Polyribosomes of Escherichia coli

BREAKDOWN DURING GLUCOSE STARVATION*

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SUMMARY

Growing cells incubated in a medium lacking glucose undergo a rapid breakdown of polyribosomes to single 70 S ribosomes. This conversion is accompanied by a loss of protein-synthetic capacity in vivo and is inhibited by chloramphenicol, but not by puromycin. The conversion of polyribosomes to 70 S ribosomes, with a half-life of $1 \frac{1}{2}$ to 2 1/2 min at $37^\circ$, appears to reflect a breakdown of messenger ribonucleic acid. The polysomes remaining during starvation remain the most active sites of protein synthesis.

The synthesis and breakdown of genetic messages in bacteria has been postulated to occur rapidly (1). Evidence for this postulate was initially obtained by Naono and Gros (2), who used 5-fluorouracil to modify the synthesis of genetic messages specific for the inducible enzyme, $\alpha$-galactosidase. More recent experiments have indicated that bacterial messenger ribonucleic acid decays exponentially with a half-life of $1 \frac{1}{2}$ to 2 1/2 min at $37^\circ$ (3-5), although other reports have demonstrated a much longer half-life for the messenger of the enzyme penicillinase in Bacillus cereus (6), and the existence of long-lived messenger RNA in sporulating cells of Bacillus subtilis (7). This report shows that depriving growing cells of glucose results in rapid conversion of polyribosomes to 70 S ribosomes and a concomitant loss of protein-synthetic capacity in vivo, with a half-life of $1 \frac{1}{2}$ to 2 1/2 min at 37°. The conversion of polyribosomes to single ribosomes is inhibited by chloramphenicol, but not by puromycin.

In the accompanying report (8) the re-formation of polysomes on recovery from glucose starvation will be examined.

EXPERIMENTAL PROCEDURE

Bacteria—Escherichia coli B was grown at $37^\circ$ in minimal medium (9) with 0.2% glucose on a rotary shaker. Under these conditions the cells had a doubling time of 55 min.

Preparation of Lysates—Polyribosome-rich extracts were prepared by the EDTA-lysozyme technique described in a previous communication (10). The following modifications of this technique were made: 1 ml of 12.5% sucrose was inserted between the EDTA (10% sucrose) and lysozyme (15% sucrose) layers, and the top layer (5% sucrose) was reduced to 0.5 ml. These modifications ensured a greater reproducibility within individual experiments. Lysis of the cells treated with EDTA and lysozyme was carried out in magnesium-Tris buffer (10) by the addition of sodium deoxycholate to 0.25%. Cell debris and unbroken cells were removed by centrifugation at 20,000 $\times$ g for 10 min at 0°.

To determine the polysome content of cells undergoing glucose starvation, exponentially growing cells were harvested by centrifugation at 20,000 $\times$ g for 5 min at 0°. The cells were rapidly resuspended in 0.1 volume of cold minimal medium (without glucose) and diluted into this medium at 37°. Aliquots of the culture were removed at various time intervals and chilled by the addition of crushed ice, and extracts were prepared as described above.

These manipulations did not in themselves lead to appreciable polysome breakdown, since the polysome contents of cells in exponential growth and of cells harvested, resuspended in medium without glucose (as described above) and immediately chilled were the same. The procedures leading up to glucose starvation, when performed rapidly, were found to be the least harmful to the cellular polysome content. Millipore filtration (of the culture volumes required for these experiments) was less rapid and yielded cells with lower polysome content than the technique described above.

It is clear that the recovery of ribosomes from cells treated by the EDTA-lysozyme method varies with the physiological state of the cell. This is not due to differences in susceptibility of the cells to attack by EDTA and lysozyme, but results from trapping of polysomes (but not monosomes) within partially lysed cells. This conclusion is supported by the finding that equivalent amounts of ribosomes are recovered from all cells when they are mildly treated with RNase. We have also determined that the recovery of soluble material (sedimenting at the top of the gradient) from cells in different physiological states is constant. Therefore, by measuring the cellular content of single ribosomes (as a function of the amount of soluble material, so as to correct for different amounts of cells in different experiments), we have obtained an additional index of polysome content. As is shown in Fig. 3, the two ways of measuring the cellular content of polysomes give essentially the same answer.

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* These investigations were supported by a research grant and a predoctoral fellowship from the National Institute of General Medical Sciences.
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Alternative Method of Lysate Preparation—The EDTA-lysozyme technique could not be used for the preparation of lysates from cells treated with proflavine. The reason for this failure is not clear. It was found, however, that a recently developed method (11), utilizing lysozyme and freezing and thawing to weaken the bacterial surface, could be used with such cells. This technique was used to obtain some of the data shown in Fig. 5.

Sedimentation of Ribosomes—Sucrose gradients from 15 to 30% sucrose (4.4 ml), containing 0.01 M magnesium acetate, 0.05 M KCl, and 0.01 M Tris-HCl, pH 7.6, at 37°C, were loaded with 0.3 to 0.5 ml of extract and centrifuged as previously described (10). Absorbance at 260 mμ was monitored in a Gilford continuous flow spectrophotometer or in a Zeiss PMQ II spectrophotometer. In all cases the observed absorbance at 260 mμ was corrected for contributions from the sucrose medium.

The proportion of ribosomes as polysomes was determined by summation of the relative areas under the polysomal and monosomal regions; radioactivity was determined as previously described (10).

Materials—Chloramphenicol was obtained from Parke, Davis and Company, and puromycin dihydrochloride from Nutritional Biochemicals. Proflavine sulfate was obtained from the National Aniline Division of the Allied Chemical Corporation. Spectinomycin was obtained from the Upjohn Company. 14C-Leucine and 14C-uracil were purchased from New England Nuclear.

RESULTS

Polyribosome Content of Starved Cells—Cells deprived of glucose undergo a net loss of ribonucleic acid (primarily from the ribosomes) at a rate of less than 5% per hour (12, 13). When cells starved for 1 hour were examined by the EDTA-lysozyme technique (Curve A in Fig. 1), a striking absence of polyribosomes was observed. Although E. coli polysomes are extremely sensitive to disaggregation by ribonuclease (14), the lack of polysomes observed in starved cells was probably not due to such enzymatic action during extraction, since starved cells (Curve A) and growing cells (Curve B) were extracted together.

Since polysomes are the major sites of protein synthesis in growing cells (14, 10), it was of interest to determine whether this was also true in starved cells. These cells were exposed to 14C-leucine for 15 sec and chilled, and extracts were prepared. Sucrose gradient centrifugation revealed that in glucose-starved cells the few remaining polysomes were the major active sites in the process of polysome breakdown (that is, after 1 to 3 min of starvation).

Kinetics of Breakdown of Polyribosomes—The polyribosome content of cells deprived of glucose for various time periods was investigated. The results of a series of five experiments are shown in Fig. 3. These data indicated that the breakdown of polyribosomes to 70 S ribosomes during starvation occurred rapidly. The cellular polyribosome content was reduced to 25% of the initial content in 5 min at 37°C.

Since polyribosomes are the major sites of protein synthesis in E. coli, their breakdown might be reflected in the rate of protein synthesis during starvation. By adding 14C-leucine to cells at the beginning of starvation, the rate of protein synthesis at any time during glucose deprival may be determined from the accu-
mulating radioactivity. The data obtained from such experiments are shown in Fig. 4. The rate of protein synthesis, as measured in this way, declines exponentially, with a half-life of approximately 1.3 to 1.7 min, to about 10% of the rate attained at the beginning of starvation. This estimate for residual protein synthesis approaches that obtained for the rate of protein synthesis in long term glucose-starved cells (5% of the normal rate) by Mandelstam and Halvorson (12), and confirmed by us (Table I).

The residual protein synthesis observed in glucose-starved cells may reflect synthesis and breakdown of messenger RNA at a rate 5 to 10% of that of the growing cell. Such synthesis cannot be detected directly since the uptake of $^{14}$C-uracil in glucose-starved cells is negligible (Table I). Indirectly, the function of this RNA, rather than its synthesis, might be analyzed. Proflavine has been shown to inhibit RNA synthesis in E. coli (15, 16); the more commonly used agent, actinomycin D, does not function in this species. When proflavine is added to glucose-starved cells, the incorporation of $^{14}$C-leucine into protein is inhibited (Table I), suggesting that the synthesis of messenger RNA does occur in glucose-starved cells. This finding is supported by earlier reports describing the synthesis of inducible enzymes in glucose-starved cells (17, 4). In this situation, therefore, the incorporation of $^{14}$C-uracil is not an adequate means for detecting RNA synthesis.

Inhibition of Protein Synthesis and Polysome Breakdown—It was of interest to determine the effect of various inhibitors of protein synthesis on the conversion of polysomes to single ribosomes during glucose starvation. Extracts prepared from cells starved with and without chloramphenicol were analyzed as shown in Fig. 5. The breakdown of polysomes during glucose starvation was not observed in the presence of chloramphenicol. This effect was probably not due to a prolongation of RNA syn-

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** Breakdown of polysomes during glucose starvation. Cells growing exponentially were rapidly harvested. The cell pellet was resuspended in 5 to 10 ml of cold glucose-free medium, and the suspension was rapidly pipetted into 100-ml glucose-free medium at 37°. Aliquots (30 ml each) were removed and chilled after various times of starvation. Extracts were prepared and examined by sucrose gradient centrifugation (as in Fig. 2). In each experiment (six separate experiments are represented here), a “zero time” aliquot was removed immediately after having been pipetted into warm medium (this procedure took 10 to 20 sec). The percentage of polysomes was determined in each case by summation of the areas under the polysome and monosome regions of the gradient, and calculated as a function of the initial polysome content, setting this equal to 100% at zero time. The ratio of monosomes to soluble material was calculated by summation of the optical densities under the monosome region and that material sedimenting more slowly than the ribosomes.

![Fig. 4](http://www.jbc.org/)

**Fig. 4.** Rate of protein synthesis during glucose starvation. Exponentially growing cells were rapidly harvested and resuspended in glucose-free medium, as in Fig. 3. The medium contained 5 μg of $^{14}$C-leucine per ml. Aliquots (1-ml) of the culture were pipetted into 4 ml of 5% trichloracetic acid at 30-sec intervals after the initiation of starvation. Hot acid-insoluble material was deposited on Millipore filters and counted. The radioactivity incorporated during the 30-sec intervals is plotted as a function of starvation time. Three separate experiments are presented as indicated.

### Table I

Incorporation of $^{14}$C-uracil and $^{14}$C-leucine in growing and glucose-starved cells

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Incorporation</th>
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<tbody>
<tr>
<td></td>
<td>Growing cells</td>
</tr>
<tr>
<td>$^{14}$C-Uracil</td>
<td>233</td>
</tr>
<tr>
<td>$^{14}$C-Uracil + 100 μg of proflavine per ml</td>
<td>22</td>
</tr>
<tr>
<td>$^{14}$C-Leucine</td>
<td>2920</td>
</tr>
<tr>
<td>$^{14}$C-Leucine + 100 μg of proflavine per ml</td>
<td>564</td>
</tr>
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themselves to be due to the use of different experimental techniques, or could indicate in the rate of protein synthesis (t_b = 1.3 to 1.7 min) may be due to the rate of polysome breakdown (t_t = 2.5 min) and the decrease in the rate of polysome breakdown. The difference between these two rates is the rate of polysome breakdown (19) (Fig. 5).

The data presented above indicate that removal of glucose from the medium of growing cells resulted in a rapid conversion of polyribosomes to 70 S ribosomes. This conversion appeared to occur exponentially; however, the techniques for measuring polysome content are not sufficiently accurate to exclude rigorously other kinetics. The breakdown of polysomes did not require the addition of agents that inhibit RNA synthesis, was accompanied by a loss of protein-synthetic capacity, and occurred at rates similar to those obtained by other investigators for the breakdown of rapidly labeled RNA (3-5). This similarity implies a close relationship between messenger RNA breakdown and polysome breakdown. The difference between the rate of polysome breakdown (k_b = 2.5 min) and the decrease in the rate of protein synthesis (k_s = 1.3 to 1.7 min) may be due to the use of different experimental techniques, or could indicate an alteration in the intrinsic capacities of polysomes.

Protein synthesis in glucose-starved cells, as in growing cells, occurs on polyribosomes. Starved cells contain predominantly single 70 S ribosomes, but these single ribosomes are not nearly as active as the remaining polysomes (Fig. 2). Although monosomes derived by RNase treatment of polysomes in vitro retain nascent protein chains (14), 70 S ribosomes created during glucose starvation in vivo apparently do not, as detected by exposing cells starved for only 2 min to ^14C-leucine for 15 sec. These newly created 70 S ribosomes did not carry labeled peptide chains, suggesting that the conversion of polysomes to monosomes during starvation does not occur by direct endonucleolytic attack on the polysomes.

The maintenance of a cellular complement of polysomes when starvation is carried out in the presence of chloramphenicol is consistent with the notion that this drug prevents movement of ribosomes along the messenger RNA backbone of the polysomes, by inhibiting the peptide-linking step (20, 21). Alternatively, the maintenance of the polysome complement may be due to a chloramphenicol-mediated acceleration of polysome assembly (21).

Acknowledgments—The authors wish to thank Dr. O. A. Scornik for innumerable valuable discussions and suggestions, and Professor B. D. Davis for his constructive criticisms during the preparation of this manuscript.

REFERENCES
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