following the confirmation of the distinct composition of SpRNA both of yeast and *E. coli* cells, our interest was focused to see whether the SpRNA of mammalian tissues also has the same features. In this study, the nucleotide composition of microsomal RNA and that of the RNA in the "pH 5 enzyme fraction" obtained from the 105,000 $\times$ *g* supernatant of rat liver was examined. Predominant localization of pseudouridylic acid in the latter RNA was evidenced. These results accord well with those reported by DUNN[21], who used the same materials. Although a lower content of uridylic acid in the RNA of the pH 5 enzyme fraction was found, no characteristic differences in adenylic acid and cytidylic acid contents were detected (Table 1, column 3). It should, however, be pointed out that the composition of the RNA of the pH 5 enzyme fraction is not very different from that of SpRNA of microbial

* Throughout this paper, we have not discussed the other additional ribonucleotide components in RNA (cf. 21–25), because we have not yet made detailed analyses of these. Examination, however, of the chromatographic patterns suggest that they also are concentrated in SpRNA.

** A comparison of the nucleotide composition of RNA's in *E. coli* cells grown in glucose with those grown in lactose media revealed another aspect of SpRNA. As seen in Table I, Column 2, the composition of RNA's from the LG and SG fractions of glucose- and lactose-grown cells were very similar. A clear difference was unexpectedly obtained in the composition of the SpRNA. The cells which are synthesizing $\beta$-galactosidase (in lactose) contained more adenylic acid and less cytidylic acid in the SpRNA, compared with those not forming the enzyme. The behavior of the SpRNA during $\beta$-galactosidase formation was closely similar to that found in chloroplast formation in *Euglena gracilis*[28,29] and, although the analyses were made only on the total RNA, higher adenylic and lower cytidylic acid contents of RNA of the adaptive chloroplast formation were reported. It is not known whether the changes in the nucleotide composition of SpRNA found in our work is in some manner connected with the general mechanism of the inductive synthesis of new proteins, or is related to the determination of the specificity of the particular protein molecule in question.

TABLE I

NUCLEOTIDE COMPOSITION OF RNA'S OF SUBCELLULAR COMPONENTS OF *Saccharomyces cerevisiae*, *Escherichia coli* B(H) AND RAT LIVER

| *Fraction* | *Distribution of RNA in cell* % | *No. of determination* | *Adenylic* | *Guanylic* | *Cytidylic* | *Uridylic* | *Pseudouridylic* |
|---|---|---|---|---|---|---|---|
| *Saccharomyces cerevisiae* | | | | | | | |
| Whole cells | 100 | 3 | 10.0*(25.6)** | 11.0 (28.2) | 7.6 (19.5) | 10.4 (26.7) | < 0.3 ( — ) |
| Cell wall | 6.4 | – | — | — | — | — | — |
| Large granules | 7.7 | 3 | 10.0 (26.4) | 10.2 (26.9) | 7.5 (19.8) | 10.2 (26.9) | < 0.1 ( — ) |
| Small granules | 69.0 | 4 | 10.0 (25.9) | 10.7 (27.7) | 7.3 (18.9) | 10.6 (27.4) | < 0.3 ( — ) |
| Supernatant | 14.3 | 4 | 10.0 (21.8) | 12.5 (27.2) | 11.9 (25.9) | 10.1 (22.0) | 1.4 (3.05) |
| *Escherichia coli*, B(H) | | | | | | | |
| Whole cells, G*** | 100 | 3 | 10.0 (23.2) | 13.8 (32.4) | 9.8 (22.8) | 9.4 (20.9) | < 0.1 ( — ) |
| L§ | | 2 | 10.0 (23.9) | 13.4 (32.0) | 9.3 (22.2) | 9.1 (21.8) | < 0.1 ( — ) |
| Large granules, G | 16.6 | 2 | 10.0 (24.6) | 12.6 (31.0) | 9.4 (23.1) | 8.7 (21.4) | < 0.1 ( — ) |
| L | | 3 | 10.0 (24.3) | 12.6 (30.6) | 9.1 (22.1) | 9.5 (23.0) | < 0.1 ( — ) |
| Small granules, G | 60.0 | 3 | 10.0 (24.2) | 13.1 (31.9) | 8.9 (21.6) | 9.3 (22.5) | < 0.1 ( — ) |
| L | | 3 | 10.0 (24.4) | 13.3 (32.4) | 8.6 (21.0) | 9.2 (22.4) | < 0.1 ( — ) |
| Supernatant, G | 23.4 | 2 | 10.0 (20.0) | 15.5 (31.0) | 14.3 (28.6) | 9.6 (19.2) | 0.61 (1.22) |
| L | | 2 | 10.0 (22.0) | 14.5 (31.9) | 11.7 (25.8) | 8.8 (19.4) | 0.41 (0.9 ) |
| Rat liver | | | | | | | |
| Microsomes | — | 2 | 10.0 (18.6) | 17.2 (31.9) | 15.6 (29.0) | 11.0 (20.4) | < 0.3 ( — ) |
| pH 5 enzyme fraction | — | 2 | 10.0 (19.7) | 14.8 (29.2) | 14.6 (28.8) | 9.2 (18.2) | 2.0 (3.95) |

* Molar ratio, Adenylic acid = 10.0.

** Moles/100 moles nucleotides. In this paper, this value is in all cases only tentative, because the additional nucleotides other than pseudouridylic acid were not taken into account.

*** G = glucose grown cells.

§ L = lactose grown cells.

cells. On the other hand, the RNA's of the particle fractions were quite different from each other.

*The relation of pseudouridylic acid content in RNA's to the ability to link leucine* in vitro

It has been shown by HECHT *et al.*[9] and PREISS *et al.*[11] that the ability of RNA to link amino acids can be estimated by measuring the incorporation of labeled amino acids into the added RNA in the presence of ATP and the cell extract containing amino acid-activating enzymes. We have constructed the similar system described above in the section entitled EXPERIMENTAL. In our system, the labeling of the added RNA with [$^{14}$C]leucine is completed within 10 min and the total count in the RNA is nearly proportional to the amount of RNA added in the 10 to 15 min incubation at 30°. The presence of enzyme is essential. The omission of ATP reduced the incorporation to about one third. When there is a considerable incorporation without the addition of ATP, this may be due to the presence of ATP in the enzyme preparation, which was used undialyzed. The addition of ribonuclease (200 $\mu$g/ml) completely abolished the activity. In these experiments the RNA prepared from pH 5.0 supernatant of the 105,000 $\times$ *g* supernatant fraction was used as the amino acid acceptor.

The fact that the labeling is almost entirely in the RNA was established by recounting the samples after treatment with hot $HClO_4$ or ribonuclease.

In the first place an examination was made of the incorporation of [$^{14}$C]leucine into RNA's prepared from different subcellular fractions and also of their nucleotide composition. As Fig. 2 shows and as HOAGLAND *et al.*[8], and HECHT *et al.*[9] also showed in their experiments on animal cells, only the RNA prepared from the supernatant fraction was active as the acceptor of leucine. On the other hand, RNA's from the large granule and small granule fractions were entirely lacking in ability to incorporate

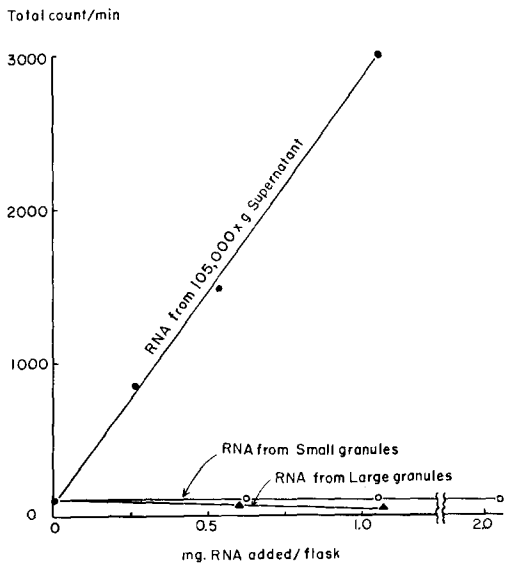


Fig. 2. Incorporation of L-[$^{14}$C]leucine into RNA's prepared from different subcellular fractions of *Saccharomyces cerevisiae*. 10 min incubation.

[$^{14}$C]leucine. Nucleotide composition analyses of these RNA preparations revealed that the active RNA contains about 4.5 % of pseudouridylic acid, whereas RNA's from the large granule and small granule fractions have only a trace of this nucleotide. The result is in good accord with the analyses done on the total RNA contained in each fraction.

It was soon found, however, that the RNA in the supernatant was not homogeneous. The specific activity of RNA prepared from the pH 5.0 supernatant is higher than that prepared from the pH 5.0 precipitate of the 105,000 × *g* supernatant (Fig. 3). It is therefore not possible to decide whether pseudouridylic acid is in the active RNA. This situation makes it necessary to correlate the pseudouridylic acid content and the amino acid-binding activity in RNA's prepared from the different subfractions of the supernatant. The quantitative estimation in one case indicated

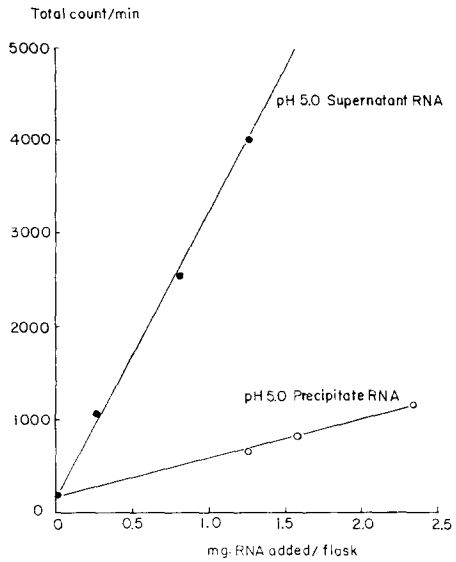


Fig. 3. Incorporation of L-[$^{14}$C]leucine into RNA's prepared from the supernatant and the precipitate at pH 5.0 of the 105,000 × *g* supernatant fraction of *Saccharomyces cerevisiae*. 15 min incubation.

that the amount of RNA present in the pH 5.0 supernatant is about equal to that in the precipitate. Although the ratio of the activity between the RNA of the pH 5.0 supernatant and the precipitate varied from one preparation to another, the specific activity of the former RNA was nearly constant throughout the preparations, whereas that of the latter varied to a considerable extent (*cf.* Fig. 3 and Table II). This probably means that a part of the fraction containing active RNA is precipitated at pH 5.0 and that the small variations of pH affect the extent of the precipitation of the active RNA along with the inactive RNA. In support of this idea it was found that the pH of the supernatant fraction adjusted to 5.0 was shifted, during the centrifugation, to between 5.05 and 5.20.

In one experiment in which the incorporation of [$^{14}$C]leucine into RNA's from pH 5.0 supernatant and precipitate was measured, the two RNA preparations were analyzed for their nucleotide composition. As Table II, Expt. 1 shows, the pH 5.0

TABLE II

INCORPORATION OF L-[$^{14}$C]LEUCINE INTO RNAs PREPARED FROM SEVERAL SUBFRACTIONS OF THE 105,000 × *g* SUPERNATANT, COMPARED WITH THE NUCLEOTIDE COMPOSITION OF THESE RNA PREPARATIONS OF *Saccharomyces cerevisiae*, STRAIN KANEKA

0.5 to 1.5 mg RNA per flask were incubated for 15 min under the conditions described in EXPERIMENTAL

| *Source of RNA* | *Nucleotide composition, moles/100 moles nucleotides* | | | | | | *Amount of L-leucine linked to RNA, mμmole/mg RNA (3)* | *(3)/(2)* |
|---|---|---|---|---|---|---|---|---|
| | *Adenylic* | *Guanylic* | *Cytidylic* | *Uridylic (1)* | *Pseudouridylic (2)* | *(1) + (2)* | | |
| Expt. 1 | | | | | | | | |
| pH 5.0 supernatant | 21.2 | 27.2 | 25.2 | 21.8 | 4.66 | 26.46 | 1.68 | 0.366 |
| pH 5.0 precipitate | 23.9 | 27.4 | 22.0 | 24.4 | 2.38 | 26.78 | 0.92 | 0.367 |
| Expt. 2 | | | | | | | | |
| Unfractionated supernatant | 21.1 | 28.3 | 24.4 | 22.1 | 4.45 | 27.55 | 1.68 | 0.368 |
| pH 5.0 supernatant | 20.8 | 27.2 | 24.3 | 22.2 | 5.57 | 27.77 | 2.01 | 0.361 |
| pH 5.3 supernatant | 20.7 | 28.0 | 24.6 | 20.9 | 5.74 | 26.64 | 2.03 | 0.354 |
| pH 5.0 precipitate | 24.8 | 28.9 | 19.5 | 25.5 | 1.32 | 26.82 | 0.47 | 0.356 |
| pH 5.3 precipitate | 25.5 | 30.3 | 17.5 | 25.9 | < 0.50 | (26.40) | 0.00 | — |
| Small granules | 25.6 | 29.0 | 18.6 | 26.8 | < 0.10 | (26.90) | 0.00 | — |

supernatant RNA was about twice as active as the precipitate RNA and the content of the pseudouridylic acid in the former was twice as much as that in the latter. It was also noticed that the more active RNA contains more cytidylic acid and less uridylic acid.

To evaluate this result more closely, several RNA preparations were made from the supernatant subfractions. The 105,000 × *g* supernatant fraction was adjusted to pH 5.0 or pH 5.3, yielding four subfractions, precipitates and supernatants at each pH. RNA's prepared from these fractions and also from the unfractionated supernatant were tested for the incorporation of [$^{14}$C]leucine and the data obtained were compared with the nucleotide composition of these RNA preparations. The treatment at pH 5.3 effectively precipitates only the inactive RNA, leaving the active RNA in the supernatant. At pH 5.0 a part of the active RNA was precipitated along with the inactive RNA. Comparison of the incorporation data with those of the nucleotide composition analyses makes it clear that the ability to incorporate [$^{14}$C]leucine into different RNA's is directly proportional to their content of pseudouridylic acid, as may be seen in Table II, Expt. 2. The most active RNA (from the pH 5.0 or 5.3 supernatant) contained about 5.6 % of this nucleotide. Only a trace of this was found in the inactive RNA (from the pH 5.3 precipitate). The RNA with intermediate activity (from the pH 5.0 precipitate) contained 1.3 % pseudouridylic acid. Comparison of the active with the inactive RNA, showed that the former had higher contents of cytidylic and lower adenylic and uridylic acids

The higher cytidylic acid content in the active RNA may be attributed in part to the terminal –pCpCpA group[9], although the occurrence of this group in our materials has not yet been examined. As Table II shows, another point of interest in the nucleotide composition is the fact that the active RNA contains less uridylic acid and more pseudouridylic acid and that the sum of these two nucleotides is nearly constant throughout these RNA preparations. The data strongly suggest that the pseudouridylic acid is a unique constituent of the RNA active for the incorporation of amino acids (at least leucine) and that some part of the uridylic acid in the active molecule is replaced by pseudouridylic acid.

There is a good deal of evidence now which indicates that amino acid is linked to the RNA which is specific for each amino acid[9,11,30,31]. It is therefore interesting to find out whether the relation of the nucleotide composition of RNA's to the leucine incorporation evidenced in this study also holds when other amino acids are used. Investigation on this question is now in progress.

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