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Transient Regulation of Protein Synthesis in *Escherichia coli* Upon Shift-Up of Growth Temperature

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Synthesis of total cellular proteins of *Escherichia coli* was studied upon transfer of a log-phase culture from 30 (or 37) to 42°C. Cells were pulse-labeled with [^{3}H]leucine, and the labeled proteins were analyzed by gel electrophoresis in the presence of sodium dodecyl sulfate. The rates of synthesis of at least five protein chains were found to increase markedly (5- to 10-fold) within 5 min after temperature shift-up and gradually decrease to the new steady-state levels, in contrast to the majority of proteins which gradually increase to the steady-state levels (about 1.5-fold the rate at 30°C). Temperature shift-down did not cause any appreciable changes in the pattern of protein synthesis as detected by the present method. Among the proteins greatly affected by the temperature shift-up were those with apparent molecular weights of 87,000 (87K), 76K, 73K, 64K, and 61K. Two of them (64K and 61K) were found to be precipitated with specific antiserum against proteins that had previously been shown to have an adenosine triphosphatase activity. The bearings of these findings on bacterial adaptation to variation in growth temperature are discussed.

Although extensive work has been devoted to elucidate the elementary processes of gene expression and its regulation in *Escherichia coli*, relatively little is known about the gross regulatory mechanisms at the cellular level. To obtain an integral picture of gene expression during cell growth, it would be important to know, for example, how many proteins are regulated in their synthesis in response to a change in growth conditions. Changes in synthesis of cellular proteins have been studied under conditions of amino acid limitation ("stringent control") (3, 16) or under conditions that give rise to lesions in DNA (6, 18).

It has been established that the relative content of macromolecules does not change appreciably when bacterial cells are grown at different temperatures in steady state, despite the wide variation in growth rate (12). However, little attention has been paid to macromolecular synthesis during transitions in growth temperature. The present paper reports some new features of regulation of *E. coli* protein synthesis that have been revealed by experiments involving transfer of a log-phase culture from a low to a high temperature.

MATERIALS AND METHODS

Materials. Prototrophic *E. coli* K-12, strain W3350, was used in most experiments. L-[^{3}H]leucine (58 Ci/mmol) and DL-[*methylene*-^{14}C]tryptophan (52 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, England. Recrystallized acrylamide and sodium dodecyl sulfate (SDS) were purchased from Wako Chemicals Co. (Osaka, Japan) and Nakarai Chemicals Co. (Kyoto, Japan), respectively. Brij 58 was obtained from Atlas Chemicals, and pancreatic deoxyribonuclease and ribonuclease were from Sigma Chemical Co. (St. Louis, Mo.). Lysozyme and sucrose were obtained from Seikagaku-Kogyo Co. (Tokyo, Japan) and Schwarz/Mann (Orangeburg, N.Y.), respectively.

Pulse-labeling of cells and analysis of protein by SDS-gel electrophoresis. Cells were grown in medium E (17) supplemented with 0.5% glucose to mid-log phase (about 2×10^8 cells per ml) and were shifted to a higher or lower temperature as indicated for each experiment. One-milliliter portions of the culture were removed at appropriate intervals, pulse-labeled with 10 μCi of L-[^{3}H]leucine for 3 min, and chased with 200 μg of L-leucine and 50 μg of L-isoleucine per ml for 2 min unless otherwise indicated. Pulse-labeled cells were chilled in ice and mixed with an equal volume of 10% trichloroacetic acid. The precipitates were collected by centrifugation and washed twice with cold 5% trichloroacetic acid and once with acetone. The precipitates were dissolved in SDS sample buffer [0.0625 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 6.8)-2% SDS-10% glycerol-10 mM dithiothreitol, 0.001% bromophenol blue] and heated at 100°C for 3 min. Electrophoresis was carried out on 15-cm-long, 1-mm-thick slab gels (5% stacking gel and 11.2% separation gel) using the discontinuous buffer system of Laemmli (11). Gels were stained with Coomassie brilliant blue. For detection of ^{3}H-labeled proteins, fluorographs were taken by ex-

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posing Fuji X-ray films to the scintillator-treated gel slabs (1).

To determine the rates of total protein synthesis during temperature shift, cells were grown in the presence of DL-[^{14}C]tryptophan (0.1 μCi/ml plus 20 μg of unlabeled L-tryptophan per ml) for several generations and pulse-labeled at each point with [^{3}H]leucine (1 μCi/ml and 1 μg/ml) for 1 min, followed by mixing with an equal volume of cold 10% trichloroacetic acid. Samples were filtered through Whatman GF/C glass fiber filters and washed three times with 5% trichloroacetic acid and twice with ethanol, and acid-insoluble radioactivities were determined by a liquid scintillation counter. The ratio of ^{3}H to ^{14}C incorporation was taken to represent the rate of protein synthesis at each point.

Immunoprecipitation of ATPase. Cells were treated with lysozyme-ethylenediaminetetraacetate and Brij 58, digested with pancreatic deoxyribonuclease and ribonuclease, and then disrupted by sonic treatment. Crude extracts were treated with antisera against adenosine triphosphatase (ATPase), essentially as described previously (14). ATPase was prepared by following a purification procedure for RNA polymerase and was separated from the latter enzyme by high-salt glycerol gradient centrifugation and DNA-cellulose column chromatography (8). Rabbit antisera were prepared by a published procedure (10).

Fractionation of cell lysate into membrane and soluble fractions. Cells obtained from a 20-ml culture were converted to spheroplasts, disrupted sonically, and centrifuged through 15% sucrose-3 mM ethylenediaminetetraacetate with a cushion of 70% sucrose-3 mM ethylenediaminetetraacetate at 60,000 rpm for 30 min in a Spinco 65 rotor, as described previously (9). A membrane fraction was collected from the 15 to 70% sucrose interphase region, and the soluble fraction was collected from the upper region of the 15% sucrose layer (9). The pooled soluble and membrane fractions were treated with 10% trichloroacetic acid, and protein precipitates were collected, washed with 5% trichloroacetic acid and acetone, dissolved in the sample buffer, and subjected to electrophoresis.

RESULTS

Effect of temperature shift-up on the rate of protein synthesis. When a log-phase culture of *E. coli* K-12, strain W3350, was transferred from 30 to 42°C, the growth rate, as determined by optical density measurement, increased about 1.5-fold (Fig. 1). To examine the rate of protein synthesis during temperature shift-up, cells that had been labeled with [^{14}C]tryptophan were pulse-labeled with [^{3}H]leucine at various times before or after the temperature shift, and the ratio of ^{3}H to ^{14}C incorporation was calculated. Since the ^{14}C radioactivity should be proportional to protein content (cell mass) of the culture, the ratios thus obtained would represent the rates (per cell mass) of bulk protein synthesis at the time of pulse-labeling. Figure 2 shows that the rate of protein synthesis increases within 30 min after the temperature shift and reaches a new steady-state value, which is about 1.5-fold the rate at 30°C. However, the initial response of the cells seems to follow more complex kinetics; the rate increases markedly, though transiently, within a few minutes, decreases somewhat, and then increases again to the new steady-state level. Similar kinetics were ob-

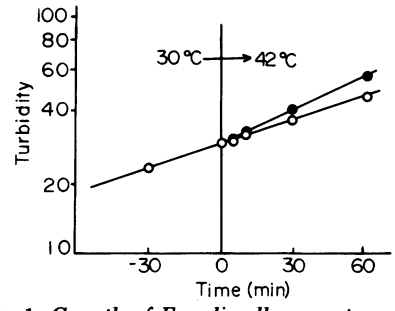

FIG. 1. *Growth of E. coli cells upon temperature shift-up. A log-phase culture of strain W3350, grown in medium E containing 0.5% glucose at 30°C, was divided into two parts (zero time) and shaken further at 30°C (○) or 42°C (●). Growth was monitored by measuring turbidity with a Klett-Summerson colorimeter (no. 54 filter).*

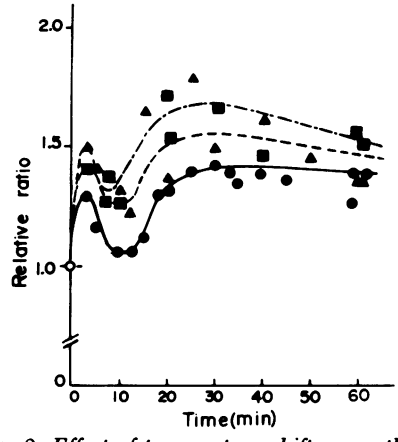

FIG. 2. *Effect of temperature shift-up on the rate of protein synthesis. Cells of strain W3350 were grown in 20 ml of medium E containing 0.5% glucose, L-tryptophan (20 μg/ml), and DL-[^{14}C]tryptophan (0.1 μCi/ml) at 30°C, divided into 0.5-ml portions, and shaken further at 42°C. At each time before (zero time) or after the temperature shift, cells were pulse-labeled with 0.5 μCi of [^{3}H]leucine (1 μCi/ml and 1 μg/ml) for 1 min. The labeling was stopped by adding trichloroacetic acid, and acid-insoluble radioactivities were determined as described in the text. The ^{3}H/^{14}C ratio for each sample was normalized to that for the zero-time sample, which was taken as unity. The data from three independent experiments are presented: (■) experiment 1; (▲) experiment 2 (●) experiment 3.*

tained in several independent experiments, including those presented in Fig. 2.

Transient stimulation of synthesis of specific proteins upon temperature shift-up. In the course of our studies with temperature-sensitive mutants of *E. coli* (20), we noticed that a temperature shift-up from 30 to 42°C causes marked and transient alterations in the differential synthesis rates of some proteins even in a wild-type strain. Thus, we analyzed the proteins synthesized under these conditions in some detail. A culture of strain W3350 was grown in minimal medium at 30°C, shifted to 42°C, and pulse-labeled with [^{3}H]leucine at various times before or after the temperature shift. The labeled cells were directly treated with trichloroacetic acid, and the proteins precipitated were analyzed by SDS-gel electrophoresis. Figure 3 shows the patterns of ^{3}H-labeled proteins as obtained by fluorography. It can be seen that synthesis of some protein chains relative to the total proteins is markedly stimulated shortly after the temperature shift-up, reaching the maximum at about 5 min, and then decreases gradually to a steady-state level comparable to the preshift level. Proteins affected most under these conditions are those with apparent molecular weights of about 87,000 (87K), 76K, 73K, 64K, and 61K. The proteins synthesized after temperature shift-up seem to remain stable, since radioactivities associated with these protein bands did not decrease appreciably during "chase" for at least 1 h (data not shown).

A rough estimation of the proportion of these "stimulated proteins" in the total proteins synthesized was made from densitometer tracings of the fluorograms (Table 1). It is clear that the proportion of each of these proteins reaches the maximum (5- to 10-fold stimulation) at about 5 min after temperature shift-up, although the extents of stimulation calculated here give only approximations. A temperature shift from 30 to 37°C (Fig. 3B) or 23 to 34°C (data not shown) gave similar but less striking effects. Temperature shift-down does not seem to cause any appreciable changes in the pattern of proteins synthesized (Fig. 4).

Relative rates of synthesis of these and the remaining classes of protein (per cell mass) were then calculated by multiplying the rate of total protein synthesis (Fig. 2) by the proportion (percentage) each protein class occupies in the total protein (Table 1). Figure 5 shows that not only the proportion in total protein but also the relative rate (per cell mass) of synthesis increases abruptly but transiently for those five protein chains, whereas the synthesis rates of other proteins increase gradually during about 30 min after temperature shift to reach the new steady-state level.

Intracellular localization of the stimulated proteins. In an attempt to localize the affected proteins to the soluble or membrane fraction, lysates of labeled cells were fractionated by the method of Ito et al. (9). Figure 6 shows the protein patterns of both soluble and membrane fractions as analyzed by SDS-gel electrophoresis. It is apparent that all species of stimulated proteins are found mainly in the soluble fraction, although some 64K and/or 61K proteins are also found in the membrane fraction. The amount of the latter proteins in the membrane fraction was estimated to be less than about 10% of that found in the soluble fraction. In this particular experiment, two pairs of closely located bands (76K/73K and 64K/61K) were not well separated from each other, presumably due to some minor differences in electrophoretic conditions. It may also be noted that synthesis of a protein found in the membrane fraction, having a molecular weight slighlty higher than that of outer membrane protein I, is appreciably stimulated after temperature shift-up. However, increased synthesis of this protein seems to persist at 42°C, and it does not belong to the "transiently stimulated" class of proteins (Fig. 3).

Identification of the 64K and 61K polypeptides as subunits of an ATPase. In our previous studies on the synthesis of DNA-dependent RNA polymerase (13), it was noted that the synthesis of a protein that copurifies with the polymerase and has an ATPase activity (7, 8) is stimulated markedly by temperature shift-up, just as observed here. We thus examined whether the ATPase protein can be found among the proteins shown to be subject to transient stimulation by the temperature shift. Crude extracts of pulse-labeled cells were treated with antiserum against ATPase, and the resulting precipitates and supernatants were subjected to electrophoresis on SDS-gels. A pair of bands with molecular weights of 64K and 61K was clearly seen in the electrofluorogram obtained with antigen-antibody precipitates (Fig. 7). In contrast, the supernatant contained little proteins corresponding to the two bands (data not shown), indicating that most of these proteins had been specifically removed by the antiserum. This pair of protein bands exactly coincides in electrophoretic mobiliy with two of the protein chains whose synthesis is stimulated by the temperature shift.

Figure 7 also shows that radioactivity in these ATPase bands increases upon temperature shift-up, although this experiment permits only rough quantitative comparisons because no correction

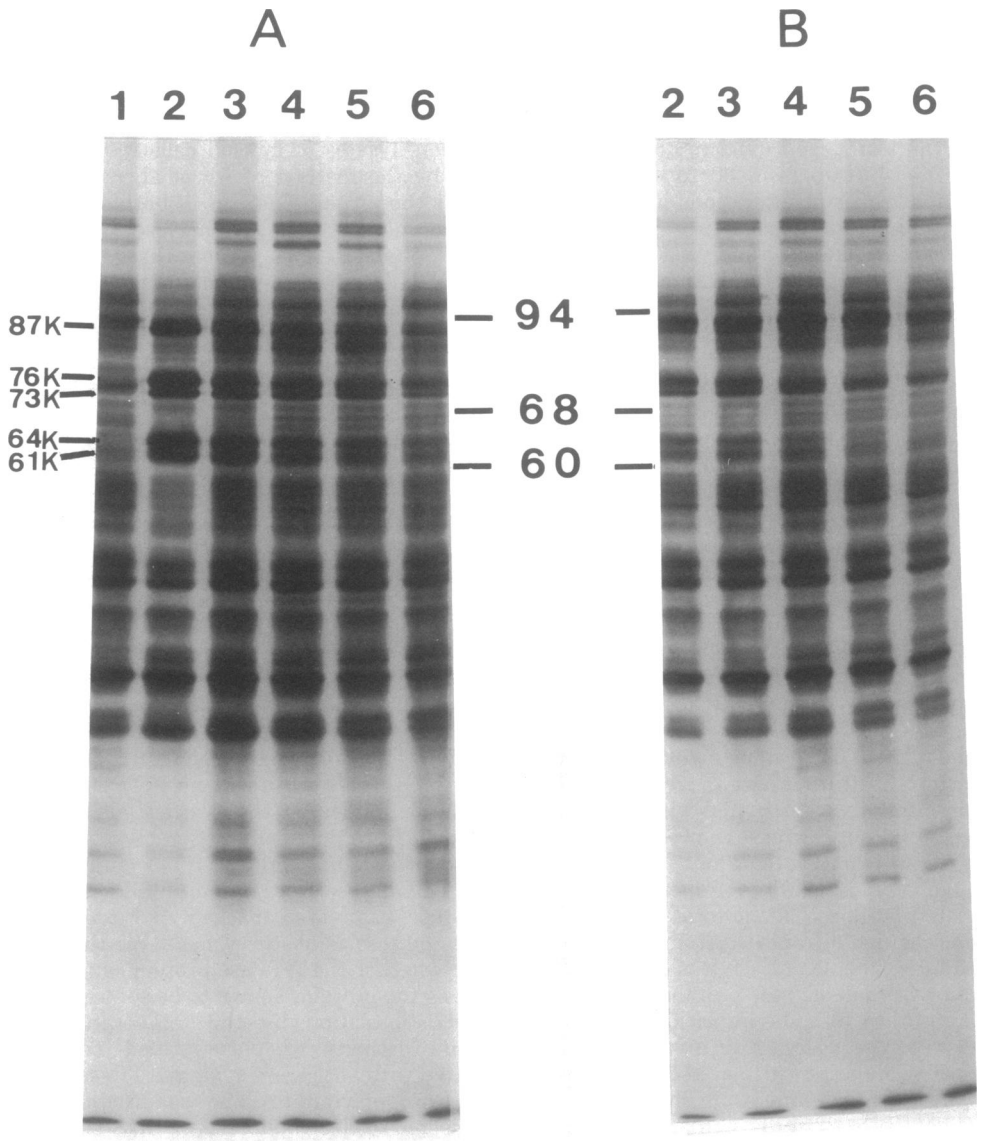

FIG. 3. *Effect of temperature shift-up on the composition of newly synthesized proteins. A culture of strain W3350 was grown in medium E at 30°C to about 2 × 10⁸ cells per ml, divided into two portions, and shifted to 37 or 42°C. One-milliliter portions were removed before (zero time) or after temperature shift, pulse-labeled with 10 μCi of [³H]leucine, chased with unlabeled leucine and isoleucine, and treated with trichloroacetic acid, and the whole protein mixtures were analyzed by SDS-gel electrophoresis followed by fluorography, as described in the text. Radioactivities applied to the gels were 1.0 × 10⁵ cpm for each column. Films were exposed for 4 days. (1) zero time (30°C); (2) 5 min; (3) 10 min; (4) 20 min; (5) 30 min; and (6) 60 min after the shift. (A) Shift from 30 to 42°C; (B) shift from 30 to 37°C. The positions of molecular weight markers (94K, 68K, and 60K; see reference 9), as well as of the protein chains affected by the temperature shift (87K, 76K, 73K, 64K, and 61K), are indicated.*

for recovery during antiserum treatment and gel electrophoresis has been made. It was possible to obtain a more quantitative estimate from our previous experiments in which purified ^{35}S-labeled ATPase was used as an internal reference (see 13); an approximately 10-fold stimulation of the ATPase synthesis was observed at 10 min after temperature shift-up (13). Although the gel

TABLE 1. *Change in proportion of each protein relative to total protein synthesized upon temperature shift-up*[a]

Protein class	Change in proportion of protein (%) at time (min) after shift					
	0	5	10	20	30	60
87K	1.3	4.1	1.9	1.6	1.7	1.0
76K	0.76	4.0	1.9	1.8	1.5	1.4
73K	0.76	4.0	1.9	2.1	1.7	1.1
64K	0.47	4.5	2.5	1.8	1.7	1.4
61K	0.47	4.5	2.5	1.7	1.4	1.1
87K + 76K + 73K + 64K + 61K	3.76	21.1	10.7	9.0	8.0	6.0

[a] Proportion of each protein class among total protein synthesized at various times after temperature shift (30 to 42°C) was estimated from densitometer tracings (Joyce-Loebl densitometer MKIII) of the fluorograms presented in Fig. 3. Each peak cut out from a tracing paper was weighed, and the weight was normalized to that of total protein. Since the bands of these proteins labeled at 30°C were rather faint and could not easily be distinguished from other adjacent bands, the values at zero time may have been overestimated.

electrophoresis system used in the previous studies gave a single protein band of this ATPase (7, 8, 13), Laemmli's system, used here, usually resolved two protein species with a purified ATPase preparation (data not shown) and with the antibody precipitates (Fig. 7). Thus, we conclude that among the five protein chains stimulated transiently by the temperature shift-up, two (64K and 61K) represent subunits of the ATPase.

DISCUSSION

The present study revealed some new features of regulation of protein synthesis that may be specifically associated with temperature shift-up in *E. coli*. When a culture was transferred from 30 to 42°C, the rate of bulk protein synthesis appeared to increase to the steady-state level characteristic of the latter temperature within 20 to 30 min, exhibiting apparently complex kinetics. The differential synthesis rates of at least five species of protein chains were found to increase 5- to 10-fold within 5 min and then decrease, whereas the rates of synthesis of other proteins increased gradually only by about 50%. The molecular weights of these protein chains have been estimated to be 87K, 76K, 73K, 64K, and 61K on the basis of their mobilities in SDS-gel electrophoresis. Cooper and Ruettinger (4) previously reported that a protein with a molecular weight of 58K is specifically produced at high temperature (42°C); they have detected this protein at 15 min after temperature shift-up but did not follow the time course of its synthesis. This protein may correspond to one of the proteins whose synthesis is transiently stimulated. Although West and Emmerson (18) observed similar temperature-induced changes in protein patterns using some mutants of *E. coli* affected in cell division, comparable results for a wild-type strain were not presented.

Each of the five species of protein chains seems to exist as soluble protein, although small portions of 64K/61K polypeptides (ATPase) may be associated with the membrane as well. The latter observation, however, might simply

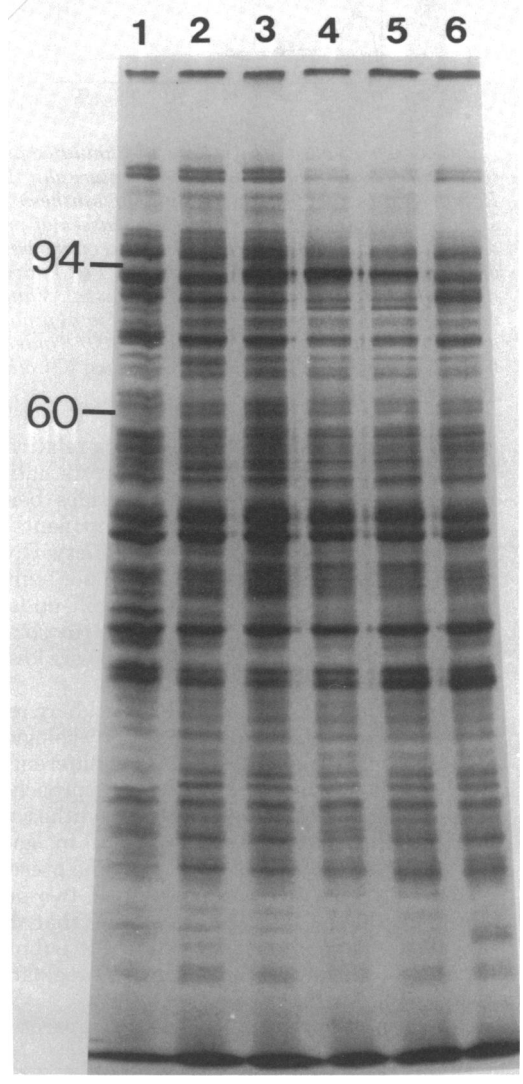

FIG. 4. *Effect of temperature shift-down on the composition of newly synthesized proteins. Experimental procedures are the same as in Fig. 3, except that the temperature shift was from 42 to 30°C. (1) zero time (42°C); (2) 5 min; (3) 10 min; (4) 20 min; (5) 30 min; and (6) 60 min after the shift.*

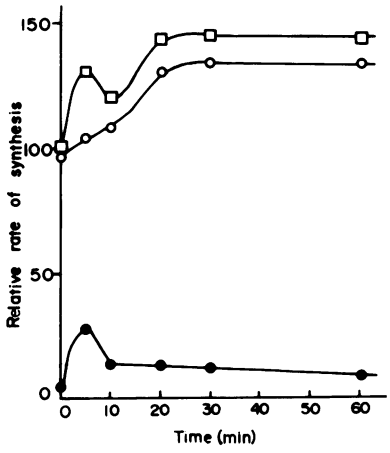

FIG. 5. *Time course of synthesis of stimulated proteins and of other proteins upon temperature shift-up. Relative rates (per cell mass unit) of synthesis of stimulated proteins (sum of the five classes of proteins) and of total protein were estimated on the basis of the values presented in Table 1 and Fig. 2 (averages from the three experiments were used). Values are presented as rates relative to that of total protein synthesis at 30°C (zero time), set as 100. Symbols: (□) total protein; (●) stimulated proteins; (○) other proteins.*

reflect the fact that the ATPase has a relatively high molecular weight (9.3×10^5) in its native form (7). The proteins or polypeptides being affected are not likely to be the components of transcriptional or translational machinery; RNA polymerase subunits α, β, and β' are not stimulated markedly by the temperature shift-up (see 13). The molecular weights of all the ribosomal proteins except S1 (65,000 in SDS-gels) are lower than 60K (19).

The 64K and 61K proteins detected here represent subunits of ATPase whose physiological role is not yet known (7, 8). The differential synthesis rate of this enzyme protein approached about 10% of the total protein synthesized shortly after temperature shift-up, in good agreement with previous data (13). The present system of gel electrophoresis resolved two protein bands of the ATPase, suggesting that this enzyme is composed of two different subunit species whose synthesis is coordinately regulated upon temperature shift-up.

It was reported recently that temperature

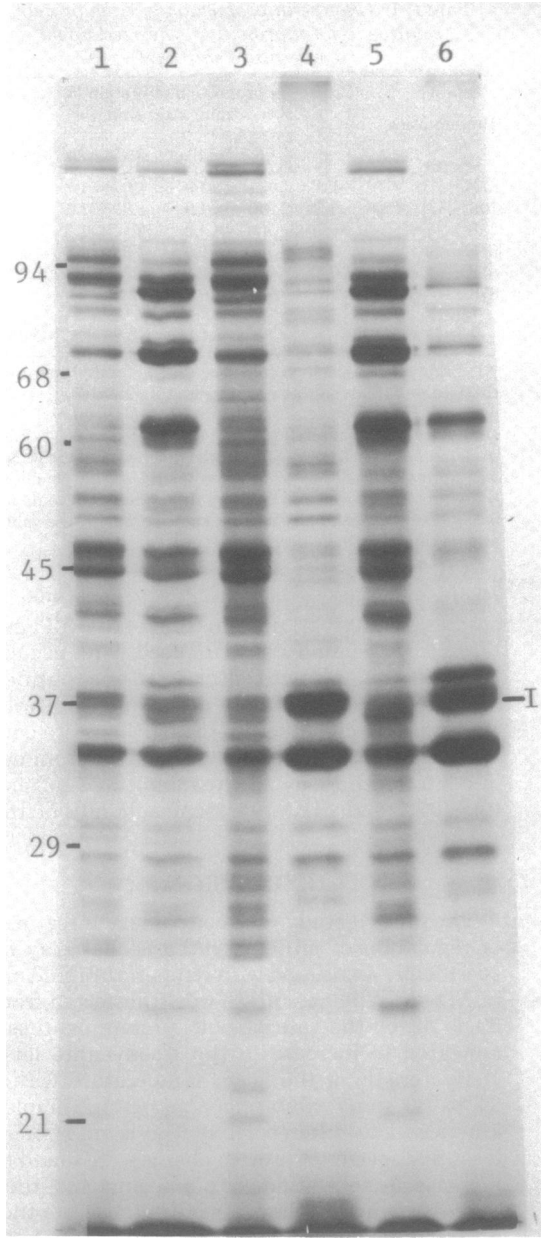

FIG. 6. *Localization of the stimulated proteins. Cells were grown in 40 ml of medium E-glucose at 30°C, and a log-phase culture was divided into two parts; one part was pulse-labeled at 30°C (columns 1, 3, and 4) with 50 μCi of [^{3}H]leucine for 3 min and chased with unlabeled leucine (200 μg/ml) and isoleucine (50 μg/ml) for 2 min, whereas the other was shifted to 42°C and pulse-labeled after 5 min of incubation at 42°C (columns 2, 5, and 6). The soluble and membrane fractions were prepared as described in the text, and proteins were solubilized with SDS and applied for SDS-gel electrophoresis. Fluorograms were taken after 3 days of exposure. Radioactivities of 10^5 cpm were applied to each column. (1 and 2) Total protein; (3 and 5) soluble fraction; (4 and 6) membrane fraction. In the present gel system, the major outer membrane protein I can be seen at the position of molecular weight = 37K.*

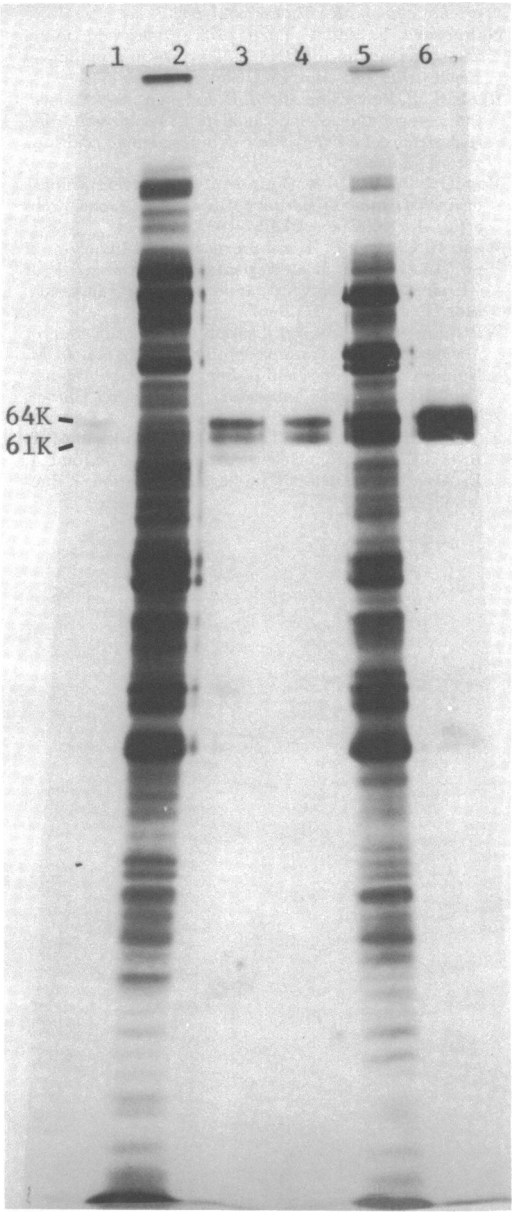

FIG. 7. *Precipitation of 64K and 61K polypeptides by specific antiserum against ATPase. A 5-ml culture of W3350 was pulse-labeled at 30°C (1, 2, and 3) or 5 min after shift to 42°C (4, 5, and 6) with 50 μCi of [^{3}H] leucine and chased with unlabeled leucine-isoleucine as described in the text. Crude extracts were prepared, and a portion (2 × 10^6 cpm) was treated with antiserum against ATPase; the resulting precipitates were dissolved in 50 μl of SDS sample buffer and analyzed by SDS-gel electrophoresis. Fluorograms were taken after 4 days of exposure. (2 and 5) Crude extract (10^5 cpm each); (1, 3, 4, and 6) antigen-antibody precipitates (1, 1,055 cpm in 5 μl; 3, 4,220 cpm in 20 μl; 4, 2,687 cpm in 5 μl; 6, 10,750 cpm in 20 μl).*

shift-up of *E. coli* cells leads to transient accumulation of guanosine tetraphosphate, a compound implicated as a key substance for the stringent control system (2, 5). Thus, guanosine tetraphosphate may have a role in the temperature-induced regulation of protein synthesis. However, starvation for amino acids, during which this nucleotide accumulates, appears to induce synthesis of proteins apparently different from those reported here (3, 16).

The sum of the five (or more, if any of the protein bands is a mixture of more than one polypeptide) species of protein chains that are subject to transient stimulation amounts to as much as about 20% of the total protein synthesized, and the stimulation of these protein chains seems to be well coordinated. Such a remarkable response of bacterial cells to the temperature shift-up may be significant in considering bacterial adaptation to variation in growth temperature. The present finding that a large proportion of such protein is associated with an intriguing enzyme having ATPase activity may provide an important clue for further analysis of such adaptation mechanisms. The present observation at least suggests the existence of a novel regulatory mechanism for gene expression in *E. coli*, namely, transient stimulation of specific protein synthesis in response to a sudden change in growth temperature.

Finally, the results presented in this paper should be borne in mind in future studies of bacterial mutants, particularly in analysis of protein synthesis with temperature-sensitive mutants.

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LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium labelled proteins and nucleic acid in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Chaloner-Larsson, G., and H. Yamazaki.** 1977. Adjustment of RNA content during temperature upshift in *Escherichia coli*. Biochem. Biophys. Res. Commun. **77**:503–508.
3. **Chao, L.** 1977. Gene expression in stringent and relaxed strains of *Escherichia coli* during amino acid deprivation. Biochem. Biophys. Res. Commun. **77**:42–49.
4. **Cooper, S., and T. Ruettinger.** 1975. Temperature dependent alteration in bacterial protein composition. Biochem. Biophys. Res. Commun. **62**:584–586.
5. **Gallant, J., L. Palmer, and C. C. Pao.** 1977. Anomalous synthesis of ppGpp in growing cells. Cell **11**:181–185.
6. **Gudas, L. J., and A. B. Pardee.** 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459–478.
7. **Ishihama, A., T. Ikeuchi, A. Matsumoto, and S. Yamamoto.** 1976. A novel adenosine triphosphatase isolated from RNA polymerase preparation of *Escherichia coli*. II. Enzymatic properties and molecular structure.

J. Biochem. **79**:927-936.

8. **Ishihama, A., T. Ikeuchi, and T. Yura.** 1976. A novel adenosine triphosphatase isolated from RNA polymerase preparation of *Escherichia coli*. I. Copurification and separation. J. Biochem. **79**:917-925.
9. **Ito, K., T. Sato, and T. Yura.** 1977. Synthesis and assembly of the membrane proteins in *E. coli*. Cell **11**:551-559.
10. **Iwakura, Y., K. Ito, and A. Ishihama.** 1974. Biosynthesis of RNA polymerase in *Escherichia coli*. I. Control of RNA polymerase content at various growth rates. Mol. Gen. Genet. **133**:1-23.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) **227**:680-685.
12. **Maaløe, O., and N. O. Kjeldgaard.** 1966. Control of macromolecular synthesis. W. A. Benjamin, Inc., New York.
13. **Nakamura, Y., T. Ikeuchi, M. Imai, and T. Yura.** 1977. Escape synthesis of RNA polymerase subunits and termination factor rho following induction of prophage lambda in *Escherichia coli*. Mol. Gen. Genet. **150**:317-324.
14. **Nakamura, Y., and T. Yura.** 1975. Evidence for a positive regulation of RNA polymerase synthesis in *Escherichia coli*. J. Mol. Biol. **97**:621-642.
15. **Nakamura, Y., and T. Yura.** 1975. Hyperproduction of the sigma subunit of RNA polymerase in a mutant of *Escherichia coli*. Mol. Gen. Genet. **141**:97-111.
16. **Reeh, S., S. Pedersen, and J. D. Friesen.** 1976. Biosynthetic regulation of individual proteins in *relA*$^+$ and *relA* strains of *Escherichia coli* during amino acid starvation. Mol. Gen. Genet. **149**:279-289.
17. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*; partial purification and some properties. J. Biol. Chem. **218**:97-106.
18. **West, S. C., and D. T. Emmerson.** 1977. Induction of protein synthesis in *Escherichia coli* following UV or γ-irradiation, Mitomycin C treatment or *tif* expression. Mol. Gen. Genet. **151**:57-67.
19. **Wittman, H. G.** 1974. Purification and identification of *Escherichia coli* ribosomal proteins, p. 93-114. *In* M. Nomura, A. Tissieres, and R. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Yamamori, T., K. Ito., T. Yura, T. Suzuki, and T. Iino.** 1977. A ribonucleic acid polymerase mutant of *Escherichia coli* defective in flagella formation. J. Bacteriol. **132**:254-261.