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Macromolecule Synthesis in Yeast Spheroplasts

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Conditions have been established for the preparation of spheroplasts of *Saccharomyces cerevisiae* which are able to increase their net content of protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA), several-fold upon incubation in a medium stabilized with 1 M sorbitol. The rate of RNA and protein synthesis in the spheroplasts is nearly the same as that occurring in whole cells incubated under the same conditions; DNA synthesis occurs at about half the whole cell rate. The spheroplasts synthesize transfer RNA and ribosomal RNA. The newly synthesized ribosomal RNA is incorporated into ribosomes and polysomes. The polysomes are the site of protein synthesis in these spheroplasts. Greater than 90% of the total RNA can be solubilized by treatment of the spheroplasts with sodium dodecyl sulfate or sodium deoxycholate. These spheroplast preparations appear to be a useful subject for the study of RNA metabolism in yeast.

In conjunction with our investigation of temperature-sensitive mutants in yeast (7), it has become desirable to undertake a study of ribonucleic acid (RNA) metabolism in this organism. Advances in the understanding of RNA metabolism in mammalian cells have come through experiments which involve the pulse labeling of cells with radioactive precursors to RNA followed by the rapid lysis of the cells with detergents (for a review, see 11). The cell wall of *Saccharomyces cerevisiae* prevents the use of this technique with whole cells, since detergents release only small amounts of RNA from the cell. The removal of the yeast cell wall with enzymes of the digestive juice of the snail and enzymes from microbial sources has been reported by many workers (5, 12). [Previous investigators have referred to these structures as protoplasts. However, since elements of the cell wall are still remaining (5, 12), a more correct term is spheroplast.]

These spheroplasts of yeast can be lysed by osmotic shock or detergents and seem, therefore, to be a desirable subject for the study of RNA synthesis. Indeed, several investigations on the incorporation of radioactive precursors into RNA and protein by yeast spheroplasts have been reported by de Kloet and co-workers (1-3). However, de Kloet reported that his preparations of spheroplasts synthesize net protein at a rate of only 2% per hour and never increase their net protein content by more than 5% (de Kloet, Thesis, Utrecht, 1961). Since whole yeast cells can double their protein content in approximately 1.5 hr, these spheroplasts are synthesizing protein at a rate and to an extent which is not comparable to that occurring under conditions of active growth. Indeed, de Kloet considered his spheroplast preparations analogous physiologically to resting whole cells (de Kloet, Thesis, Utrecht, 1961). Eddy and Williamson, on the other hand, had mentioned, in one of the earliest studies on yeast spheroplasts (5), that their preparations increased RNA and protein content several-fold; however, no data were presented on the actual rate or extent of macromolecule synthesis. Furthermore, McLellan and Lampen reported the synthesis of the enzymes invertase and phosphatase in spheroplasts of yeast (8). We wish to report the preparation of spheroplasts of *S. cerevisiae* which upon incubation in a medium stabilized osmotically with 1 M sorbitol increased their net content of protein, RNA, and deoxyribonucleic acid (DNA) several-fold. These spheroplasts synthesize RNA and protein at nearly the same rate as that occurring in whole cells incubated under the same conditions; DNA increases at about half the whole-cell rate. Furthermore, the spheroplasts synthesize transfer RNA and ribosomal RNA. The newly synthesized ribosomal RNA is incorporated into ribosomes and polysomes. The polysomes are shown to be the site of protein synthesis in these spheroplasts. In short, the spheroplasts behave physiologically much like whole cells and constitute, therefore, a suitable subject for studies on RNA metabolism in yeast.

MATERIALS AND METHODS

Yeast strain. The haploid yeast strain of *S. cerevisiae* used in this study, is designated A364A (a, ade-1$^-$, gal-1$^-$, ura-1$^-$, tyr-1$^-$, his-7$^-$, lys-2$^-$, tryp-1$^-$).

Media. The media designated YM-1 and YM-5 have been described previously (7). Synthetic medium contained, per liter: 6.7 g of Yeast Nitrogen Base (Difco), 20 g of glucose, 0.04 g of tyrosine, 0.04 g of lysine, 0.01 g of adenine, and 0.01 g of uracil.

Chemicals. Sodium dodecyl sulfate (SDS) and sodium deoxycholate (DOC) were obtained from Matheson Coleman and Bell (Norwood, Ohio), and Atlas Brij 58 from Atlas Powder Co. (Wilmington, Del.). All radioactive compounds were the products of Schwarz Bioresearch Inc. (Orangeburg, N.Y.). Glusulase is a commercial preparation of snail digestive juice, obtained from Endo Laboratories (Garden City, N.J.) which contains 170 mg of protein/ml.

Whole-cell counts. The number of intact whole cells in spheroplast preparations was determined by counting in a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). Samples were diluted into 0.5% SDS and then into saline, or were diluted directly into saline containing 0.025% SDS. An alternate method involved dilution into 0.5% SDS and counting in a Petroff-Hausser counting chamber in a phase-contrast microscope. Both of these methods give essentially identical results.

Radioactive labeling. Unless otherwise indicated, all labeling was done by incubation in YM-5 to which radioactive compounds had been added. In cases where a radioactive label was not employed, the cells were grown in YM-1. Determination of radioactivity incorporated into macromolecules was performed as described previously (7) except that 15% trichloroacetic acid was used to precipitate macromolecules from solutions containing SDS. The ^{14}C-adenine used in these experiments had a specific activity of 45 mc/mmole.

Sucrose gradient centrifugation. The methods for sucrose gradient centrifugation of RNA and polysomes were adapted from those of Darnell and coworkers (6, 9). Sucrose gradients of free RNA were prepared by extracting spheroplasts with a buffer containing 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.4, 0.1 M NaCl, 0.001 M ethylenediaminetetraacetic acid (EDTA), and 0.5% SDS (SDS-buffer). One ml of this extract was layered on a 5 to 20% (w/w) sucrose gradient containing the same buffer (with SDS) and centrifuged for 15 hr (unless otherwise indicated) at 24,000 rev/min and 20 C in the SW25 head of a Beckman L-2 ultracentrifuge. Polysome gradients were prepared by extracting spheroplasts with a buffer composed of 0.01 M Tris, *p*H 7.4, 0.1 M NaCl, and 0.0015 M $MgCl_2$, and sequentially adding DOC and Brij to 0.5% (DOC-buffer). One ml of this extract was layered on a 15 to 30% sucrose gradient containing the same buffer (without detergents) and centrifuged for 2 or 3 hr at 24,000 rev/min and 2 C in the SW25 head. The gradients were fractionated by pumping 60% sucrose into the bottom of the tubes and collecting drops from the top of the tube.

RESULTS

Conditions for the production of spheroplasts. We have attempted to find conditions which provide a maximal yield of spheroplasts without damaging the ability of the spheroplasts to synthesize macromolecules, particularly RNA. Eddy and Williamson (5) produced spheroplasts by incubating yeast cells in Glusulase with sorbitol added as an osmotic stabilizer. The use of a reducing agent such as 2-mercaptoethylamine has been suggested as a means of increasing the yield of spheroplasts with Glusulase (4). With this in mind, we tested the effects of several reducing agents on the ability of whole cells to synthesize RNA after a 10-min treatment in a 1 mM concentration of the reducing agent. All agents tested (2-mercaptoethanol, 2-mercaptoethylamine, Cleland's reagent, cysteine, and sodium bisulfite) caused a severe reduction in the ability of the cells to synthesize RNA subsequent to the treatment. Preliminary experiments also showed that the addition of any one of a number of salts (NaCl, $MgCl_2$, $(NH_4)_2SO_4$), or buffers (Na succinate, *p*H 6.3; Tris-chloride, *p*H 8.0) at a concentration of 0.005 M to the incubation mixture strongly inhibited the production of spheroplasts. Consequently, we have adopted the procedure of incubating whole cells in a solution of sorbitol and Glusulase without the addition of reducing agents or buffers.

Next, we undertook a study of the effect of Glusulase concentration, cell concentration, and the culture density at which the cells were harvested. Part A of Table 1 presents the results obtained when cells were incubated with various concentrations of Glusulase. Cells from growing cultures were washed once with distilled water by centrifugation, resuspended in 1 M sorbitol, and incubated for various periods of time with Glusulase. It can be seen that the number of remaining whole cells decreased with both increasing times of incubation and increasing concentrations of Glusulase. At a Glusulase concentration of 1% and an incubation time of 60 min, 97% of the cells were converted to spheroplasts; a further increase in Glusulase concentration did not increase the yield. Using a Glusulase concentration of 1%, we next examined the effect of cell concentration upon the yield of spheroplasts (Table 1, part B). At cell concentrations of up to 2×10^8 cells/ml, there were less than 2% whole cells remaining after 60 min of incubation; a further increase in cell concentration caused a drastic reduction in the fraction of the population converted to spheroplasts. In part C of Table 1, data are presented which deal with the effect of the density of the culture from which the cells were harvested before treatment. The fraction of the population remaining as whole cells after a 30-min incubation in Glusulase increases rapidly when the culture density is allowed to go above 3.5×10^7 cells/ml. Therefore, we routinely harvest cells

TABLE 1. *Per cent whole cells remaining as a function of time of incubation with Glusulase at various enzyme concentrations, cell concentrations, and culture densities*

Glusulase concn	Cell concn during Glusulase treatment	Density of sampled culture	Time of incubation (min)						
			0	5	10	15	20	30	60
%	*cells/ml*	*cells/ml*							
Part A									
10.0	350×10^6	25×10^6	100	70	39	—	—	7.4	4.1
2.0	350×10^6	25×10^6	100	52	25	—	—	4.6	1.6
1.0	350×10^6	25×10^6	100	60	33	—	—	6.7	2.5
0.2	350×10^6	25×10^6	100	87	74	—	—	41	25
0.1	350×10^6	25×10^6	100	98	100	—	—	62	44
0.0	350×10^6	25×10^6	100	97	—	—	—	98	105
Part B									
1.0	6.4×10^6	22×10^6	100	21	5.2	—	—	1.2	1.2
1.0	21×10^6	22×10^6	100	25	6.0	—	—	1.4	1.2
1.0	77×10^6	22×10^6	100	20	8.6	—	—	1.4	1.4
1.0	222×10^6	22×10^6	100	50	27	—	—	4.2	1.5
1.0	714×10^6	22×10^6	100	77	61	—	—	37	20
Part C									
1.0	271×10^6	3.3×10^6	100	—	17	—	7	5	—
1.0	309×10^6	15×10^6	100	—	26	—	11	6	—
1.0	290×10^6	35×10^6	100	—	38	—	21	16	—
1.0	262×10^6	43×10^6	100	—	—	61	47	34	24
1.0	292×10^6	78×10^6	100	—	69	—	58	51	—

at a density of 2.0×10^7 cells/ml, which corresponds to a Klett reading of 100 (red filter).

Osmotic stabilization of spheroplasts. Spheroplasts are osmotically fragile structures which lyse in medium which is not supplemented with an osmotic stabilizer such as sorbitol. A concentration of 1 M sorbitol was used in previous experiments, as this concentration was sufficient to preserve the spheroplasts from lysis. Figure 1 presents the results of an experiment to determine an optimal sorbitol concentration for the stabilization of yeast spheroplasts with respect to RNA synthesis. It can be seen that increasing concentrations of sorbitol cause a decrease in the rate of RNA synthesis in whole cells. This inhibition amounts to about 20% in 0.9 M sorbitol and more than 50% for 1.3 M sorbitol. Essentially no RNA was synthesized, however, by spheroplasts in a medium lacking sorbitol, verifying that spheroplasts are, in fact, osmotically fragile. Increasing the sorbitol concentration to 0.9 M caused an increase in the amount of RNA synthesized, but a further increase to 1.3 M resulted in an inhibition of RNA synthesis. A direct comparison of the curves for spheroplasts and whole cells in this experiment cannot be made, as the cell concentration in the spheroplast preparation is not known. This uncertainty arises from the fact that 20 to 40% of the spheroplasts are lysed during the process of centrifuging them out of the Glusulase mixture and resuspending them in media. A comparison of the synthetic activity of spheroplasts with that of whole cells will be made in a subsequent experiment. As a result of this experiment, 1 M sorbitol has been chosen for the osmotic stabilization of yeast spheroplasts. Other experiments demonstrated that 0.4 M $MgSO_4$ and 1.0 M mannitol can also be used.

Length of incubation in Glusulase. The time of incubation of whole cells with Glusulase which is required to produce high yields of spheroplasts varies with the activity of the Glusulase preparation. Since in some cases it might be necessary to incubate cells longer than 1 hr, it is desirable to know whether extended incubation is detrimental to the subsequent ability of spheroplasts to synthesize RNA. Log-phase cells were centrifuged and resuspended in 1 M sorbitol containing 1% Glusulase at a density of 2×10^8 cells/ml. Samples of this mixture were taken at various times, diluted 40-fold into YM-5 medium containing 1 M sorbitol and ^{14}C-adenine, and incubated for an additional 4 hr. Dilution into medium prevents further Glusulase action both by dilution of the enzyme preparation and by inhibition of its activity by the salts in the medium. The results of this experiment demonstrated that the amount of radioactivity incorporated into RNA in 4 hr by these cultures was constant for times of incubation in Glusulase from 30 min to 4 hr. Therefore, incubation for as long as 4 hr in 1 M sorbitol with Glusulase is not detrimental to the subsequent

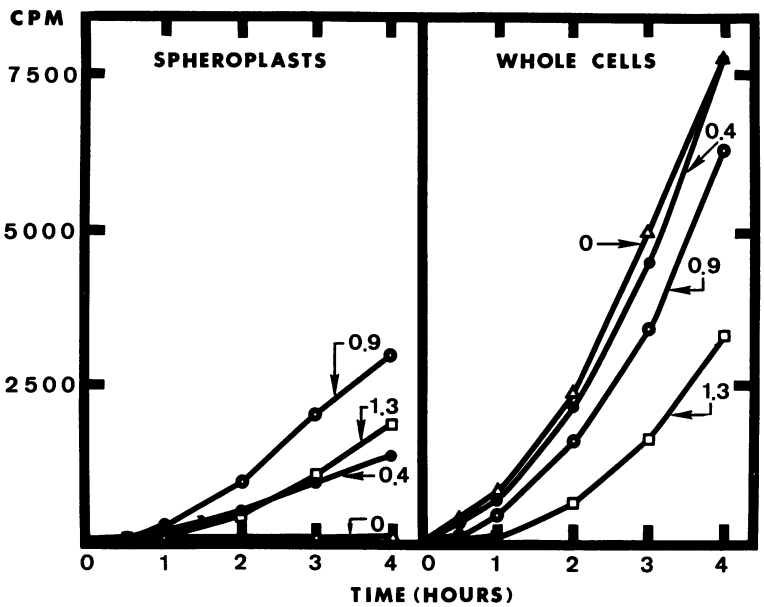

FIG. 1. *RNA synthesis in spheroplasts and whole cells as a function of sorbitol concentration. Log-phase cells were washed with distilled water and resuspended in various concentrations of sorbitol with (spheroplasts) and without (whole cells) Glusulase. The cells were centrifuged and resuspended at a density of 1 × 10⁷ cells /ml in medium containing sorbitol at the same concentration as in the incubation and ¹⁴C-adenine (0.05 μc/ml). Samples were removed at various times, and the amount of radioactivity in RNA was determined.*

ability of the spheroplasts to synthesize RNA. Furthermore, 98% of the RNA synthesized by the spheroplasts was released from the cells by the addition of 0.5% SDS after incubation in Glusulase for 30 min or 4 hr. Only approximately 10% of the RNA was released from whole cells after treatment with 0.5% SDS.

In summary, the optimal conditions for spheroplast formation appear to be the following. Cells are grown in YM-1 medium and harvested at a density of 2×10^7 cells/ml or less. The cells are washed once in distilled water by centrifugation and resuspended in 1 M sorbitol at a density of 2×10^8 cells/ml or less. Glusulase is added to a concentration of 1% and the mixture is incubated at 23 C until less than 2% whole cells remain. Although 1 hr is usually sufficient, a 4-hr incubation is not detrimental to the spheroplasts.

Macromolecule synthesis in spheroplasts. The next experiment was designed to give a quantitative estimate of the course of protein, RNA, and DNA synthesis in spheroplasts. Cells were grown from a small inoculum in YM-5 media containing ¹⁴C-adenine and ³H-lysine. Spheroplasts were prepared from the uniformly labeled cells. Spheroplasts and whole cells were then incubated in YM-5 medium containing 1 M sorbitol, ¹⁴C-adenine, and ³H-lysine. Since the yeast strain employed is an adenine and lysine auxotroph, all of the cell protein is uniformly labeled with ³H, and the RNA and DNA are uniformly labeled with ¹⁴C. Thus an increase in the amount of radioactivity in RNA, DNA, or protein is directly proportional to the net increase in that macromolecular component. Figure 2 shows the time course of macromolecule synthesis in spheroplasts and whole cells. Examination of the curves for whole cells indicates that RNA, DNA, and protein increased normally after an initial lag of about 2 hr. A similar lag is seen for spheroplasts; then RNA and protein began increasing at rates comparable to those observed in whole cells. DNA increased at about half the whole-cell rate, achieving a 2.3-fold increase in 18 hr. RNA increased 2.4-fold in 9 hr and then remained essentially constant. Protein increased nearly sixfold in 15 hr with no further increase at 18 hr. The eventual cessation of RNA, DNA, and protein synthesis is not due to a deficiency of some component in the medium, as dilution into fresh medium does not lead to a further increase in these components. In other experiments, increases in RNA and protein of about eightfold and in DNA of about threefold were recorded. In all cases, spheroplasts approximated the behavior of growing whole cells between about 2 and 9 hr after their formation, yet remained osmotically sensitive.

RNA from spheroplasts. RNA may be extracted

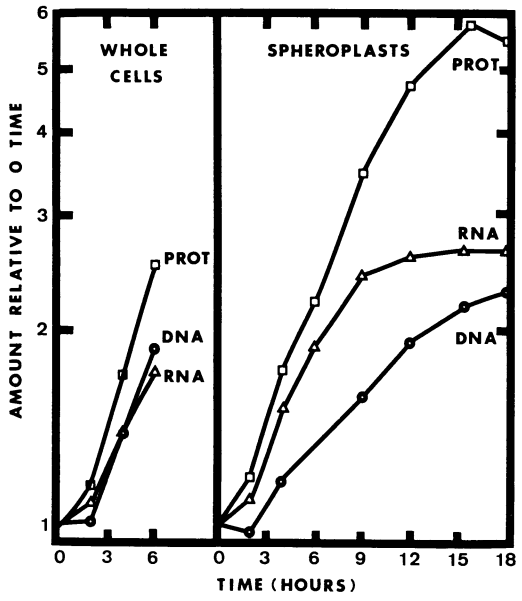

FIG. 2. *Macromolecule synthesis in spheroplasts and whole cells in a medium containing 1 M sorbitol. Cells were grown in YM-5 containing ^{14}C-adenine (0.1 μc/ml) and ^{3}H-lysine (0.3 μc/ml) for 16 hr at 23 C. Half of the cells were converted to spheroplasts. The whole cells and the spheroplasts were then resuspended in the same medium containing 1 M sorbitol (t = 0) and incubated at 23 C. Samples were taken at various times and analyzed for radioactivity in protein, RNA, and DNA. The amount of radioactivity per milliliter of culture in each of the macromolecules was normalized to the value at time zero.*

from spheroplasts by treatment with SDS, DOC, or osmotic shock. More than 99% of the RNA in the spheroplasts is solubilized by SDS. Figure 3 presents the results of a sucrose gradient centrifugation of the RNA extracted with SDS-buffer from spheroplasts made from cells which had been labeled with ^{14}C-adenine for 4 hr prior to spheroplast formation. *Escherichia coli* ribosomes were added as an optical density (OD) marker. A normal pattern is seen, indicating the presence of transfer RNA (tRNA) and two ribosomal RNA (rRNA) components. The sharpness of the peaks and the fact that they sedimented in the usual positions relative to *E. coli* rRNA (10) constitutes good evidence that yeast RNA does not suffer extensive degradation during the preparation of spheroplasts and extraction with SDS buffer. However, about one-third of the RNA originally present in the whole cells is not recovered in the spheroplasts. This loss may be due to breakage of some spheroplasts during the centrifugation, or perhaps to the leakage of material from intact spheroplasts. We have observed this effect several times.

A comparison is made in Fig. 4 of RNA synthesized after spheroplast preparation with that synthesized before. In this experiment, spheroplasts were prepared and incubated for 4 hr in medium containing ^{14}C-adenine. Thus, the radioactive material represents RNA made by spheroplasts, whereas the total RNA is represented by the OD at 260 mμ. The large amount of ultraviolet-absorbing material at the top of the gradient is protein and other cellular components extracted into the SDS-buffer along with the RNA. Since the cells were incubated for only 4 hr after spheroplast formation, less than one-third of the total RNA was new material. Figure 4 indicates, then, that the sucrose gradient pattern of newly made rRNA corresponds closely to that of rRNA made by whole cells. The OD pattern of tRNA is obscured by the material at the top of the gradient, but nonetheless a peak of radioactivity is seen which indicates that spheroplasts were making tRNA. Thus, the RNA made by spheroplasts appears to be normal with respect to its sedimentation properties in a sucrose gradient.

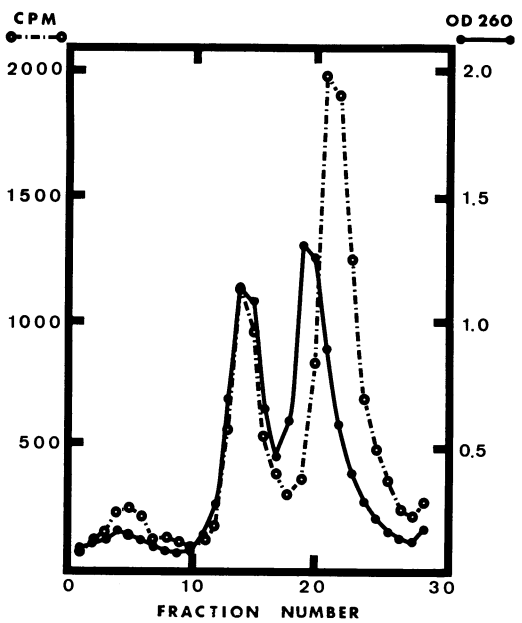

FIG. 3. *Sucrose gradient pattern of RNA from whole cells which was extracted by spheroplast formation. Log-phase cells were labeled for 4 hr with ^{14}C-adenine (0.3 μc/ml) in YM-5 medium at 23 C. The cells were converted to spheroplasts and lysed with SDS-buffer. Purified Escherichia coli ribosomes were added to the extract before centrifugation. The dashed line represents radioactivity in yeast RNA and the solid line is the OD 260 due to E. coli RNA.*

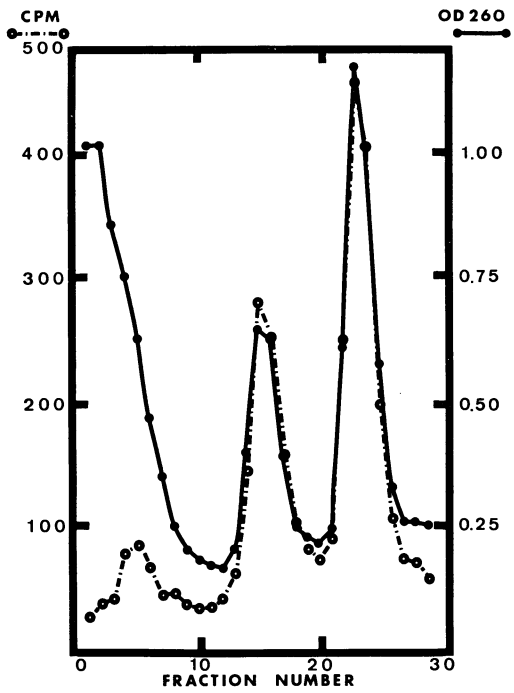

FIG. 4. *Sucrose gradient pattern of RNA synthesized in spheroplasts. Spheroplasts were incubated for 4 hr in YM-5 medium containing ^{14}C-adenine (0.05 μc/ml) and 1 M sorbitol at 23 C. RNA was extracted in SDS-buffer and centrifuged. The solid line is the OD 260, which is due mostly to RNA synthesized in whole cells before spheroplast formation. The dashed line is the radioactivity, which represents RNA synthesized by spheroplasts.*

Polysomes from spheroplasts. Extraction of spheroplasts with SDS frees the RNA from essentially all attached protein. DOC, on the other hand, allows intact ribosomes to be isolated free from microsomal membranes and other cytoplasmic contamination. Approximately 90% of the RNA in spheroplasts is solubilized by treatment of the spheroplasts with DOC-buffer. These facts allow us to examine the fate of newly synthesized rRNA. In the experiment presented in Fig. 5, spheroplasts were labeled with ^{14}C-adenine for 4 hr after formation, then chilled rapidly in the presence of cycloheximide (100 μg/ml). The resulting pellet was extracted with DOC-buffer containing Bentonite (50 μg/ml) to inhibit nuclease action, layered on a sucrose gradient, and centrifuged for 2 hr. The OD profile in Fig. 5 indicates the presence of a single ribosome peak and a broad area of more rapidly sedimenting material, which is presumably polysomes. The labeled material (RNA synthesized by the spheroplasts) is seen to follow a pattern closely similar to the OD pattern. In this experiment, 94% of the total labeled RNA sedimented with the single ribosomes and polysomes, and 83% of this RNA was in the polysome region. This constitutes good evidence that rRNA synthesized by spheroplasts is incorporated into ribosomes and that these ribosomes, in turn, are incorporated into polysomes. It is further noted that polysomes can be isolated in high yield from spheroplasts by use of very simple and rapid procedures.

Figure 6 presents the results of an experiment to determine whether the polysomes isolated from spheroplasts are functional in protein synthesis. Spheroplasts which had been preincubated in unlabeled growth medium were pulse-labeled for 2 min with a mixture of radioactive amino acids. One half of the culture was collected immediately and the other half was chased for a 10-min period with unlabeled amino acids. Incubation in labeled medium should result in the incorporation of labeled amino acids into nascent protein attached to the polysomes. Further incubation in unlabeled medium should allow the completion and release of the labeled protein from polysomes. That this occurs is seen in Fig. 6. The graph of polysomes from spheroplasts which were collected immediately after labeling shows a large amount of

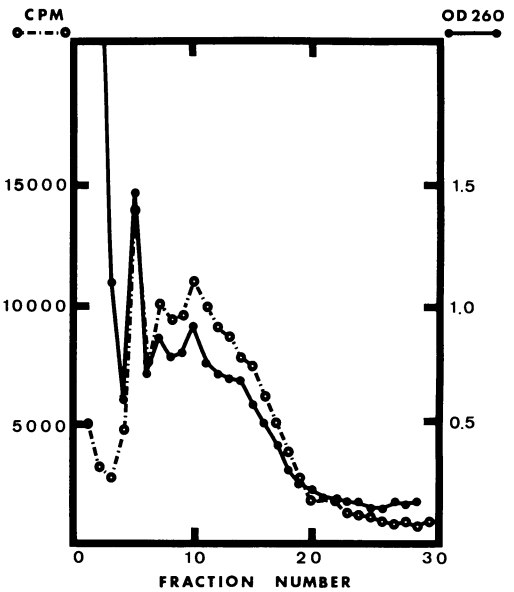

FIG. 5. *Sucrose gradient pattern of polysomes extracted from spheroplasts. Spheroplasts were incubated in YM-5 medium containing ^{14}C-adenine (0.05 μc/ml) and 0.4 M $MgSO_4$ for 4 hr at 23 C. Cell extracts were prepared in DOC-buffer and the extract was centrifuged on a sucrose gradient for 2 hr. The solid line is the OD 260, and the dashed line is the radioactivity in RNA.*

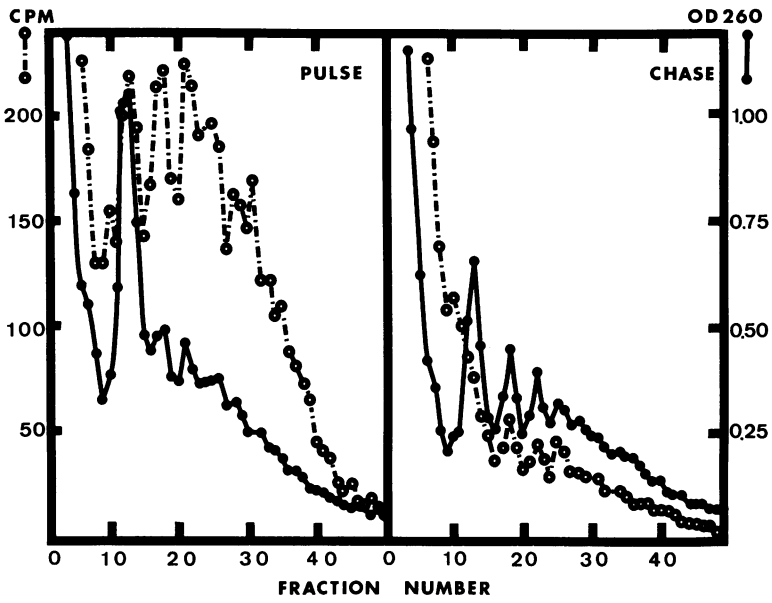

FIG. 6. *Pulse-labeling of polysomes from spheroplasts with radioactive amino acids and chase of radioactivity with unlabeled amino acids. Spheroplasts were incubated for 4 hr after formation in unlabeled YM-5 medium containing 1 M sorbitol, then centrifuged and resuspended for 2 min in a small volume of synthetic medium containing a mixture of ^{14}C amino acids (^{14}C-reconstituted protein hydrolysate, 0.5 $\mu C/ml$) and 1 M sorbitol. One half of the culture was collected and resuspended in DOC buffer immediately (PULSE) while the other half was centrifuged and resuspended for 10 min in unlabeled medium containing 3% Casamino Acids (CHASE). The extracts were layered on sucrose gradients and centrifuged for 3 hr. The solid line in each case represents OD 260 and the dotted line is radioactivity.*

radioactivity in the polysome region. The chase with unlabeled amino acids led to a fourfold reduction in the specific activity of the polysome region. These results are consistent with those obtained with many other organisms which indicate that the polysomes are the site of protein synthesis. The recovery of ribosomes and polysomes was 25% less in the culture that was chased with cold amino acids. This loss is due to the additional centrifugation step which this culture underwent, and confirms the previous observation of a loss of material from spheroplast preparations upon centrifugation.

Pulse-labeled RNA from spheroplasts. Incubation of cells with a radioactive precursor of RNA for a very short period of time leads to a different labeling pattern than does incubation over a long period of time. In a short pulse experiment, the amount of label incorporated into an RNA component is roughly proportional to the rate of its synthesis, irrespective of its rate of degradation, whereas in a long-term experiment the amount of label incorporated into an RNA component is proportional to the total amount of that component in the cell. Figure 7 presents the results of an experiment in which a spheroplast preparation was labeled with ^{14}C-adenine in synthetic medium for 1 min, extracted with SDS-buffer, and centrifuged on a sucrose gradient. It can be seen that the curve of radioactivity is quite different from the OD 260 curve which represents stable RNA. It will not be possible to interpret the radioactivity profile until a more detailed investigation of RNA metabolism in these yeast spheroplast preparations is carried out.

DISCUSSION

The purpose of this study was to prepare yeast spheroplasts which are physiologically normal as far as RNA metabolism is concerned. The spheroplast preparations which we have obtained increase their net contents of protein, RNA, and DNA more than twofold, and in some cases as much as eightfold. The initial rate of RNA and protein synthesis is almost the same as that of whole cells. Furthermore, these spheroplasts synthesize the same size of ribosomal RNA components as do whole cells, and the newly formed ribosomal RNA is incorporated into ribosomes and polysomes. Polysomes are the site of protein

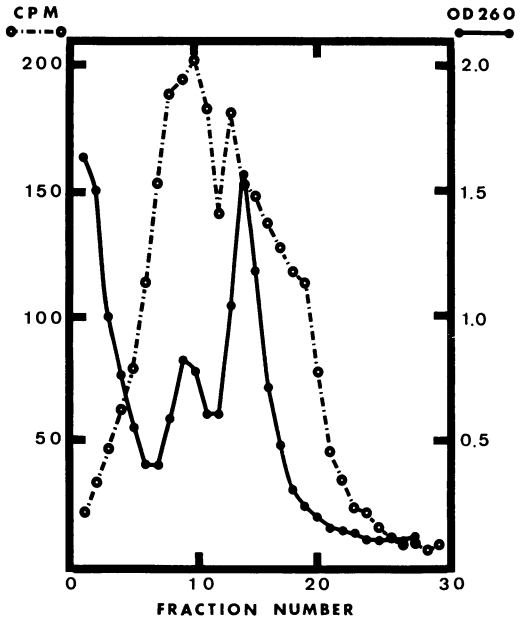

FIG. 7. *Sucrose gradient pattern of pulse-labeled RNA from spheroplasts. Spheroplasts were prepared and preincubated in YM-5-containing 1 M sorbitol for 2 hr, centrifuged, and resuspended in synthetic media minus adenine.* ^{14}*C-adenine (0.3 μc/ml) was added and the spheroplasts were incubated for 1 min at 23 C. An extract was prepared in SDS-buffer and centrifuged on a sucrose gradient. The solid line is OD 260 and the dashed line is radioactivity in RNA.*

synthesis in the spheroplasts. These facts constitute good evidence that RNA metabolism is normal in spheroplasts for a period of several hours after their formation.

The spheroplasts eventually stop synthesizing macromolecules altogether. Several explanations for this failure to display continuous growth can be imagined. Our inability to recover all the RNA from a culture of spheroplasts by centrifuging the spheroplasts down indicates that the spheroplasts may be leaky. The leakage of essential cellular components may account for the cessation of macromolecule synthesis. Another possibility arises from the fact that spheroplasts appear not to divide but merely to increase in size; this observation was made earlier by Williamson and Eddy (5). The resulting changes in the surface-to-volume ratio could be responsible for the cessation of macromolecule synthesis. Finally, the spheroplasts might be slowly permeable to sorbitol. This would result in a slow dilution of the internal contents of the cell, which in turn might limit the synthetic capacity of the cell.

The reason for the different behavior of our spheroplast preparations which increase their protein contents six- to eightfold and those of de Kloet which increase protein content by only 5% is not immediately apparent. However, several differences in technique exist. We used a nonclumpy haploid strain of *S. cerevisiae*, whereas de Kloet used a diploid strain of *S. carlsbergensis*. Our preparations were incubated in 1 M sorbitol at 23 C, whereas he used approximately 0.6 M mannitol with 20 μM citrate buffer at 30 C. In spite of the quantitative difference in the rate of macromolecule synthesis, the two preparations behave qualitatively similar with respect to protein and RNA synthesis. Thus, de Kloet and co-workers have shown with their spheroplast preparations that protein synthesis occurs on polysomes (3), that ribosomal RNA can be made and incorporated into ribosomes (2), and that pulse-labeled RNA is heterogeneous in size (1). We have confirmed these results with our spheroplast preparations.

These spheroplast preparations should be useful for the study of RNA metabolism in yeast, since high yields of both total and pulse-labeled RNA can be quickly released from the cells with detergents or osmotic shock. We anticipate employing this technique in conjunction with studies on certain temperature-sensitive mutants of yeast that appear to have defects in RNA synthesis (7).

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