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Levels of Ribosomal Protein S1 and Elongation Factor G in the Growth Cycle of *Escherichia coli*

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The relative levels of ribosomes, ribosomal protein S1, and elongation factor G in the growth cycle of *Escherichia coli* were examined with two-dimensional polyacrylamide gel electrophoresis. Nonequilibrium pH gradient polyacrylamide gel electrophoresis was used in the first dimension, and polyacrylamide gradient-sodium dodecyl sulfate gel electrophoresis was used in the second dimension. The identities of protein spots containing S1 and elongation factor G were confirmed by radioiodination of the proteins and peptide mapping of the radiolabeled peptides. The levels of ribosomes and ribosomal protein S1 were coordinately reduced during transition from exponential phase to stationary phase. There was no accumulation of S1 in the stationary phase. In marked contrast, the level of elongation factor G showed no significant change from exponential phase to stationary phase. The relative level of elongation factor G compared with ribosomes or S1 increased by about 2.5-fold during transition from exponential phase to stationary phase. The results show that there are differences between the regulation of the levels of elongation factor G and of ribosomal proteins, including S1, apparent during the transition from exponential to stationary phase.

The relative rates of synthesis of ribosomal components remains balanced when *Escherichia coli* are growing exponentially at a variety of growth rates (6, 16). The pools of free ribosomal components are very small (9, 10, 16). Bacteria have regulatory mechanisms to coordinate the synthesis of each ribosomal component at various growth rates and accomplish the production of the components in the same stoichiometry as that found in the ribosomes (6, 9, 10, 16). Elongation factor G (EF-G) and the unusual ribosomal protein S1 (see below) are also synthesized coordinately with ribosomes at a variety of growth rates (11, 21), and both are under stringent control (2, 7, 28). The regulation of EF-G and of S1 differs from that of typical ribosomal proteins and ribosomal RNA in relaxed cultures (28).

The coordinate regulation of EF-G is consistent with its location in an operon containing genes for ribosomal proteins S7 and S12 (14). It was shown recently that the synthesis of both S7 and EF-G is regulated by an autogenous translational feedback mechanism in which S7 acts as a repressor through interaction with the mRNA (5).

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Protein S1 is atypical among ribosomal proteins. It is more acidic and has a higher molecular weight (32). It is a component of the replicase of bacteriophage Qβ (31). It is freely exchangeable with unbound S1 (19), and it has been reported that free cytoplasmic S1 accumulates in stationary-phase cells (26). The mechanism by which the biosynthesis of S1 is regulated is not yet established, and the location of the gene for S1 is distinct from those for the other 47 ribosomal proteins that have been determined (24).

In the work reported here, we compared the relative levels of ribosomes, S1, and EF-G in the growth cycle of *E. coli* during the transition from exponential to stationary phase. We examined the protein composition of cells during the growth cycle using the high sensitivity and resolution provided by the two-dimensional polyacrylamide gel separation described by O'Farrell and co-workers (22, 23). This system, involving nonequilibrium isoelectric focusing in the first dimension, has been used in this laboratory to identify cross-links to S1 (3). We found that S1 did not accumulate but that the level of EF-G was elevated relative to S1 and ribosomes during the transition from exponential to stationary phase.

MATERIALS AND METHODS

Materials. Ampholines (pH range 3 to 10) were purchased from Bio-Rad Laboratories, and Nonidet P-

40 was from British Drug Houses. $Na^{125}I$ (carrier free, 100 mCi/ml) was from Amersham Corp. Thermolysin was obtained from Sigma Chemical Co., and V-8 protease came from Boehringer Mannheim Corp. Coomassie brilliant blue R-250 and Iodo-gen were products of Pierce Chemical Co. Silica gel thin-layer sheets (0.25 mm) were from Macherey-Nagel. All other reagents were commercially obtained and were of analytical grade.

Growth of bacteria. *E. coli* MRE600 was grown in yeast extract-glucose media (12). One-liter cultures were grown in a shaker bath at 37°C, and growth was monitored by following the absorbance at 550 nm (A_{550}). Under these conditions, the doubling time in exponential phase was 22 min. Bacteria were harvested by centrifugation at 0°C.

Preparation of cell extract. All steps were performed at 0°C. Cells were lysed by grinding with alumina (2 g/g of cells) and were suspended in 10 mM Tris-hydrochloride (pH 7.4)–100 mM NH_4Cl–10 mM $MgCl_2$–14 mM 2-mercaptoethanol–1 mM EDTA (5 ml/g of cells). The buffer contained phenylmethylsulfonyl fluoride (0.1 mM) added from a freshly prepared stock solution (50 mg/ml in ethanol). DNase (final concentration, about 1 μg/ml) was added, and the suspension was allowed to stand for 15 min. The homogenate was centrifuged at 12,000 × *g* for 30 min to remove alumina and unbroken cells and then at 100,000 × *g* for 20 min to clarify the cell extract. Ribosomes were separated by centrifugation at 350,000 × *g* for 3 h. Protein concentrations were estimated by the method of Lowry et al. (20).

Two-dimensional polyacrylamide gel electrophoresis. Proteins in the cell extracts were analyzed by modification of the two-dimensional gel electrophoresis technique described by O'Farrell et al. (23), in which the first dimension is a nonequilibrium pH gradient (pH 3 to 10) electrophoresis (3). The procedures used in preparation of the gels were as described by Howe and Hershey (13). The samples (50 to 60 μl and containing 75μg of protein) were subjected to electrophoresis in two dimensions as described previously (3). Proteins were stained with Coomassie brilliant blue R-250 in methanol-acetic acid-water (5:1:5, vol/vol). The protein-bound dye was quantified by the method of Fenner et al. (8).

Radioiodination of proteins eluted from polyacrylamide gels. Sections of polyacrylamide gel containing stained proteins of interest were pulverized, and the proteins were extracted with 0.5 ml of 1% (wt/vol) sodium dodecyl sulfate (SDS)–50 mM triethanolamine-hydrochloride (pH 8.0)–0.1 mM EDTA–0.2 mM dithioerythritol (30). Iodoacetamide (1 mM) was added to alkylate any possible protein sulfhydryl groups, and after 2 h at 25°C, gel fragments were removed by filtration. After extraction, free sulfhydryl groups were undetectable by assay (15). The proteins were radioiodinated with 0.025 μmol of iodide containing 200 μCi of ^{125}I in the presence of 18 μg of Iodo-gen (30). The reaction was for 1 min at 0°C; other details were as described previously (30). Radiolabeled proteins (about 500,000 cpm/μg) were recovered with 40 μg of carrier total 50S ribosomal protein.

Peptide mapping of radioiodinated proteins. Radiolabeled protein samples were submitted to electrophoresis in one-dimensional polyacrylamide-SDS gel slabs (0.75 mm thick) of 10% acrylamide (17). The gel ensured a pure preparation of radiolabeled protein for peptide mapping, separating background traces of contaminating radioactive bands of different mobilities. Most of the radioactivity (≥90%) comigrated with the proteins of interest.

Radiolabeled protein bands (approximately 2.5 by 1 by 0.75 mm, containing 150,000 to 300,000 cpm) were excised from the gel and submitted to peptide mapping by two different techniques. The first method was one-dimensional peptide mapping in polyacrylamide-SDS gels, employing partial digestion with V-8 protease by the method of Cleveland et al. (4). The gel pieces containing the radiolabeled proteins were equilibrated with 125 mM Tris-hydrochloride (pH 6.8)–0.1% (wt/vol) SDS and were inserted into wells of an acrylamide gradient gel slab (12.5 to 20%). The samples were overlayered with 0.25 μg of V-8 protease, and electrophoresis was performed as described previously (4). The gels were dried and submitted to radioautography for 2 days with Kodak Noscreen medical X-ray film to locate radioactive peptides. The second method of analysis was two-dimensional peptide mapping on silica gel thin-layer sheets (1). Radiolabeled proteins were eluted from the pieces of acrylamide gel in the presence of 500 μg of glyceraldehyde-3-phosphate dehydrogenase carrier protein (30). The resulting protein samples were alkylated with iodoacetamide and digested with thermolysin (12.5 μg) as described previously (18). One-tenth of the digest (50 μg; 5,000 to 10,000 cpm) was applied to silica thin-layer sheets (10 by 10 cm) and submitted to two-dimensional peptide mapping (1). Electrophoresis in the first dimension was with 2% (vol/vol) pyridine–4% (vol/vol) acetic acid–15% (vol/vol) acetone (pH 4.4) at 200 V for 1 h at 1°C. Chromatography (second dimension) was with butanol-water-pyridine-acetic acid (15:12:10:3, vol/vol) for 1.5 h at 25°C. The peptides of glyceraldehyde-3-phosphate dehydrogenase were stained with ninhydrin-cadmium acetate (18), and radiolabeled peptides were located by radioautography for 3 to 11 days.

RESULTS AND DISCUSSION

Figure 1 shows two-dimensional polyacrylamide gels of cell extracts from *E. coli* at different stages of the growth cycle (growth curve shown in Fig. 2). The samples for each gel contained 75 μg of protein. In the early exponential phase (Fig. 1a), the small basic ribosomal proteins seen at the lower left of the gel clearly form a substantial fraction of the soluble proteins, a point that is well established (6, 9, 10, 16). Ribosomal proteins L2, S4, S7, and L13 and the small acidic proteins L7 and L12 are identified in the figure. It is clear by comparison of Fig. 1c and d with Fig. 1a and b that the proportion of ribosomal proteins is drastically reduced after transition to the stationary phase. The synthesis of ribosomal proteins is suppressed as the cells reach the stationary phase, but many other gene products continue to be made. Ribosomal proteins L7 and L12 (Fig. 1) are identical, except that L7 has an acetylated amino terminal. It was shown previously that the degree of acetylation increases in the stationary phase (27), and indeed, Fig. 1 shows more

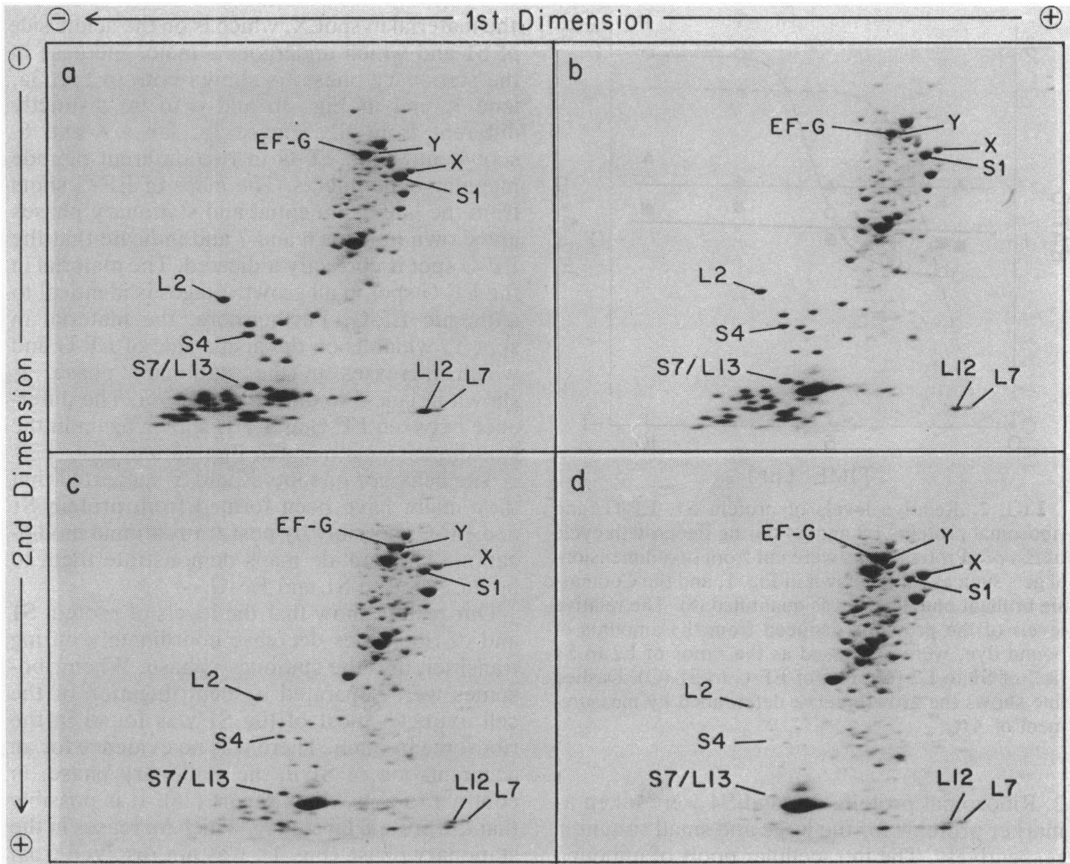

FIG. 1. Analysis of cell extracts at different stages in the growth cycle of *E. coli* by two-dimensional polyacrylamide gel electrophoresis. The first dimension (12.5 by 0.2 cm) was nonequilibrium pH gradient electrophoresis run towards the cathode at 400 V for 3 h with ampholines in the pH range of 3 to 10 (3). The second dimension was a polyacrylamide-SDS gel slab (13 by 10 by 0.15 cm) containing an acrylamide gradient (10 to 16%), with a stacking gel (13 by 1 by 0.15 cm) of 5% acrylamide, run towards the anode at 150 V for 4 h (17). Proteins were stained with Coomassie brilliant blue R-250, and ribosomal proteins were identified by the method of Tolan et al. (30). Samples of cell extract (75 μg of protein), prepared as described in the text, were as follows: (a) Early exponential phase (A_{550}, 0.25); (b) late exponential phase (A_{550}, 2.5); (c) transition to stationary phase (A_{550}, 6.5); (d), stationary phase (A_{550}, 7.1). Refer to Fig. 2 for the growth curve. The dark spot below S4 in all the panels is RNase added in the sample preparation.

L12 than L7 in the exponential phase (Fig. 1a and b) and more L7 than L12 in the stationary phase (Fig. 1d).

The protein spots corresponding to ribosomal protein S1 and to EF-G are also indicated in Fig. 1. Their identities were deduced by reference to the work of Pedersen et al. (25), and the assignments were confirmed by peptide mapping (see below). The relative level of S1 in the stationary phase (Fig. 1d) was clearly much reduced when compared with the extracts from cells in the early exponential phase (Fig. 1a). Quite a different result was seen in the case of EF-G. The protein spot corresponding to EF-G is approximately constant in the four gels shown in Fig. 1. Thus, the proportion of EF-G synthesized, compared with the total synthesis of soluble proteins, remained about the same in the exponential phase and the stationary phase. However, in the exponential phase a large proportion of total protein synthesis is ribosomal protein synthesis, whereas in the stationary phase the bulk of protein synthesis is of nonribosomal proteins (Fig. 1).

The changes illustrated in Fig. 1 were quantified by measuring the amount of dye bound to the proteins of interest. Since different proteins may not bind Coomassie brilliant blue in proportion to their size, the results of these measurements were expressed as ratios as shown in Fig.

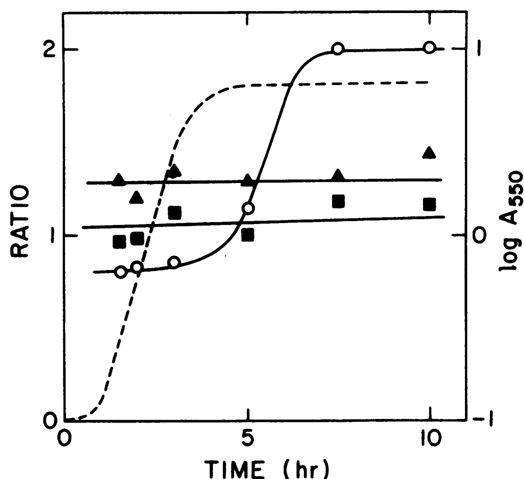

FIG. 2. Relative levels of protein S1, EF-G, and ribosomal proteins L2 and S4 during the growth cycle of *E. coli*. Protein spots were cut from two-dimensional gels such as those shown in Fig. 1, and the Coomassie brilliant blue R-250 was quantified (8). The relative levels of the proteins, deduced from the amounts of bound dye, were expressed as the ratios of L2 to S4 (▲), of S1 to L2 (■), and of EF-G to S1 (○). Dashed line shows the growth curve determined by measurement of A_{550}.

2. Ribosomal proteins L2 and S4 were taken as marker proteins for the large and small subunits, respectively. The intracellular pools of unbound ribosomal proteins are negligible (9). The ratio L2/S4 remained constant throughout the growth cycle (Fig. 2). The ratio S1/L2 also remained approximately constant, suggesting that the synthesis of S1 and ribosomal subunits were coordinately controlled during transition from the exponential phase to the stationary phase. The ratio EF-G/S1 increased from 0.8 in the exponential phase to over 2.0 in the stationary phase (Fig. 2). Thus, the levels of EF-G compared with those of ribosomes and to S1 increased by about 2.5-fold during the transition from exponential phase to stationary phase. This is in striking contrast to the coordinate regulation of EF-G and ribosomes in exponentially growing cells, which are in a 1:1 ratio under many different growth conditions (11).

To be certain that the protein spots labeled S1 and EF-G in Fig. 1 were correctly identified, proteins were eluted from the stained spots and were compared with preparations of authentic S1 and EF-G by peptide mapping. Radioiodine was used to label tyrosine and histidine residues. The results of one- and two-dimensional peptide mapping are shown in Fig. 3. Figure 3a, lanes 1 and 2, shows authentic S1 and material from spot S1 in Fig. 1, respectively, and indicates that the S1 spot is correctly identified. Furthermore, the material in spot X, which is on the acidic side of S1 and which undergoes a major increase in the stationary phase, is shown both in Fig. 3a, lane 3, and in Fig. 3b and c to be distinctly different from S1. Figure 3a, lanes 4 and 8, shows authentic EF-G in two different peptide mapping experiments. The maps of EF-G spots from the late exponential and stationary phases are shown in lanes 6 and 7 and indicate that the EF-G spot is correctly indicated. The material in the EF-G spot in all growth stages is identical to authentic EF-G. Furthermore, the material in spot Y, which is on the acidic side of EF-G and which increases in the stationary phase, is shown in lane 5 to differ from EF-G. The difference between EF-G and Y is shown again in the two-dimensional maps in Fig. 3d and e.

The behavior of spots X and Y suggested that they might have been formed from protein S1 and EF-G, possibly by post-translational modification. The peptide maps demonstrate them to be unrelated to S1 and EF-G.

Our results show that the levels of protein S1 and of ribosomes decrease coordinately during transition into the stationary phase. When ribosomes were separated by centrifugation of the cell extracts, most of the S1 was found in the ribosome fraction. There was no evidence for an accumulation of S1 in the stationary phase, in contrast to a previous report (26). It is possible that the protein labeled X, which increases in the stationary phase (Fig. 1), was not resolved from S1 in the previous work, which used electrophoretic techniques with less resolution than those used here. It was also reported that free ribosomal proteins L7 and L12 accumulated in the stationary phase (26). There was no evidence for this in our work (Fig. 1). Inspection of Fig. 1 shows a protein of small molecular weight appearing in the stationary phase at a position just underneath that of L12. It is possible that this protein, characteristic of the stationary phase, was not resolved from L12 in the previous work (26).

The results presented here show clearly that the level of EF-G behaves differently from the levels of ribosomal proteins in cells entering the stationary growth phase. In particular, its behavior differs from that of S7, despite the facts that coordinate behavior of EF-G and S7 has been reported under a variety of growth conditions (6, 11) and that the syntheses of both S7 and EF-G are under many conditions regulated by S7 through autogenous translational control (5). The level of EF-G increases in stationary growth, whereas the levels of ribosomal proteins, including S7, decrease. The level of S1 also decreases in the stationary phase in parallel with the decrease in the levels of the typical small basic ribosomal proteins.

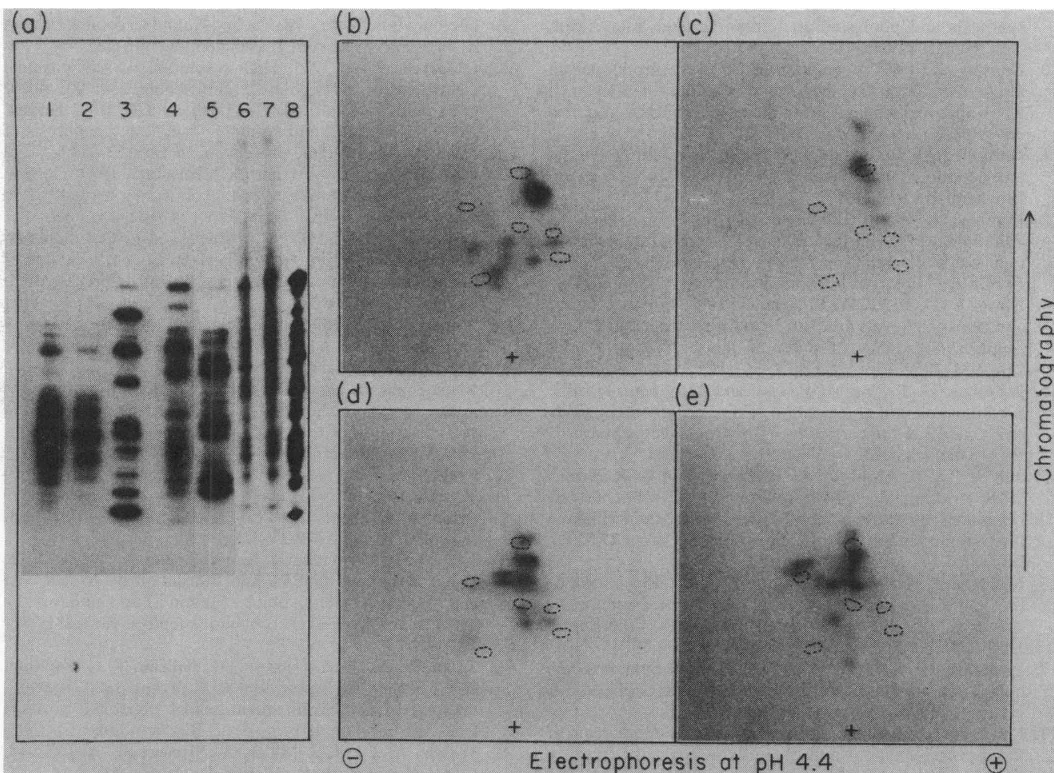

FIG. 3. Radioautographs of peptide maps of radioiodinated proteins extracted from two-dimensional polyacrylamide gels. (a) Radioautographs of one-dimensional polyacrylamide-SDS gel peptide maps (4). Radioiodinated proteins were digested with V-8 protease. Experiment 1, lanes 1 through 5; experiment 2, lanes 6 through 8. Protein samples were as follows: Lane 1, authentic S1 prepared by the method of Tal et al. (29) from purified 30S ribosomal subunits; lane 2, protein extracted from stained spots labeled S1 in Fig. 1; lane 3, protein extracted from stained spots labeled X in Fig. 1; lane 4, authentic EF-G, which was kindly provided by J. W. B. Hershey; lane 5, protein from stained spots labeled Y in Fig. 1; lane 6, protein from EF-G spot in transition to stationary phase; lane 7, protein from EF-G spot in stationary phase; lane 8, authentic EF-G. (b through e) Radioautographs of two-dimensional thermolytic peptide maps of radioiodinated proteins run on silica gel thin-layer sheets (10 by 10 cm). Glyceraldehyde-3-phosphate dehydrogenase was used as carrier and marker, and about 45 peptides were visible with ninhydrin-cadmium acetate. Each map gave an identical pattern, and positions of six of the stained peptides are shown in the radioautographs by dotted outlines. The protein samples were as follows: (b) Authentic S1; (c) protein extracted from stained spots labeled X in Fig. 1; (d) authentic EF-G; (e) protein from stained spots labeled Y in Fig. 1.

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