NONIDENTITY OF RIBOSOMAL STRUCTURAL PROTEINS IN GROWING AND STARVED *TETRAHYMENA*

RICHARD L. HALLBERG and CLAUDIA A. SUTTON

From the Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14853

ABSTRACT

We have examined the ribosomal structural proteins isolated from vegetatively growing *Tetrahymena pyriformis* and from cells that had been starved of all nutrients for 24 h. Reproducible, nonartifactual differences in protein complement, primarily associated with the large ribosomal subunit, were found. The kinetics of change in ribosomal protein complement were followed both in refed and in newly starved cells. Furthermore, attempts at correlating a certain protein "phenotype" with a particular functional state of the ribosome were made. It was concluded that the alterations seen could not be correlated with a specific stage in the normal ribosome cycle. We did show, however, that the change in protein complement could occur as a result of altering preexisting ribosomes. In addition, we showed that the change correlates with a decrease in growth rate rather than being caused by the starvation conditions themselves. Speculations as to the functional significance of the protein changes are presented.

KEY WORDS ribosomal proteins · nutritional deprivation · *Tetrahymena*

We have recently shown (9) that in *Tetrahymena pyriformis* the ribosome contents of growing cells and nongrowing (starved) cells are regulated at distinct and reproducible levels. Furthermore, the transition from one state (starvation) to the other (exponential growth) involves regulation of ribosome metabolism in a way which indicates that the control of ribosome accumulation in these cells is significantly different than that seen in bacteria. Specifically, we showed that the rate of ribosome synthesis remains constant at a time when the rate of protein synthesis increases some 10- to 15-fold. Also, it appears as if, during this time, ribosomal protein and ribosomal RNA are noncoordinately synthesized. In continuing our studies of ribosome metabolism in *T. pyriformis*, we have examined further the changes in ribosome number and fraction of active ribosomes in cells shifted either from starvation conditions to growth conditions or vice versa. An unexpected finding of this research is that the proteins of isolated ribosomal subunits from starved and growing cells show reproducible quantitative and qualitative differences when compared by polyacrylamide gel electrophoresis.

There have been a number of reports that changes in ribosome structure and/or function can occur in bacteria (e.g., 7, 8, 12) and in eukaryotes (e.g., 6, 10, 15) either during the ribosome cycle or as a response to changes in physiological conditions. We sought to document more carefully the differences we observed, thus hoping we might be able to correlate structural changes with functional ones. We first determined that the differences seen were not the result of an isolation artifact. We than examined the kinetics of change in ribosomal protein "phenotype" when cells were shifted from growth to starvation conditions and

vice versa. Our results indicate that the changes in ribosomal protein complement can come about as a consequence of the alteration of preexisting ribosomes. This transformation appears to involve the exchange of proteins into and out of old subunits. Experiments have also been carried out in an effort to correlate these changes with ribosome function. The results of these experiments are discussed.

MATERIALS AND METHODS

Growth Conditions

Strain B IV was grown, and starved cells were prepared as described previously (9). All labeling conditions were as described previously, and specific details are given in appropriate figure legends.

Ribosome Isolations

PROCEDURE 1. Frozen cells were thawed on ice and then suspended in a 8- to 20-fold excess of 0.01 M NaCl, 0.01 M Tris, pH 7.5, 0.0015 M $MgCl_2$ (RS-buffer). Cells were broken by homogenization in a tight-fitting Dounce homogenizer (20 strokes; Kontes Co., Vineland, N. J.). Triton X-100 and deoxycholate (DOC) were then added to final concentrations of 1 and 0.5%, respectively, using twenty times stock solutions. The cell homogenate was centrifuged for 15 min at 15,000 *g*; the resulting supernate was recovered and subjected to centrifugation at 100,000 *g* for 90 min. The resulting ribosome pellet was rehomogenized in 0.5 M KCl, 0.01 M Tris, pH 7.5, 0.01 M $MgCl_2$, and then spun at 15,000 *g* for 15 min. Ribosomes and ribosomal subunits were pelleted from the resulting supernate by centrifugation at 150,000 *g* for at least 5.0 h. This was necessary because most ribosomes are dissociated into subunits under these conditions. Ribosomal subunits were prepared from the 0.5 M KCl-washed ribosomes by centrifugation on a sucrose gradient in the absence of Mg^{++}. These conditions cause the complete dissociation of ribosomes. Pellets of 0.5 M KCl-washed ribosomes were homogenized in a small volume of distilled water, and enough 1.0 M KCl in 0.1 M Tris, pH 7.5, was added to give a final concentration of 0.2 M KCl. The solution was then clarified by centrifugation at 15,000 *g* for 10 min. The resulting ribosome solution was overlaid on 15–30% sucrose gradients containing 0.1 M KCl and 0.01 M Tris, pH 7.5, and spun for 15 h at 23,000 rpm and 5°C in a SW27 rotor (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). Fractions were collected, and their absorbances read at 259 nm to determine the locations of the large and small ribosomal subunits.

PROCEDURE 2. To prepare ribosomal subunits washed in 0.7 M KCl, cells were homogenized in 0.2 M KCl, 0.01 M $MgCl_2$, 0.02 M Tris, pH 7.5 (P-buffer), in the presence of 1.0% Triton X-100 and 0.5% DOC and then spun at 20,000 *g* for 15 min. The supernate was spun at 100,000 *g* for 2–3 h to pellet all ribosomes. The resulting pellet was resuspended in 0.7 M KCl, 0.01 M $MgCl_2$, 0.01 M Tris, pH 7.5, and centrifuged at 12,000 *g* for 10 min. The resulting supernate was overlaid on 15–35% sucrose gradients containing the same concentrations of ions as the resuspending buffer and spun at 23–25 k-rpm for 13 h in an SW27 rotor. The remainder of this procedure was the same as procedure 1.

Ribosomal subunits were precipitated by pooling the appropriate fractions from a sucrose gradient, adding $MgCl_2$ to 0.01 M, and then adding 0.7 vol of cold 95% ethanol. The solution was kept on ice for 20–30 min; the flocculent precipitate that formed was pelleted by centrifugation at 15,000 *g* for 15 min. The supernate was discarded and, the pellet rinsed and frozen.

Polysome Isolations

Polysome isolations for quantitative determinations were carried out as previously described (9).

The procedure for preparative isolation of polysomes was as follows. Frozen cells (stored in liquid N_2) were thawed in P-buffer which had been treated with diethylpyrocarbonate and contained 5 μg/ml polyvinyl sulfate (PVS), and then lysed by the addition of Triton X-100 to 1.0% and DOC to 0.5%. After two or three strokes with a Dounce homogenizer, the cell lysate was spun at 27,000 *g* for 15 min. The supernate was recovered, and aliquots containing 40–60 OD_{260} U were layered on 37 ml of 15–50% (wt/vol) RNase-free sucrose gradients containing 0.1 M KCl, 0.005 M $MgCl_2$, 0.01 M Tris, pH 7.5. These gradients were spun in an SW27 rotor at 5°C for 3 h at 35 k-rpm. 2-ml fractions were collected, absorbances read at 259 nm, and appropriate fractions of both monosomes and polysomes were pooled. Only polysomes containing three or more ribosomes were used for preparing polysomal ribosomes. Pooled fractions were then centrifuged at 35 k-rpm at 5°C for at least 20 h in a 42.1 rotor in order to pellet either polysomal or monosomal ribosomes. The resultant pellets were rehomogenized in 0.5 M KCl, 0.01 M Tris, 0.01 M $MgCl_2$, spun once at 27,000 *g* for 10 min, and then the ribosomes were pelleted by centrifugation at 50 k-rpm in a 65 rotor for 3.5–4 h at 3°C. The remainder of the procedure for ribosomal subunit isolation was as previously described.

Electrophoresis

Electrophoresis of ribosomal proteins following the procedure of Laemmli (11) was as described (9). All gels contained 18% acrylamide except the one shown in Fig. 2*b* which was a gradient gel containing 7.5–15% acrylamide.

RESULTS

We previously presented the kinetics of change in ribosome content and percentage of functioning ribosomes in starved cells which had been refed

(9). To complete a description of the cycle, the same measurements were made on exponentially growing cells which were transferred to starvation media. Fig. 1 shows that upon entering starvation there is a rapid decrease in the percentage of functioning ribosomes which plateaus at the 5–10% level within 1.5 h. The decrease in ribosome number per cell, however, is more gradual, taking about 4–5 h to plateau. This decrease occurs primarily as a result of degradation of ribosomes, because the cell number increases by no more than 5% during this time. This is in contrast to the micronucleate strain HSM which increases 100% during a 24-h starvation (4). This level of ribosomes per cell remains constant for at least 30 h of starvation.

To determine whether this plateau level of ribosomes is a dynamic equilibrium, as the data of Connor and Koroly (5) would suggest, 2-h pulses of [^{3}H]uridine and [^{14}C]leucine were administered to separate groups of cells starved for 18–20 h. Ribosomal subunits were prepared from each group of cells, and the radioactivity associated with them was monitored (Fig. 2 *a* and *b*). When the proteins from the ribosomal subunits of the [^{14}C]leucine-labeled cells were separated on gradient SDS-acrylamide gels, most of the proteins could be seen to be radioactive (Fig. 3). Also, when the RNA from the large and small subunits was isolated, electrophoresed and fluorographed, the 25S and 17S rRNAs were found to be radioactive (data not shown). Clearly, complete ribosome synthesis and assembly must be occurring in starved cells.

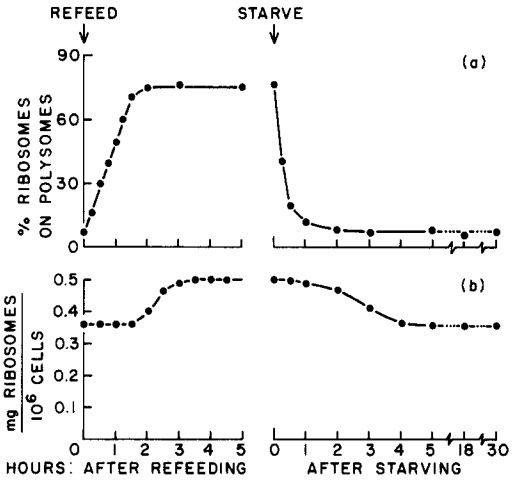

FIGURE 1 Changes in levels of functioning ribosomes per cell (*a*) and amounts of ribosomes per cell (*b*) in starved and subsequently refed cells, and exponentially growing cells transferred to starvation conditions. The measurements were made as previously described (9), and all values are averages of three or more determinations. The data for the starved and then refed cells are taken from a previous publication (9). The level of ribosomes per cell is corrected for recovery as described in reference 9.

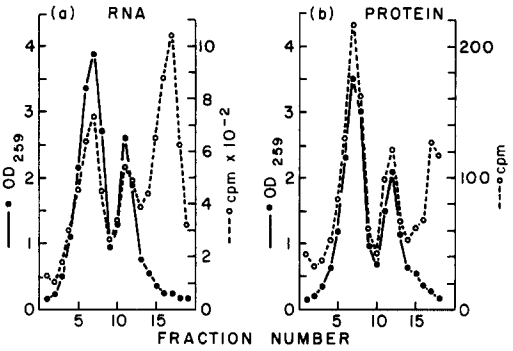

FIGURE 2 Synthesis of ribosomal RNA and ribosomal protein in starved cells. Cultures of cells starved in 50 mM Tris for 18 h (*a*) and 20 h (*b*) at about 100,000 cells/ml were given (*a*) [^{3}H]uridine (New England Nuclear, Boston, Mass., 40 Ci/mmol) at 1.0 μCi/ml or (*b*) [^{14}C]leucine (New England Nuclear, 0.27 Ci/mmol) at 0.2 μCi/ml for 2 h. Cells were collected at the end of this pulse and ribosomal subunits prepared on sucrose gradients using procedure 1 given in Materials and Methods. Aliquots of each fraction were TCA-precipitated and counted.

To examine the ribosomal protein complements of the two cell populations, ribosomal subunits were isolated from exponentially growing cells and from 18- to 20-hr-starved cells. Fig. 4 shows that distinct quantitative and qualitative differences are seen when large ribosomal subunit proteins from these cells are compared. Some variability in relative band intensities is sometimes seen but the basic patterns are highly reproducible. Quantitative differences in some bands are sometimes apparent in small subunit preparations, but qualitative differences are not seen. Inasmuch as these gels separate proteins according to molecular weight, the differences seen must be due to changes in relative quantities of different-sized polypeptides in the ribosomal subunits. The additions and losses do not result in any great change in total mass of protein per ribosome, as the densities of the large subunits from the vegetative and starved cells are approximately the same (data not shown).

Two immediately obvious explanations for

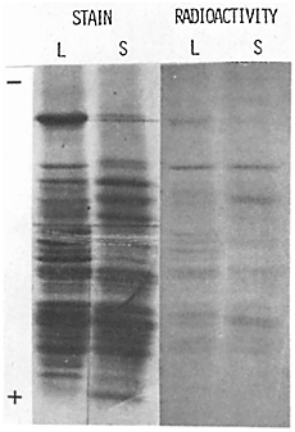

FIGURE 3 Assembly of newly synthesized ribosomal proteins into ribosomes of starved cells. The large (*L*) and small (*S*) subunits were isolated from the [^{14}C]leucine-labeled cells shown in Fig. 2. They were electrophoresed on 7.5–15% linear gradient SDS-polyacrylamide gels. Coomassie Blue-stained gels were dried and exposed to X-ray film for 4 wk.

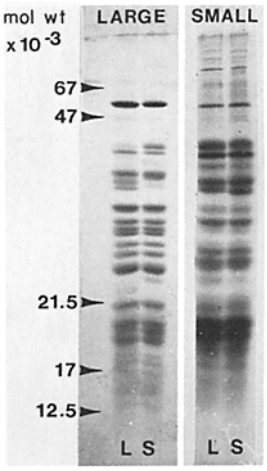

FIGURE 4 Proteins of large and small ribosomal subunits. Ribosomal subunits were prepared by procedure 1 from cells in exponential growth or from cells that had been starved in 50 mM Tris for 24 h. The proteins were extracted and run on 18% SDS-polyacrylamide gels as described. The molecular weight markers used were bovine serum albumin, ovalbumin, soybean trypsin inhibitor, equine myoglobin, and equine cytochrome C. *L*, growing (log) cells; *S*, starved cells.

these differences are that (*a*) they are due to an artifact of isolation—the cellular milieu of the two cell populations is different enough to cause alterations in the proteins associated with ribosomal subunits; and (*b*) the observation is biologically meaningful, i.e., the differences in subunit-associated proteins actually exists *in situ*, but what is seen is the result of differential sampling of ribosomes from the two cell populations.

To rule out the first possibility, the following experiments were conducted. Equal numbers of starved and vegetative cells were mixed, homogenized, and their ribosomes were coisolated and subunits prepared. Electrophoresis of the large subunit proteins of such a mixture is seen in Fig. 5*a*. From a visual inspection, a simple additivity of proteins is seen for the mixture. This result says that if the differences are the result of a modification during isolation, then whatever is causing the modification is not in great excess. The most likely

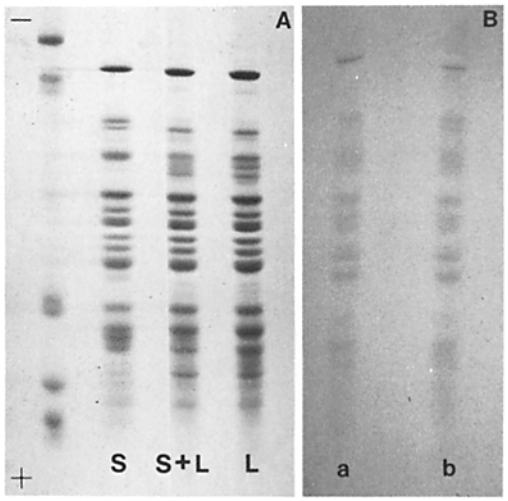

FIGURE 5 Electrophoretic profiles of large ribosomal proteins extracted from mixtures of starved (*S*) and log (*L*) phase cells. (A) Freshly isolated cells were mixed in equal numbers, and ribosomal subunits were then prepared by procedure 1 from homogenates of this mixture. The proteins of the large ribosomal subunits were extracted and run on 18% SDS-polyacrylamide gels. The molecular weight markers were the same as those in Fig. 4. (B) Autoradiographs of [^{14}C]leucine-labeled large subunit proteins. Cells in vegetative growth were labeled with [^{14}C]leucine (0.2 μCi/ml) for 9 h (~3.5 doublings). The cell culture (100 ml at 93,000 cells/ml) was divided in half, and each half was added to 450 ml of 24-h-starved cells at 155,000 cells/ml, or to 450 ml of log cells at 147,000 cells/ml. The cell mixtures were harvested and ribosomal subunits prepared. The proteins of the large ribosomal subunits of both mixtures were subjected to SDS-gel electrophoresis, stained, dried, and exposed to X-ray film for 40 days. (*a*) [^{14}C]leucine-labeled cells + log phase cells; (*b*) [^{14}C]leucine-labeled cells + starved cells.

candidate for modification of ribosomal proteins which would be detected by SDS-gel electrophoresis is proteolytic activity. As this would most likely be higher in starved cells, because the rate of protein turnover is much higher (9), we mixed a small quantity of [^{14}C]leucine-labeled vegetative cells with a 15-fold excess of cold starved cells and also with a 15-fold excess of cold vegetative cells. Large ribosomal subunits were isolated from both these mixtures, and the proteins obtained from each were autoradiographed (Fig. 5*b*). Since no detectable alteration of the vegetative ribosomal proteins in the presence of starved cells was seen, we conclude that the differences observed between starved and vegetative cells are not generated during the isolation procedure itself. These results do not rule out the possibility that some of the differences are due to proteolytic modifications which naturally take place within the cell. What is ruled out is the induction of proteolysis during ribosome isolation.

Another possibility which had to be considered was that of differential nonribosomal protein contamination. Although our standard isolation procedure involves washing ribosomes through a 0.5 M KCl solution, the subunits themselves are separated on Mg^{++}-free, 0.1 M KCl-containing sucrose gradients. Because a large percentage of vegetative cell ribosomes are polysomal and because 0.5 M KCl does not completely dissociate active ribosomes at the $MgCl_2$ concentration (0.01 M) used in the washing buffer, it was possible that our results might be due to incomplete removal of nonribosomal proteins. As all ribosomes are dissociable in 0.7 M KCl and 0.01 M $MgCl_2$ (C. A. Sutton, unpublished data), we isolated subunits from vegetative and starved cells on sucrose gradients containing 0.7 M KCl and extracted and electrophoresed the large ribosomal subunit proteins. The same differences were observed as seen previously (Fig. 6). As 0.7 M KCl is a higher ionic strength than is usually used for washing eukaryotic ribosomal subunits, this result indicates that the differences seen are due to differences in ribosomal structural proteins (operationally defined) and not to differential contamination with nonribosomal proteins. The remaining experiments were carried out with the isolation procedure utilizing the 0.1 M KCl sucrose gradients.

The electrophoretic pattern of large ribosomal proteins was then monitored in cells that had just been refed or in growing cells that had been transferred to starvation conditions. Fig. 7*a* shows that

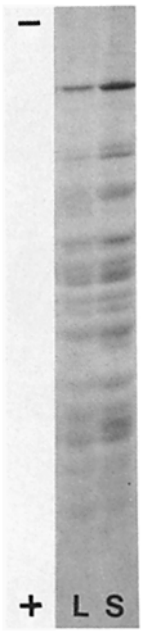

FIGURE 6 Comparison of the proteins of the large ribosomal subunits from log phase (*L*) and starved (*S*) cells that had been washed in 0.7 M KCl. Subunits were prepared by procedure 2 as described in Materials and Methods.

the transition from the starved phenotype to the vegetative phenotype occurs within the first 60–75 min after refeeding. By simple visual analysis, the changes in the different protein bands appear to occur simultaneously. Moreover, all of the changes occur before any change in ribosome number per cell. It is interesting to note that the change parallels the change in percentage of active (i.e., polysomal) ribosomes (Fig. 1). The significance of this observation will be dealt with shortly. Inasmuch as the rate of ribosome accumulation in cells at this time is less than 1/20th the rate in vegetative cells (9), this changeover would not appear to be due to a turnover and replacement of the whole ribosome population. Thus, assuming that there is no sampling bias in our isolation procedure, the changeover must occur as a result of modification of preexisting ribosomes. To more directly show that the changeover can occur in the absence of new ribosome synthesis, starved cells were treated with Actinomycin at 20 μg/ml for 15 min (which inhibits >95% rRNA synthesis and ~70% non-rRNA synthesis) and were then refed in the presence of the drug. Cells were collected at 1.5 and 3 h after refeeding and their ribosomes

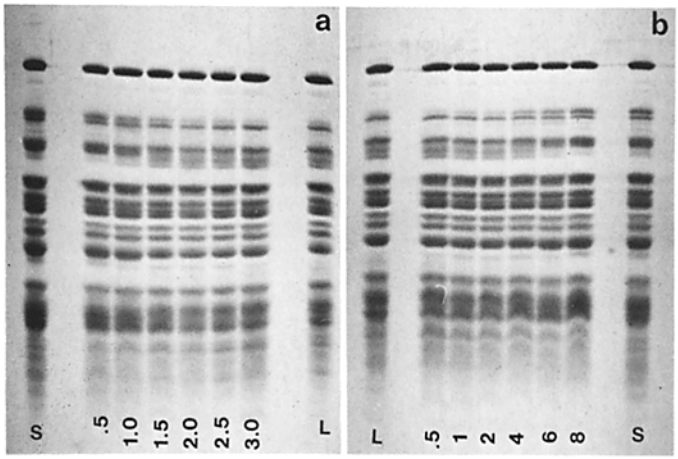

FIGURE 7 Electrophoretic patterns of large subunit ribosomal proteins isolated by procedure 1 from (*a*) starved cells that had been refed or (*b*) vegetative cells that had been starved. The numbers below the individual patterns are the times in hours either after being fed (*a*) or after having been starved (*b*). *L*, log phase cells; *S*, 24-h-starved cells.

isolated. In the Actinomycin D-treated cells, there was no change in ribosome number per cell whereas the control showed an increase of about 30%. However, the large ribosomal proteins of both cell populations showed identical patterns (Fig. 8). Thus, the appearance and disappearance of proteins occurs on old ribosomes.

The change in ribosome "phenotype" was also followed after exponentially growing cells were transferred to starvation media. In this case (Fig. 7*b*), the change occurred more gradually and did not correlate, as it had done with refed cells, with the fraction of ribosomes engaged in protein synthesis (Fig. 1). Whether the changeover in protein complement in this case occurs as a result of the modification of old ribosomes or is due to a replacement with new ribosomes remains to be seen. By studying the synthesis of the ribosomal proteins in cells transferred to starvation conditions, it should be possible to answer this question. Such work is in progress.

Because all the results presented so far could still be explained by differential sampling of ribosomes (yields are typically 40% for both log and starved cells [9]), polysomal and nonpolysomal ribosomes of starved and log phase cells were isolated and their proteins compared. It can be seen in Fig. 9 that active (polysomal) and nonactive (monosomal) ribosomes in a given cell show identical "phenotypes" with respect to their ribosomal protein complements. Thus, whatever functional changes occur as a result of these protein changes, the ability to function in protein synthesis is not altered. This is not to imply, however, that some step in the ribosome cycle is not altered. It simply says that the ability for a ribosome to cycle is not abolished.

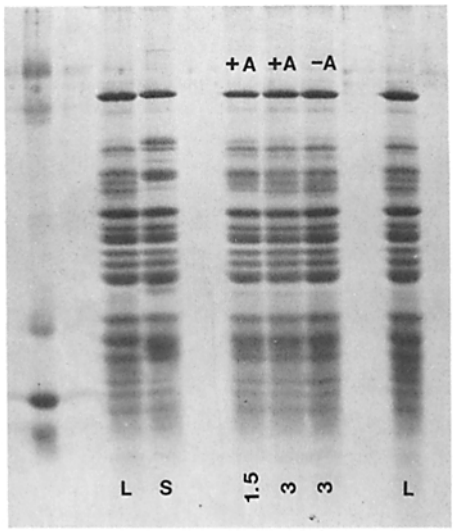

FIGURE 8 Structural proteins of large ribosomal subunits from starved cells refed in the presence (+A) and absence (−A) of Actinomycin D. 24-h-starved cells (*S*) were treated as described in the text, and cells refed for 1.5 and 3.0 h were collected and their ribosomes isolated. Ribosomes from vegetatively growing cells are included for comparison (*L*). The molecular weight markers are the same as in Fig. 4.

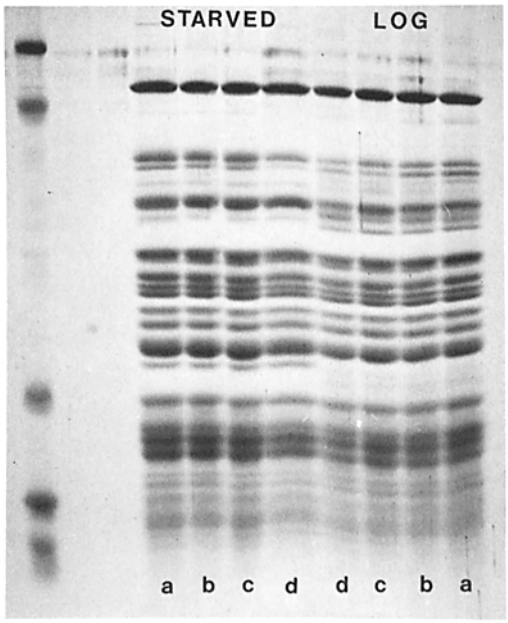

FIGURE 9 Comparison of the electrophoretic patterns of large ribosomal proteins from total unfractionated ribosomes and from polysomal and monosomal ribosomes of either starved or vegetative cells. Subunits were prepared by procedure 1. (*a*) Total ribosomes prepared by standard technique, (*b*) total ribosomes prepared by the polysomal isolation technique but combining both polysomal and monosomal ribosomes, (*c*) monosomal ribosomes prepared from a polysome isolation, (*d*) polysomal ribosomes.

That the alteration is not a response to the starvation conditions per se and possibly reflects changes in cellular ribosome levels or fraction of functioning ribosomes is shown by the following experiments. Cells that become overgrown decrease their growth rate and enter a plateaulike stage (cell division does not cease completely [14]). However, cells in this near-plateau phase existence show decreases in the fraction of functioning ribosomes and in the number of ribosomes per cell to nearly the same extent as starved cells, although the kinetics of change are different (unpublished data). Ribosomes were isolated from cells that had been in plateau phase for 2 days—at least a day longer than necessary to reduce ribosomal levels to that seen in starved cells. The proteins from ribosomes of these cells were identical to those in starved cells. This result would indicate that decreasing either the fraction of functioning ribosomes per cell and/or the total number of ribosomes per cell may be sufficient conditions to bring about the ribosome alteration. Thus, the difference appears to reflect a growth versus nongrowth condition.

Depending on the starvation conditions used, *Tetrahymena* either can simply stop growing (starvation in 50 mM Tris) or can be induced to mate within 2 h (starvation in 10 mM Tris; 3), we examined the ribosomal proteins of cells starved in 50 mM Tris, 10 mM Tris, or two different mating types starved together in 10 mM Tris for 18 h. In all cases, the ribosomal protein phenotypes were identical. Although the protein synthetic activities of 50 mM starved cells, 10 mM starved cells (stimulated to mate), and mixed stimulated cells (in the process of mating) are both quantitatively and qualitatively distinct from one another (W. Wellnitz and P. Bruns, unpublished data), the alterations in ribosomal proteins are identical. Thus, the change is not associated with a particular protein synthetic capacity.

DISCUSSION

Our results clearly indicate that *Tetrahymena* ribosomes can exist in at least two forms with regard to their overall protein complement, at least as defined by *our* isolation conditions. The change from one protein complement to the other in cells transferred from starvation to growth conditions occurs either by the removal and addition of several polypeptides on the large ribosomal subunit or by the alteration of affinities of different proteins for that subunit in the two cell types. This latter change could conceivably come about as a result of a conformational change in the ribosomal subunits. Either form of ribosome can participate in protein synthesis as demonstrated by the fact that polysomal ribosomes from starved and vegetative cells show the same protein differences as seen when the proteins from the entire ribosome population of the two cell types are compared. The change that occurs when growing cells are starved seems to be associated with a change in level of actively functioning ribosomes rather than the starvation condition itself.

However, these observations allow no conclusion with regard to the functional significance of the changes that are seen. Several earlier reports indicate possible roles for the protein differences. One possibility is that the protein changeover confers (or, alternatively, reflects) a different stability on the ribosome itself. Both Weber (18) and Abelson et al. (1) have shown that whereas ribosomal RNA is stable in growing mammalian cells, it turns

over with a half-life of some 50–75 hs in stationary cells. It is known that rRNA and ribosomal protein turnover together so that if certain proteins can block (or expose) nuclease-sensitive sites or are themselves more (or less) resistant to proteolysis, then this could confer a greater (or lesser) stability on the ribosome. If the alterations do affect ribosome turnover rate, one would predict that the ribosomes from plateaued *Tetrahymena*, which have undergone the protein change, should not be stable but should turn over with the same half-life as those in starved cells. Preliminary experiments indicate that rRNA in growing cells is stable, whereas rRNAs in starving and plateaued cells turnover with similar, if not identical, half-lives (C. A. Sutton and R. L. Hallberg, unpublished data). In addition, refeeding of starved cells causes cessation of ribosomal RNA turnover, adding further evidence to the claim that the changes in protein complements of ribosomes reported in this paper must occur on old ribosomes. However, whether the alterations in protein complements can be shown to correlate with and be functionally related to ribosome stability remains to be seen. A more detailed analysis of rRNA synthesis and turnover is in progress.

An alternative possibility is that, although the change (whether it be cause or effect) does not alter the capacity for a ribosome to participate in protein synthesis, it may affect some step in the ribosome cycle. Henshaw et al. (10) showed that the subunits of polysome-derived ribosomes and the subunits of monomeric ribosomes (those not engaged in protein synthesis) differed in that their subunit affinities were different. More recently, Cooper et al. (6) have confirmed and extended these findings. It was hypothesized by Henshaw et al. that the large ribosomal subunit underwent a functional alteration at some time between its release from a polysome and its reassociation with a small subunit containing initiation factor, such that its affinity for a small subunit without initiation factor was reduced. Without this change, the large and small ribosomal subunits would be more likely to come together to form an inactive monomeric ribosome. The data of Cooper et al. suggest rather the absence of a factor on monomeric ribosomes that is necessary for their dissociation. In either case, however, the formation of monomeric ribosomes is considered essentially a sidetracking of subunits from the ribosome cycle. If the changes we observe cause (or reflect) increased affinities of ribosomal subunits for one another, then this would increase the frequency of monomeric ribosome formation and cause a subsequent lowering of the concentration of cycling ribosomal subunits. This could help maintain the rate of protein synthesis at a low level by virtue of changing the equilibrium between monomeric ribosome formation and ribosome cycling. It is certainly possible that some other step in the ribosome cycle is affected by the changed proteins, with the same net result. In any case, it should be possible to test various parameters of the protein synthetic apparatus from vegetative and starved cells to determine whether the protein changes we see can be correlated with some specific physiological differences. It will also be of interest to know whether a similar phenomenon occurs on ribosomes of growing and stationary mammalian cells in which these other aspects of ribosome metabolism and function have already been measured.

It is noteworthy that Adelman and Lovett (2) have reported that, in *Blastocladiella* zoospores, newly synthesized ribosomal proteins are found associated with ribosomes at a time when no (or very little) ribosome synthesis is occurring. The pattern of labeling they see indicates that not all ribosomal proteins are labeled. It is hard to tell, however, just how many different polypeptides are being made. At any rate, this synthesis occurs at a time when ribosomes are undergoing a "packaging" for later use during zoospore germination (13). It is tempting to postulate that a phenomenon similar to what we report here may be occurring.

Warner has clearly shown both in Hela cells (16) and in yeast (17, 19) that some ribosomal proteins exchange in the cytoplasm. These exchanges could well be involved in the functional changes that accompany ribosome cycling, though this has not been proved. Although it is possible that the changes we see affect some ribosome cycle step, they clearly are not a normal alteration associated with cycling itself. If they were, one would expect to see differences within the ribosome population of a given cell type. That we do not see these differences suggests that the changeover may be in ways analogous to the changes reported in ribosomes of sporulating bacteria (7, 8, 12). As the functional significance of ribosome alteration in bacteria undergoing sporulation is also unknown, it remains to be seen whether the analogy between the two systems can be extended any further. Only additional work will answer that question.

We thank Bill Wellnitz for running the gradient gel. We also thank Dr. Peter Bruns for the kind use of his *Tetrahymena* facilities. The technical assistance of T. Shelley is greatly appreciated.

This work was made possible by grant HD-6448 from the National Institutes of Health (NIH). Dr. Sutton was supported by an NIH training grant.

Received for publication 24 February 1977, and in revised form 13 June 1977.

REFERENCES

1. ABELSON, H. T., L. F. JOHNSON, S. PENMAN, and H. GREEN. 1974. Changes in RNA in relation to growth of the fibroblast. II. The lifetime of mRNA, rRNA and tRNA in resting and growing cells. *Cell.* **4**:161–165.
2. ADELMAN, T. G., and J. S. LOVETT. 1972. Synthesis of ribosomal protein without *de novo* ribosome production during differentiation in *Blastocladiella emersonii*. *Biochem. Biophys. Res. Commun.* **59**:1174–1182.
3. BRUNS, P. J., and T. BRUSSARD. 1974. Pair formation in *Tetrahymena pyriformis*, an inducible developmental system. *J. Exp. Zool.* **188**:337–344.
4. CAMERON, I. L., E. E. GRIFFIN, and M. J. RUDDICK. 1971. Macromolecular events following refeeding of starved *Tetrahymena*. *Exp. Cell Res.* **65**:265–272.
5. CONNER, R. L., and M. J. KOROLY. 1974. Relationship of cellular energetics to RNA metabolism in *Tetrahymena pyriformis* W. *J. Protozool.* **21**:177–182.
6. COOPER, H. L., L. S. BERGER, and R. BRAVERMAN. 1976. Free ribosomes in physiologically nondividing cells: human periferal lymphocytes. *J. Biol. Chem.* **251**:4891–4900.
7. DOMOTO, T., K. KOBAYASHI, and Y. KOBAYASHI. 1975. Erythromycin-resistant, conditional asporogenous mutant of *Bacillus subtilis*. *In* Spores VI. P. Gerhardt, R. N. Costilow, and H. L. Sadoff, editors. American Society for Microbiology, Washington, D.C. 307–313.
8. FORTNAGEL, P., R. BERGMANN, B. HAFEMANN, and C. LEGELSEN. 1975. Structural and functional alterations of *Bacillus subtilis* ribosomes. *In* Spores VI. P. Gerhardt, R. N. Costilow, and H. L. Sadoff, editors. American Society for Microbiology, Washington, D.C. 301–306.
9. HALLBERG, R. L., and P. J. BRUNS. 1976. Ribosome biosynthesis in *Tetrahymena pyriformis*: regulation in response to nutritional changes. *J. Cell Biol.* **71**:383–394.
10. HENSHAW, E. C., D. G. GUINEY, and C. A. HIRSCH. 1973. The ribosome cycle in mammalian protein synthesis. I. The place of monomeric ribosomes and ribosomal subunits in the cycle. *J. Biol. Chem.* **248**:4367–4376.
11. LAEMMLI, U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* **227**:680–685.
12. LEIGHTON, T. J. 1974. Sporulation-specific translational discrimination in *Bacillus subtilis*. *J. Mol. Biol.* **86**:855–863.
13. LOVETT, J. S. 1975. Growth and differentiation of the water mold *Blastocladiella emersonii*: cytodifferentiation and the role of ribonucleic acid and protein synthesis. *Bacteriol. Rev.* **39**:345–404.
14. SZYSZKO, A. H., B. L. PRAZAK, C. F. EHRET, W. J. EISLER, JR., and J. J. WILLE, JR. 1968. A multi-unit sampling system and its use in the characterization of ultradian and infradian growth in *Tetrahymena*. *J. Protozool.* **15**:781–785.
15. VANVENROOIJ, J., and A. P. M. JANSSEN. 1976. Heterogeneity of native ribosomal 60-S subunits in Ehrlich ascites tumor cells cultured *in vitro*. *Eur. J. Biochem.* **69**:55–60.
16. WARNER, J. R. 1966. The assembly of ribosomes in HeLa cells. *J. Mol. Biol.* **19**:383–398.
17. WARNER, J. R., and S. A. Udem. 1972. Temperature sensitive mutations affecting ribosome synthesis in *Saccharomyces cerevisiae*. *J. Mol. Biol.* **65**:243–257.
18. WEBER, M. J. 1972. Ribosomal RNA turnover in contact inhibited cells. *Nat. New Biol.* **235**:58–60.
19. ZINKER, S., and J. R. WARNER. 1976. The ribosomal proteins of *Saccharomyces cerevisiae*: phosphorylated and exchangeable proteins. *J. Biol. Chem.* **251**:1799–1807.