Isolation of Polyribosomes from Yeast During Sporulation and Vegetative Growth

DALLICE MILLS

Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Illinois 60680

Received for publication 5 March 1974

Exponentially growing and sporulating cells of *Saccharomyces cerevisiae* have been subjected to a variety of conditions which mechanically disrupt the cell in an effort to establish conditions which permit the recovery of intact polyribosomes. Grinding cells for 10 s with glass beads in a Bronwill cell homogenizer was sufficiently gentle to yield a polyribosome content in exponentially growing cells which was similar to values obtained from yeast spheroplasts. Polyribosome patterns in sporulating yeast were similar to those from exponentially growing cells. This technique is fast, reproducible over a wide range of cell concentrations, and eliminates the need to make spheroplasts to recover intact polyribosomes.

The yeast, *Saccharomyces cerevisiae*, is particularly amenable for studying macromolecular synthesis throughout the vegetative cell cycle (2, 3, 10) and during meiosis and sporulation (1, 6, 7). However, some of the biochemical techniques which have been used to measure protein and ribonucleic acid (RNA) synthesis during vegetative growth have been ineffective with the sporulating cell. Sporulating yeast apparently are more impermeable than the vegetative cell. In a previous communication (9), the rise in pH of the sporulation medium which accompanies the sporulation process was shown to have an inhibitory effect on the cell's ability to take up precursors of RNA and protein. It was clear that the incorporation of radioactive precursor may not reflect the true rate of RNA or protein synthesis but may reflect, instead, the rate at which it enters the sporulating cell.

Another technical problem which has limited our understanding of transcription and translation in the sporulating cell has been the inability to recover polyribosomes by using conventional techniques. Understanding the genetic control of protein and RNA synthesis has been greatly aided by observing changes in the polyribosome content in spheroplasts of temperature-sensitive cell division cycle mutants (3–5). Although the technique of lysing spheroplasts has been aptly used with vegetative cells, there are two reasons why it is an unsatisfactory method for obtaining polyribosomes from sporulating yeast. First, although spheroplasts of some strains have been induced to sporulate, the kinetics of spore formation is retarded and the maximum percentage of sporulation is low. Second, the time required to convert a sporulating culture to spheroplasts becomes increasingly longer as sporulation ensues. Consequently, pulse-label experiments cannot be used to measure macromolecular synthesis. An alternative method for obtaining polyribosomes from vegetative and especially sporulating yeast was essential to further our understanding of the genetic control of protein and RNA synthesis. In this report, vegetative and sporulating cells were subjected to a variety of conditions of mechanical disruption, and the polyribosome profiles are described.

MATERIALS AND METHODS

Yeast strain, media, and culture conditions. *S. cerevisiae* Z186, a homothallic diploid strain that was used in this study, YEP presporulation medium, liquid sporulation medium, and culture conditions were described previously (9).

Cell breakage and preparation of homogenates. A sporulating culture (30 ml) at 5 × 10⁷ cells/ml or an exponentially growing culture at a cell density of 1 to 5 × 10⁹ cells/ml was harvested by filtration on membrane SSWP 24-mm diameter, 3-µm pore, filters (Millipore Corp.). The cells were washed from each filter with 0.5 ml of lysing buffer, containing 0.05 M triethanolamine (pH 7.4), 0.5 M KCl, and 0.01 M MgCl₂, and collected in a 70-mm diameter porcelain mortar. Either prewashed sand, previously heat-treated at 180°C for 12 h, or granular dry ice was added to cover the cell suspension. The mortars were held on ice, and the cells were disrupted by vigorous grinding for 45 s with an ice-cold pestle. An additional 1 to 2 ml of cold lysing buffer was added to each sample followed by 15 s of vigorous grinding. The contents of each mortar were centrifuged at 12,000 × g...
for 10 min to sediment cellular debris and sand. A 0.5-to 0.9-ml sample from the supernatant of each sample was layered on a 10 to 40% sucrose gradient (11 ml) made with lysing buffer and containing a 0.65-ml cushion of 50% sucrose. The gradients were centrifuged in a Spinco SW41 rotor at 150,000 × g for 90 min at 4 C. The polyribosome profiles were obtained by measuring the absorbance at 260 nm in a Gilford Model 2000 spectrophotometer equipped with a 1-cm path length flow cell.

Cell homogenates were also obtained by using the Micro Chamber provided with the Bronwill cell homogenizer model MSK. Routinely, 15 to 30 ml of cells ranging from 10^7 to 5 × 10^7 cells/ml of a sporulating or exponentially growing culture was harvested by centrifugation at 2,000 rpm for 3 min in a Sorvall GLC-1 bench top centrifuge. The cell pellets were suspended in 0.2 ml of lysing buffer. Two grams of glass beads (0.45 to 0.5 mm in diameter) was added, and the cells were disrupted with either 10 or 15 s of grinding at 4,000 cycles/s. The homogenate was diluted with 1 or 2 ml of lysing buffer and centrifuged at 12,000 × g for 10 min to sediment glass beads and cellular debris. Sucrose gradients and centrifugation conditions were as previously described for sand- and dry ice-homogenized preparations.

Polysome content was determined by using a plasmimeter purchased from Gleman 'Instruments Co.

Chemicals. Cycloheximide was obtained from Sigma Chemical Co.

RESULTS

Polyribosomes in vegetative cells. The polysome content in vegetative yeast spheroplasts has been reported to be 90% of the cell's total ribosome population (3). In an effort to obtain polyribosome patterns similar to those from spheroplasts, a culture of exponentially growing cells was divided into six equal portions and subjected to a variety of conditions of mechanical disruption (Table 1). After disruption, the supernatants of the samples were brought to an equal volume, the absorbance at 260 and 280 nm was measured to determine the extent of cell breakage, and the polyribosome content was measured to determine whether the grinding procedure disrupted the polyribosomes. Homogenates which had been obtained by grinding with sand or dry ice in a mortar had an average absorbance at 260 or 280 nm, which was only 60% of that obtained by grinding with glass beads in the MSK homogenizer (Table 1). Although grinding with sand or dry ice disrupted fewer cells as indicated by the absorbance of the supernatant at 260 or 280 nm, this procedure resulted in a large absorbance in the subunit region of the gradient (Fig. 1a,c). The addition of cycloheximide to the growth medium, which blocks polypeptide chain elongation, stabilizes the polyribosomes as indicated by a twofold increase in the polyribosome content (Fig. 1b; Table 1). Much of the material sedimenting in the polyribosome region was present as tetramers or smaller, indicating that some of the heavier polyribosome material may have been sheared. The 80S monosomes were not clearly separated from the 60S ribosomal subunit in most gradients, and, therefore, material sedimenting heavier than 80S was used to determine the minimum polyribosome content (Table 1). However, an estimate of 80S material was obtained by measuring absorbance, which was in the shoulder on the heavy side of the 60S subunit material and, when included with the polysome material, represented the maximum polysome content in the cell (Table 1). In all gradients except one, the 80S material was

| Sample | Grinding apparatus | Abrasive material | Grinding time (s) | Cycloheximide^a | Absorbance of supernatant | Percentage of ribosomes as polyribosomes |
|--------|--------------------|------------------|------------------|-----------------|--------------------------|--------------------------------------|
|        |                    |                  |                  |                 | A_260/mg | A_280/mg | Excluding 80S | Including 80S |
| Vegetative cells | MP^c | Sand | 60 | - | 8.30 | 4.30 | 24 | 37 |
|        | MP | Sand | 60 | + | 7.45 | 3.40 | 68 | 82 |
|        | MP | Dry ice | 60 | - | 6.40 | 3.90 | 40 | 53 |
|        | MSK | Glass beads | 15 | - | 11.30 | 5.80 | 39 | 43 |
|        | MSK | Glass beads | 15 | + | 12.40 | 6.40 | 75 | 88 |
|        | MSK | Glass beads | 10 | + | 12.90 | 6.70 | 75 | 88 |
| Sporulating cells | MSK | Glass beads | 10 | - | 10.9 | 6.8 | 16 | 57 |
|        | MSK | Glass beads | 10 | + | 6.7 | 3.7 | 38 | 50 |
|        | MSK | Glass beads | 10 | + | 6.0 | 3.4 | 58 | 75 |

^a Symbols: - , absent; +, added to media and buffers at a concentration of 100 µg/ml.

^b A_260 = absorbancy at 260 nm; A_280 = absorbancy at 280 nm.

^c MP, mortar and pestle; MSK, Bronwill cell homogenizer model MSK.
Fig. 1. Polyribosome patterns obtained after mechanical disruption of exponentially growing yeast. Polyribosomes in Fig. a to c were obtained by grinding cells with a mortar and pestle with (a) sand, (b) sand plus cycloheximide, and (c) dry ice plus cycloheximide. In Fig. d to e, polyribosomes were obtained by grinding with glass beads in the MSK homogenizer for (a) 15 s, (b) 15 s with cycloheximide, and (c) 10 s with cycloheximide.

estimated to be approximately 13% of the ribosome population. Polyribosome profiles of cells disrupted for 10 or 15 s in an MSK homogenizer are presented in Fig. 1d–f. Even though more cells were disrupted as indicated by a greater absorbance at 260 or 280 nm (Table 1), this procedure is gentle enough to preserve the polyribosomes intact. The addition of cycloheximide stabilized the polyribosomes, and about 75% of the ribosome population sedimented as
material heavier than 80S (Table 1).

Polyribosomes in sporulating yeast. Cells were harvested after 3 h in sporulation medium and were disrupted by grinding for 10 s with glass beads in the MSK homogenizer. No effort was made to keep the volume of the supernatants constant, since polyribosome content is independent of volume. Polyribosome content in cells harvested without using cycloheximide to stabilize the polysomes was 16%, whereas the addition of the drug to a final concentration of 100 ng/ml 5 min before harvesting the cells increased the yield of polysomes to 38% (Fig. 2a,b; Table 1). Centrifugation of sporulating cells and resuspension in sporulation medium at pH 6.0 (a technique which enhances the uptake of precursors of RNA and protein), before the addition of cycloheximide, increased the polyribosome content to 58% (Fig. 2c; Table 1). Polysome content under these conditions is 75% when the 80S material is included.

DISCUSSION

Vegetative and sporulating yeast have been subjected to a variety of conditions which mechanically disrupt the cell in an effort to recover intact polyribosomes. Grinding for 10 s in the MSK homogenizer resulted in nearly twice as much cell disruption as grinding with dry ice or sand with a mortar and pestle, and the technique was sufficiently gentle to routinely yield 8 to 10 peaks in the polyribosome region of the gradient. However, on many occasions we have observed that polyribosome shearing does occur with grinding in excess of 15 s. The addition of cycloheximide was necessary to stabilize the polyribosomes, presumably by preventing the ribosomes from finishing a round of translation and subsequently dissociating from the messenger RNA (3, 4). When added to sporulating yeast, cycloheximide was most effective in preventing polyribosome decay when the pH of the medium was adjusted to 6.0.

We have used 0.5 M KCl in our buffers to help distinguish between free monosomes and monosomes which were bound to messenger RNA. High salt has been shown to dissociate the ribosomes into their subunit species, but does not affect monosomes bound to messenger RNA (8). The sedimentation of primarily subunit material in samples ground with sand or dry ice occurred in the 80S monosome region,
thus making it difficult to measure the true polyribosome content. However, when vegetative cells were disrupted in the Bronwill cell homogenizer in high salt after exposure to cycloheximide, 75% of the ribosomes were in material which was heavier than 80S. When the 80S monosomes were included, the polysome content was 88%, which is in good agreement with values obtained in spheroplasts (3). The corresponding values obtained from cells harvested 3 h after inoculation into sporulation media were 58 and 75%, with very little evidence of polyribosome shearing.

We have used the technique of Martin (8), which does not necessitate having intact polyribosomes, to measure the polyribosome content in sporulating and meiotic mutant strains of yeast (K. Frank, S. Purtock, and D. Mills, Amer. J. Bot., 60:20, 1973; D. Mills and K. Frank, Genetics, 74:182, 1973). Their technique has been invaluable for determining the temporal pattern of polyribosome content during the sporulation process. However, the recovery of intact polyribosomes should prove useful for studying transcription and translation at different stages of development and prompted this search for a satisfactory method of cellular disruption in vegetative and sporulating cells. Cell disruption in the MSK homogenizer has two attractive features: (i) gene products and nascent protein can be recovered from intact polyribosomes with ease and brevity without exposing cells to Glusulase treatment and (ii) intact polyribosomes have been obtained from as few as 5 × 10⁸ cells or as many as 2 × 10¹⁸ cells with only 10 s of grinding. We are currently using this technique to examine the gene transcripts present in vegetative and sporulating yeast polyribosomes.

ACKNOWLEDGMENTS

Technical assistance by Donna Mirkes and the helpful criticisms of Benjamin Hall and Herschel Roman are greatly appreciated.

Support for this investigation, provided in part by Public Health Service postdoctoral fellowship 5-FO2-GM-37, 151-02 from the National Institute of General Medical Sciences and Public Health Service grant 5-RO1-AI-00328 from the National Institute of Allergy and Infectious Diseases to H. Roman and by grant GM-11895 from the Institute of General Medical Sciences to B. Hall, is greatly appreciated.

LITERATURE CITED

1. Esposito, M. S., R. E. Esposito, M. Arnaud, and H. O. Halvorson. 1969. Acetate utilization and macromolecular synthesis during sporulation of yeast. J. Bacteriol. 100:180-186.
2. Hartwell, L. H. 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. 93:1662-1670.
3. Hartwell, L. H., and C. S. McLaughlin. 1968. Temperature-sensitive mutants of yeast exhibiting a rapid inhibition of protein synthesis. J. Bacteriol. 96:1664-1671.
4. Hartwell, L. H., and C. S. McLaughlin. 1969. A mutant of yeast apparently defective in the initiation of protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 66:882-889.
5. Hartwell, L. H., C. S. McLaughlin, and J. R. Warner. 1970. Identification of ten genes that control ribosome formation in yeast. Mol. Gen. Genet. 109:42-56.
6. Kadowaki, K., and H. O. Halvorson. 1971. Appearance of a new species of ribonucleic acid during sporulation of Saccharomyces cerevisiae. J. Bacteriol. 105:826-830.
7. Kadowaki, K., and H. O. Halvorson. 1971. Isolation and properties of a new species of RNA synthesized in sporulating cells of Saccharomyces cerevisiae. J. Bacteriol. 105:831-836.
8. Martin, T. E. 1973. A simple general method to determine the portion of active ribosomes in eukaryotic cells. Exp. Cell Res. 80:496-498.
9. Mills, D. 1972. Effect of pH on adenine and amino acid uptake during sporulation in Saccharomyces cerevisiae. J. Bacteriol. 112:519-526.
10. Tauro, P. E. Schweizer, R. Epstein, and H. Halvorson. 1969. Synthesis of macromolecules during the cell cycle in yeast, p. 101. Academic Press Inc., New York.