Studies on the Conversion of 60s Yeast Ribosomes into a 50s Component

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The process of conversion of the larger (60s) subunit of yeast ribosomes into a 50s component was studied. The release of any RNA or protein material during conversion was assayed by using ^{32}P - or ^{35}S -labelled ribosomes; ribosomal RNA distributions of the particles were examined and protein/RNA ratios of subunits were determined. The change in sedimentation coefficients was found to be due, not to loss of material, but to a structural change. The change in structure was shown by electron microscopy.

The transformation of ribosomal subunits to slower-sedimenting components has been shown by many workers, some of whom have attributed it to loss of material (Elson, 1961) and others to unfolding of ribonucleoprotein strands (Gavrilova, Ivanov & Spirin, 1966; Gesteland, 1966; Miall & Walker, 1969; Natori, Maruta & Mizuno, 1968). With yeast cells Morgan (1962) showed that a new 50s component was formed from the larger 60s subunit on removal of Mg^{2+} . The present paper reports that this change in sedimentation coefficient is due, not to loss of material, but to a structural change.

EXPERIMENTAL

Materials. Exponentially multiplying cells of *Saccharomyces cerevisiae* were collected from 4l. of growth medium [1% peptone (Bacteriological, Oxoid Ltd., London, E.C.4), 4% maltose, pH 5, cultured at 30°], washed twice with water and resuspended in 10ml. of TCM_3 buffer (5mm-MgSO₄-10mm-tris-chloride buffer, pH 7.4). The crude extract was prepared by the method of Chao (1957). The purified ribosome preparation was obtained by the usual procedure of alternate low-speed and high-speed centrifugations at 10000g and 100000g respectively. All steps were performed in the cold (0–5°). The ribosome concentration was measured from the extinction at 260nm., taking $E_{1\%}^{1\text{cm}}$ as 113 (Yin & Bock, 1960).

Ribosomal subunits were obtained by dialysing the ribosomes in TCM_3 buffer against TC buffer (10mm-tris-chloride buffer, pH 7.4) for several hours or TCM_5 buffer (0.05mm-MgSO₄-10mm-tris-chloride buffer, pH 7.4) overnight. Always 1ml. of a sample was dialysed against 1l. of buffer without stirring.

Radioactivity study. The release of any RNA or protein material during conversions of 60s subunits into 50s units was measured by labelling the ribosomes with ^{32}P or ^{35}S (^{32}P orthophosphate and sodium ^{35}S sulphate obtained from Atomic Energy Establishment, Trombay, Bombay, India). Cells were grown up to the exponential phase in the presence of [^{32}P]phosphate (2 μC /ml.) or [^{35}S]sulphate

(0.5mc/ml.), collected and mixed with three times their number of exponential-phase carrier cells. Ribosomes were obtained as described above. Radioactivity counts were taken in a Geiger-Müller counter. Counting efficiencies for ^{32}P and ^{35}S are 100% and 4% respectively.

Sedimentation analysis. The ribosomal distribution was examined in a Spinco model E analytical ultracentrifuge with schlieren optics. All runs were made at 50740 rev./min. at room temperature. Sedimentation coefficients are reported as $S_{20,w}^0$ values.

Ribosomal subunits were separated by centrifugation in a linear 5–20% (w/v) sucrose gradient made in TCM_5 buffer by using the inverted-gradient technique of Britten & Roberts (1960). After centrifugation at 37000 rev./min. for 90 min. with a SW39 rotor, 2-drop fractions were collected and diluted with 2ml. of TCM_5 buffer.

Determination of RNA and protein content. (1) RNA. Ribosomal RNA was isolated by using the sodium dodecyl sulphate technique described by Kurland (1960).

(2) Protein/RNA ratio. The protein content of the subunit preparations was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) on 0.75 ml. of each fraction obtained after sucrose-density-gradient centrifugation, with bovine serum albumin as standard. The RNA content was determined by measuring the extinction at 260 nm. of the remaining portion of each fraction.

Electron microscopy. Electron microscopy was done by the pre-shadowed replica technique of Hall (1960). Electron micrographs were taken in a Siemens Elmiskop 1 at electronic magnifications of $\times 8000$.

RESULTS AND DISCUSSION

The ultracentrifugal pattern of yeast ribosomes taken in TCM_3 buffer is shown in Plate 1(a), where 90% of the particles are in the 80s form. Plates 1(c) and 1(d) show the course of dissociation of the 80s component into a mixture of 60s and 40s subunits, and also the conversion of 60s particles into 50s units, during dialysis of the ribosomes against TC buffer for 1 hr. and 6 hr. respectively. To

Table 1. *Assay of radioactivity during dialysis of ribosomes against Mg²⁺-free buffer*

Ribosomes labelled with ³²P or ³⁵S were taken in TCM₃ buffer and dialysed against TC buffer for different times to ensure increased conversion of 60s particles into 50s units. Duplicate 6mg. samples of labelled ribosomes were precipitated by centrifuging for 2½ hr. at 39000 rev./min. and radioactivity of the sediment, supernatant and dialysis buffer was determined.

Samples	Time of dialysis (hr.)	Radioactivity (c.p.m./ml.)		
		Sediment	Supernatant	Dialysis buffer
³² P-labelled ribosomes in TCM ₃ buffer	0	39469	582	32
	1	40123	539	28
	6	39752	573	24
³⁵ S-labelled ribosomes in TCM ₃ buffer	0	878	78	23
	1	764	83	22
	6	748	89	22

test whether there was release of RNA or protein during these processes ribosomes labelled with ³²P or ³⁵S were prepared and treated as above. They were then precipitated as described in Table 1 and the radioactivity of sediment, supernatant and dialysis buffer was determined.

The results in Table 1 show that all the radioactivity was in the sediment fraction. Neither the dissociation of the 80s ribosomes nor the transformation of 60s subunit is associated with release of materials that remain in the supernatant or become converted into dialysable fractions.

Characterization of 50s particles

(1) *RNA*. Samples of 80s ribosomes in TCM₃ buffer, when treated with 1% sodium dodecyl sulphate by the method of Kurland (1950), show distinct 28s and 18s peaks (Plate 1b). Plate 1(e) shows a subunit preparation in which all of the 60s particles were converted into 50s units, and Plate 1(f) presents the corresponding RNA distribution. It is clear that the ribosomal RNA of 60s particles retains its integrity even after their conversion into 50s particles. Plate 1(g) shows the sedimentation pattern when the 50s particles from the sample of Plate 1(e) were transformed back into 60s particles by altering the Mg²⁺ concentrations back to 5mM by dialysis, and Plate 1(h) depicts the ultracentrifugal pattern of the RNA extracted from these re-formed particles. Here also RNA gives the same peaks without any sign of degradation. There is always a trace of a third peak sedimenting behind the 18s peak that does not change its area during the dissociation and conversion procedure. This is

presumably due to pre-existing breaks in the 28s RNA.

(2) *Protein and RNA percentage*. Since the radioactivity assays did not indicate any possibility of loss of protein or RNA and isolated RNA preparations showed normal undegraded patterns, no special attempt was made to determine the exact protein/RNA ratio of the subunits, e.g. fixation of particles with formaldehyde to avoid further loss of protein or sedimentation through caesium chloride to avoid contamination from foreign particles were not performed. The protein/RNA ratio was found to be fairly constant for all subunits, giving about 57 ± 2% protein and the rest RNA for each peak fraction.

(3) *Electron microscopy*. The electron micrograph of a purified 50s preparation (Plates 2b and 2c) shows particles having a loose structure in contrast with the intact 60s particles (Plate 2a). The 60s particles are of uniform diameter and do not show any characteristic substructure, whereas projection of the 50s particles shows three distinct units.

The above results clearly indicate that there is no loss of material accompanying formation of 50s particles from 60s particles. It can therefore be concluded that this transformation arises out of a conformational change giving rise to unfolding of the ribonucleoprotein strand. It is noteworthy that the 40s subunit cannot unfold itself under the same conditions.

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EXPLANATION OF PLATE I

(a) Ultracentrifuge diagram of yeast ribosomes in TCM₃ buffer showing large 80s peak and a trace of the 40s peak, after centrifugation for 12min. The concentration of ribosomes was 12mg./ml. (b) Ultracentrifuge diagram of RNA obtained from ribosomes in TCM₃ buffer by treatment of 0.5ml. of extract, containing 2mg. of ribosomes, with 0.5ml. of 2% sodium dodecyl sulphate, after centrifugation for 35min. (c) and (d) Ultracentrifuge diagrams of ribosomes in TCM₃ buffer dialysed against TC buffer for 1hr. (c) and 6hr. (d), after centrifugation for 14min. The concentration of ribosomes was 6mg./ml. (e) Ultracentrifuge diagram of ribosomes in TCM₃ buffer in which all of the 60s particles were converted into 50s units, after centrifugation for 13min. The concentration of ribosomes was 6mg./ml. (f) Ultracentrifuge diagram of RNA obtained from ribosomes of (e), after centrifugation for 25min. The amount of ribosomes was 3mg. (g) Ultracentrifuge diagram of ribosomes in TCM₃ buffer dialysed against TCM₃ buffer overnight, after centrifugation for 15min. The concentration of ribosomes was 8mg./ml. (h) Ultracentrifuge diagram of RNA obtained from the sample of (g), after centrifugation for 25min. The amount of ribosomes was 2mg.

EXPLANATION OF PLATE 2

(a) Electron micrograph of 60s ribosomes showing particles of uniform diameter and without any characteristic substructure. Magnification $\times 60000$. (b) and (c) Electron micrographs of 50s unit showing particles of loose structure; some projections show three clear units (a, b, c, d). Magnification $\times 60000$.

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