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Effect of Bacteriophage Lambda Infection on Synthesis of *groE* Protein and Other *Escherichia coli* Proteins

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We used two-dimensional gel electrophoresis to quantitate the changes in rates of synthesis that follow phage λ infection for 21 *Escherichia coli* proteins, including *groE* and *dnaK* proteins. Although total protein synthesis and the rates of synthesis of most individual *E. coli* proteins decreased after infection, some proteins, including *groE* protein, *dnaK* protein, and stringent starvation protein, showed increases to rates substantially above their preinfection rates. Infection by λ Q^- affected host synthesis in the same way as infection by λ^+, whereas infection by λ N^- showed no detectable effect on host synthesis. Deletion of the early genes between *att* and *N* abolished the effect, and shorter deletions in this region gave intermediate effects. By this sort of deletion mapping, we show that a large part, though not all, of the effect of λ infection on host protein synthesis can be ascribed to the early region that contains phage genes *Ea10* and *ral*. We compared the changes in protein synthesis after infection with the changes that occur in uninfected cells upon heat shock or amino acid starvation. The spectrum of changes that occurred on infection was very different from that seen after heat shock but quite similar to that seen during amino acid starvation. Despite this similarity of the effects of λ infection and starvation, we did not detect any increase in the level of guanosine tetraphosphate during infection. We show that the *groE* protein is the same protein as B56.5 of Lemaux et al. (Cell **13**:427–434, 1978) and A protein of Subramanian et al. (Eur. J. Biochem. **67**:591–601, 1976).

Infection of *Escherichia coli* by one of its bacteriophages usually causes major changes in the synthesis of host-specific macromolecules. For a virulent phage, such as T4, host-specific synthesis is shut off rapidly and virtually completely through a variety of different mechanisms, including degradation of *E. coli* DNA (1). For the temperate phage λ, which we deal with here, shutoff is not nearly so extensive, although it is clear that *E. coli* DNA, RNA, and protein synthesis are all altered by λ infection (5).

Our interest in this problem arose from our studies of *groE* protein (*groEL* protein by the nomenclature of Tilly et al. [37]), an *E. coli* protein that is required for correct assembly of the virions of several coliphages, including λ, T4, T5, and others (6, 12, 13, 30, 31, 35–37, 44). We report here studies on the changes in the rate of synthesis of *groE* protein that occurred after λ infection. We also examined synthesis rates for 20 other *E. coli* proteins after infection. Our results show that regulation of *E. coli* protein synthesis after λ infection is more complex than has generally been supposed, and they suggest that the phage may be activating pre-existing cellular control circuits.

MATERIALS AND METHODS

Media and buffers. RG medium, described previously (12), was supplemented with 0.4% glucose or maltose. M9 medium was as described by Kiger et al. (20). LLA medium, for labeling with [^{3}H]leucine, was RG glu medium supplemented with 0.02 mg of cysteine and methionine per ml but lacking leucine, isoleucine, and valine. LPM, for phosphate labeling, contained 10 mM Tris-hydrochloride (pH 7.5), 1.0 mg of NH_4Cl per ml, 0.5 mg each of NaCl and KCl per ml, 3 μM $FeCl_3$, 0.14 mM Na_2HPO_4, and 1 mM $MgSO_4$. For infection experiments this was supplemented with the amino acids in RG medium plus 0.02 mg of cysteine and methionine per ml and 0.4% maltose. For amino acid starvation, LPM was supplemented with 0.4% glucose.

λ dilution buffer contained 6 mM Tris-hydrochloride (pH 7.5), 1 mM $MgCl_2$, 70 mM NaCl, and 0.005% gelatin. Cell lysis buffer was 10 mM Tris-hydrochloride (pH 8.0), 5 mM $MgCl_2$, 0.1 mM EDTA, 50 mM NaCl, 0.1 mM dithiothreitol, and 0.6 mg of egg white lysozyme per ml (Sigma Chemical Co., St. Louis, Mo.). RIPA buffer was 10 mM Tris-hydrochloride (pH 7.5), 0.15 M NaCl, 1.0% (wt/vol) sodium deoxycholate, 1.0% Triton X-100, and 0.1% sodium dodecyl sulfate (SDS). Bovine serum albumin-ovalbumin solution was 20 mg each of bovine serum albumin and ovalbumin per ml in RIPA buffer.

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Bacterial and phage strains. All experiments described used the *sup*^{0} *E. coli* K-12 strain 594. λ *c*I857 *S*am7 was used as wild type. Other phage strains from our collection were λ *N*am7 *N*am53 *c*I857 *S*am7, λ *Q*am73 *Q*am501 *c*I857 *S*am7, λ *bio*10 *c*I857, λ *gt*-Ec·groE; from the collection of W. Szybalski were λ *bio*7-20 *c*I857, λ *bio*11 *c*I857, λ *bio*252 *c*I857.

All phage stocks used in radioactive labeling experiments were purified by banding in a CsCl density gradient and dialyzed extensively against λ dilution buffer. Failure to carry out this step in earlier experiments led to poor reproducibility between different stocks of the same phage.

Preparation of radioactive bacterial proteins. (i) Phage infection. *E. coli* 594 was grown in RG mal at 37°C to 2×10^8 cells per ml, pelleted, and suspended in fresh RG mal at the original volume. The culture was grown at 37°C for 15 to 20 min and split into aliquots. Half of these were simultaneously infected, and to each of the others a volume of sterile λ dilution buffer, equal to the volume of the phage suspension, was added. Within 20 s after infection 0.3 mCi of either $H_2{}^{35}SO_4$ or [^{35}S]methionine per ml (New England Nuclear Corp., Boston, Mass.) was added to each of two aliquots of culture, one infected and one uninfected. After 10 min, incorporation was stopped by the addition of 12.5 mM NaN_3 and 12.5 mg each of unlabeled cysteine and methionine per ml. This labeling procedure was repeated on different aliquots for subsequent 10-min periods. Cells were then pelleted, suspended in cell lysis buffer at 1/20 the original volume, and lysed by freeze and thawing 5 to 10 cycles in either liquid nitrogen or a dry ice-acetone bath. DNase and RNase were added to 0.1 mg/ml, and the lysate was incubated at room temperature for 10 to 15 min. Cell debris was again removed by low-speed centrifugation, and samples were prepared for electrophoresis as described below.

(ii) Temperature shift. *E. coli* 594 was grown in RG glu at 30°C to 2×10^7 cells per ml, pelleted, suspended in fresh medium to the original density, and grown at 30°C for an additional 15 min. The culture was then divided into 10 aliquots. Five were maintained at 30°C, and five were pipetted into prewarmed tubes and incubated at 42°C. Immediately after temperature shift, 0.3 mCi of $H_2{}^{35}SO_4$ was added to a 30 and a 42°C aliquot. After 10 min, incorporation was stopped, and cells were harvested, lysed, and prepared for electrophoresis as described for infected cells. Aliquots for subsequent time periods were treated in the same way.

(iii) Amino acid starvation. *E. coli* 594 was grown in M9 glu to 2.5×10^8 cells per ml, pelleted, suspended in fresh medium, grown an additional 15 min at 37°C, and separated into 10 aliquots. The amino acid analog, β-2-thienyl-D-L-alanine, was added at 5 μg/ml to half of the samples to cause false feedback inhibition of aromatic amino acid biosynthesis (9). Immediately 0.035 mCi of [^{35}S]methionine per ml (Amersham Corp., Arlington Heights, Ill.) was added to one of the thienylalanine-treated aliquots and to one of the untreated aliquots. After 10 min, incorporation was stopped, as described for cultures labeled during phage infection. At the same time two more aliquots were labeled for the next 10 min, then similarly stopped. This continued to 50 min after starvation had begun. Cells were harvested, lysed, and prepared for electrophoresis as described for infected cultures.

(iv) [^{3}H]leucine labeling. *E. coli* 594 (λ *c*I857*S*am7) was grown at 30°C in LLA glu to 1.0×10^8 cells per ml. [^{3}H]leucine (0.10 mCi) (New England Nuclear; 5.0 mCi/ml) was then added to 5.0 ml of culture, and growth was continued for two generations at 30°C. At this point the culture was induced by shifting to 42°C for 10 min and grown for an additional 20 min at 37°C. This permitted better cell lysis and recovery of ^{3}H-labeled proteins. Cells were pelleted, suspended in 0.5 ml of cell lysis buffer, and subjected to freeze and thaw in liquid nitrogen for three to five cycles. DNase and RNase were added to 0.1 mg/ml, and the lysate was incubated at room temperature for 15 min. Debris was removed by centrifugation, and portions of the supernatant were added to samples being prepared for electrophoresis as an internal recovery standard.

One-dimensional gel electrophoresis. Proteins were separated in a slab gel apparatus (32) by a discontinuous SDS buffer system (21). Samples were solubilized by heating in sample buffer at 100°C for 1 min. After electrophoresis, gels were dried onto filter paper under vacuum (23) and placed in contact with Kodak SB-5 X-ray film for autoradiography.

Two-dimensional gel electrophoresis. (i) Sample preparation. Labeled and unlabeled cell lysates containing primarily cytoplasmic proteins were prepared as described above. The specific activity of each extract was determined by measuring trichloroacetic acid-precipitable radioactivity and total protein concentration (2). Generally, a ^{35}S-labeled sample containing 50,000 to 500,000 cpm or an unlabeled sample with not more than 40 μg of total protein was found to provide optimal results. The appropriate sample volume was lyophilized, suspended in 50 μl of buffer A (25), and loaded with a micropipette on a prerun focusing tube gel.

For immunoprecipitates, the pellet was suspended in buffer A, supplemented with additional Nonidet P-40 to a final concentration of 4.0%. This mixture was heated at 37°C for 10 min, blended in a Vortex mixer, and centrifuged to separate *Staphylococcus aureus* cells from released *groE* protein. The supernatant was loaded directly on a prerun focusing tube gel.

(ii) General technique. Two-dimensional gels were run essentially as described by O'Farrell (26) with minor modifications.

(iii) Quantitation of proteins. Dried gels were aligned with the corresponding autoradiograms, using radioactive ink markers for positioning. Protein spot positions were marked directly on the gels and cut out with a razor blade. Most of the filter paper was removed from the back of each cut-out spot, and the gel pieces were soaked individually in 0.5 ml of 0.2% (wt/vol) SDS for 10 to 15 h at 42°C. Beckman Ready Solv EP scintillation fluid (3 ml) was added to each sample, which was then blended in a Vortex mixer to a clear suspension. The gel fragments were removed, and the samples were counted after standing at least 15 min.

The rate of synthesis of a given protein was expressed as the ratio R_N. This is the ratio of ^{35}S to ^{3}H for that spot from the gel of the infected (heat-shocked, starved) culture, normalized to the same ratio for the uninfected culture. Thus for protein *i*: $R_N = (^{35}S_i/^3H_i)$ infected/$(^{35}S_i/^3H_i)$ uninfected.

Immunological methods. (i) Preparation of antiserum. Anti-*groE* serum was prepared from female white mice, weighing approximately 25 g. Three intra-

peritoneal injections were given, each containing 10 μg of purified *groE* protein. The first was mixed with Freund complete adjuvant to a final volume of 0.2 ml. The second, administered 1 week later, was mixed with incomplete adjuvant to a volume of 0.1 ml. The third was identical to the second and given 2 weeks afterward. At 1 week after the final injection, the mice were bled from behind the eye, and serum was clarified of erythrocytes by centrifugation.

Antisera directed against A protein, P66, and S1 were the generous gift of H. Liebke and were prepared by A. Whaba.

(ii) Immunoprecipitation. Antibody precipitation of various proteins was as described by S. Jonsson and G. Kronvall (18) as modified by S. Kessler (19) and R. Burgess (personal communication). Antigen-antibody complexes were usually formed by incubating 15 μl of antiserum with up to 100 μl of antigen at 4°C for 2 h. After incubation the antigen-antibody complexes were adsorbed to formaldehyde-fixed *S. aureus* cells (Pansorbin; Calbiochem-Behring Corp., La Jolla, Calif.) for 30 min at 4°C. The best results were obtained when the Pansorbin cells were pelleted and suspended in RIPA buffer, then preadsorbed just before use with 50 μl of bovine serum albumin-ovalbumin solution per ml of cell suspension. After adsorption of the antigen-antibody complexes to the *S. aureus*, cells were pelleted and washed three times in RIPA buffer and prepared for electrophoresis as described above.

Measurement of ppGpp pool size. *E. coli* 594 was grown in LPM mal overnight at 37°C. The cells were subcultured and grown to 1.5×10^8 cells per ml. These cells were pelleted, suspended in fresh medium, and grown for an additional 10 min. $H_3{}^{32}PO_4$ (New England Nuclear) was added to 100 μCi/ml, and growth continued to 3×10^8 cells per ml. The culture was then split into aliquots, and 50-μl samples were withdrawn from each into 50 μl of 2 N formate on ice. After 10 min, one of the aliquots was infected or amino acid starved under the same conditions described above. At the appropriate times, 50-μl samples were again withdrawn from each aliquot into formate. After 1 h on ice, all samples were centrifuged at 5,000 × *g* for 5 min, and 1 μl of the supernatant was spotted onto thin-layer chromatographic plates (PEI-cellulose; Schleicher & Schuell Co., Keene, N.H.). These were chromatographed with 1.5 M KH_2PO_4 (pH 3.4), dried, and autoradiographed. The guanosine tetraphosphate (ppGpp) spots were cut out and counted, and the values for nanomoles of ppGpp at an optical density of 240 nm were then determined.

A two-dimensional separation procedure (10) was used for verification of MS1 spot authenticity. For this nucleotides were separated in the first dimension in 2 M formate plus 1.5 M LiCl, rinsed for 10 min in methanol, dried, and then chromatographed in 1.5 M KH_2PO_4 (pH 3.4), as before.

RESULTS

Location of gp *groE* on two-dimensional gels. We wished to use two-dimensional polyacrylamide gels (25) to follow the synthesis of *groE* protein after infection of *E. coli* by phage λ. To identify the *groE* spot in the two-dimensional pattern, we made a radioactively labeled extract of *E. coli* and precipitated the *groE* protein from it, using anti-*groE* antibodies and formaldehyde-fixed *S. aureus* cells (19). Figure 1a shows the autoradiogram of a two-dimensional gel of the immune precipitate, and Fig. 1b shows the autoradiogram of the gel of the original extract. This identifies a prominent spot in the gel pattern of the whole extract as *groE* protein. Its position indicates an isoelectric point of about 4.4. The intensity of the spot is consistent with the known abundance of *groE* protein in *E. coli*. (Note that in many of our gels, *groE* protein runs as a major spot with a satellite below it. This is apparently an artifact due to insufficient reducing agent in the sample [41].)

As an alternative method of identifying the *groE* spot, we mixed radioactive *groE* protein, purified as described by Hendrix (16), with a nonradioactive *E. coli* extract and ran a two-

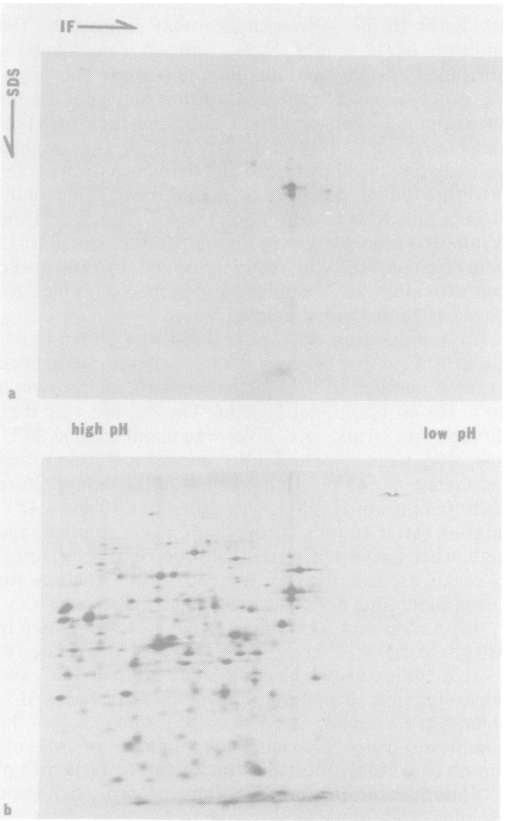

FIG. 1. Identification of *groE* protein on two-dimensional gels. A radioactive extract of uninfected *E. coli* 594 was treated with anti-*groE* antibodies and *S. aureus* cells, and the immunoprecipitate was run on a two-dimensional gel. Autoradiograph of the gel of the immunoprecipitate. (b) Autoradiograph of a gel of the untreated extract. IF, Isoelectric focusing dimension.

dimensional gel. Superposition of the stained gel and its autoradiogram identified the same spot as did the experiment shown in Fig. 1 (data not shown).

Identity of *groE*, B56.5, A protein, and P66. The identification of *groE* protein with a specific spot on the two-dimensional gel pattern allowed us to show its identity to *E. coli* proteins that have been studied in other contexts. The gel position of *groE* protein and its relative abundance in the cell argue that it is the same spot as protein B56.5 of Lemaux et al. (22). These authors have shown that B56.5 is identical to "A protein" (also known as S1A [38]), an acidic protein that copurifies with ribosomes under the conditions described by Subramanian et al. (33). A comparison of the published amino acid compositions of *groE* protein (16) and A protein (33) shows them to be very similar and probably identical.

In Fig. 2 we show the immunoprecipitation of proteins from a radioactive extract made after the infection of *E. coli* with the *groE* transducing phage λ *gt-Ec·groE* (17). The antibodies used were directed against *groE* protein, A protein, ribosomal protein S1 (with which *groE* protein has sometimes been confused), and P66. P66 is an ATPase that commonly copurifies with RNA polymerase, and we have argued elsewhere (16) that *groE* protein and P66 are the same protein. The gel shows that anti-*groE* protein, anti-A protein, and anti-P66 all specifically precipitated *groE* protein, whereas anti-S1 precipitated a band in the position expected for protein S1. All of the other bands in the precipitates were also present in the precipitate made without antiserum ("blank").

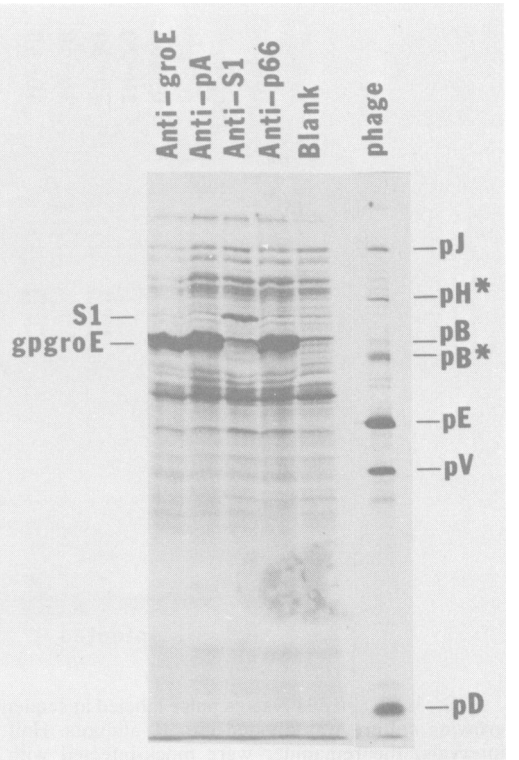

FIG. 2. SDS-polyacrylamide gels of immunoprecipitates of radioactive cell extract, using the indicated antibodies. The extract was from cells infected with λ *gt*-Ec·*groE*. Radioactive λ virions were included for size markers. For the lane marked "blank," buffer was added in place of antiserum to measure nonspecific binding to the *S. aureus* cells used to precipitate the antibodies.

We have also compared our *groE* protein with authentic A protein, P66, and S1, by the methods of two-dimensional gel electrophoresis and peptide mapping (4) (data not shown). We again found here that *groE* protein, A protein, and P66 are identical by these criteria and different from S1.

Neidhardt et al. (24) have also recently compared B56.5, A protein, and *groE* protein by a number of criteria. Their results and ours agree in showing the identity of these proteins.

Regulation of host protein synthesis after λ infection. To investigate the effect of λ infection on synthesis of *groE* protein and other host proteins, we infected *E. coli* cells with λ *c*I857 *S*am7 and labeled the proteins in different aliquots for successive 10-min intervals. SDS-polyacrylamide gels of the labeled proteins are shown in Fig. 3, with parallel results from mock-infected control cells. It is evident that the total incorporation into cellular proteins was reduced in the infected cells. Some phage-coded proteins can be seen in the infected cells, most notably gp*E*, which was synthesized starting in the 10- to 20-min interval. A band in the *groE* protein position shows interesting changes in its rate of synthesis, with a maximum rate in the 10- to 20-min labeling interval. Similar kinetics can be seen for a slightly less prominent band in the higher-molecular-weight position expected for the *dnaK* protein. (The *dnaK* protein is protein B66.0 of Pedersen et al. [27]. This identification has been made by Georgopoulos et al. [14].)

The lysates used in Fig. 3 were treated with anti-*groE* antibodies and *S. aureus* cells, and the immune precipitates were run on a gel (data not shown). As expected, the immunoprecipitated *groE* band from the infected cells varied in intensity in the same fashion as the band tentatively identified as *groE* in Fig. 3. The immunoprecipitated *groE* band from the uninfected cells remained constant.

To obtain accurate quantitation of the rate of *groE* protein synthesis after λ infection, we prepared pulse-labeled extracts of infected cells

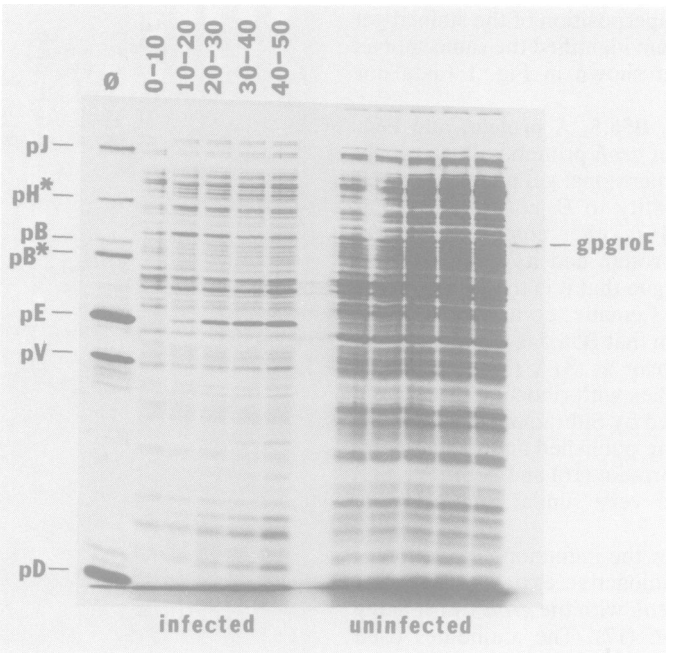

FIG. 3. *E. coli* 594 lysates pulse labeled at sequential intervals after λ *c*I857 *S*am7 infection. An exponentially growing culture was divided into 10 aliquots. Half were infected and pulse-labeled for the indicated 10 min intervals; the remainder were mock-infected with λ dilution buffer and similarly labeled. Aliquots of the supernatant of each lysate were subjected to electrophoresis on a 12.5% SDS-polyacrylamide slab gel. Purified phage and *groE* protein were included as markers.

such as those shown in Fig. 3 and separated their components on two-dimensional gels. Before running the gels, these ^{35}S-labeled extracts were each mixed with a measured aliquot of a [^{3}H]leucine-labeled extract of uninfected cells. The autoradiogram of these gels for uninfected cells and for cells labeled in the first three 10-min intervals after infection is shown in Fig. 4, and the *groE* protein spot in each is indicated with an arrow. After electrophoresis, the *groE* protein spot, as well as 20 other spots, was cut out of each gel and eluted, and the $^{3}H/^{35}S$ ratios were determined. In this method, which is essentially that described by Reeh et al. (28), the tritiated proteins act as internal recovery standards for each ^{35}S-labeled protein. We express the rates of synthesis of the various proteins by the ratio R_N ("relative α_R" of reference 28), which is the rate of synthesis of a given protein during the time interval in question, normalized to its rate of synthesis before infection.

Figure 5 shows the rate of *groE* protein synthesis as a function of time after λ infection. During the first 10-min interval after infection, *groE* protein synthesis decreased by about a factor of 2. Then it increased sharply so that the average rate during the 10- to 20-min interval was more than two times the preinfection rate. After this, it gradually decreased over the next 30 min until at 40 to 50 min it was back down to the preinfection rate.

Figure 6 shows the same data for the other 20 proteins that were cut out of the two-dimensional gels and quantitated. All of these proteins showed rather striking responses to infection, and the responses fell into several classes. The *dnaK* protein was the only one of the proteins we monitored that behaved essentially the same as *groE* protein: its rate of synthesis decreased during the first 10 min after infection, increased to about twofold above preinfection levels during the 10- to 20-min interval, and then gradually fell. Proteins D24.0 (stringent starvation protein [SSP]) and, to a lesser extent, A41.0 increased dramatically during the first 10 min and then fell, but remained substantially above the preinfection rate throughout the experiment. Proteins A12.0 and D21.0 showed slight increases followed by moderate declines. All of the other proteins examined decreased during the first 10 min and remained below their preinfection rates thereafter, but even among these there were differences in the details of the patterns.

To interpret these data as rates of synthesis, as we do, requires that the proteins not be metabolically unstable on a time scale compara-

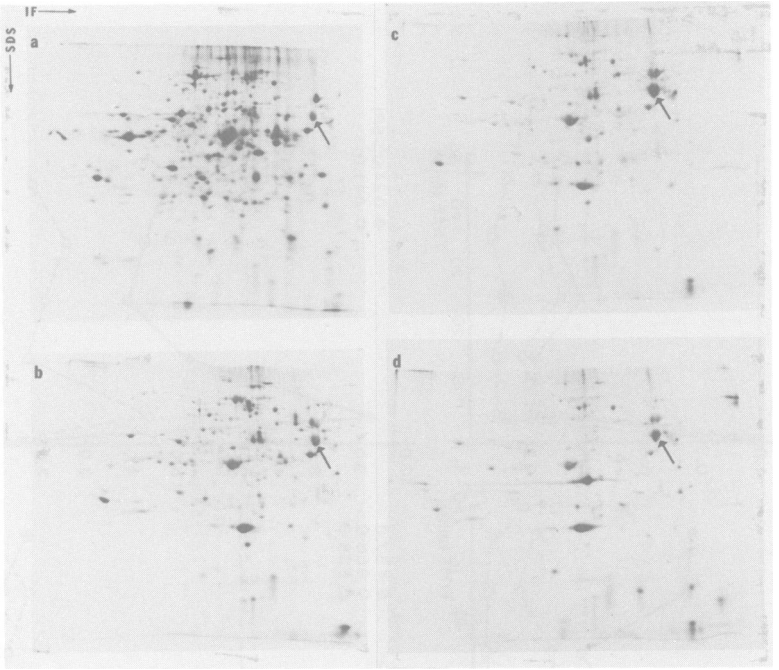

FIG. 4. Autoradiograph of two-dimensional gels of pulse-labeled λ *c*I857 *S*am7-infected *E. coli* 594 extracts. Arrows indicate the *groE* protein spot. (a) Labeled 10 min before infection; (b) labeled 0 to 10 min postinfection; (c) labeled 10 to 20 min postinfection; (d) labeled 20 to 30 min postinfection. IF, Isoelectric focusing dimension.

ble to the 10-min pulse-labeling time, or at least that their half-lives not change significantly during infection. To test this point, we took extracts of cells labeled for 10 min at various times before and after infection (as described above) and compared them by one-dimensional electrophoresis with extracts labeled for 10 min and chased for 20 min (data not shown). We detected no significant differences between the chased and unchased patterns. Thus, for *groE* and *dnaK* proteins, which were resolved on the one-dimensional gels, we can be certain that the data represent rates of synthesis. For the other proteins this is not explicitly proven but is very likely true for at least most of them.

Effect of λ genotype on host protein regulation. Can the regulation of *groE* protein, *dnaK* protein, and the other host proteins be ascribed to a particular phage gene? To investigate this question we repeated the labeling experiments described above with a series of phage mutants. The first mutant examined was λ Q^-. The *Q* gene codes for a positive regulator of the phage late proteins; thus in a λ Q^- infection early proteins are expressed normally, but late proteins are greatly reduced. We found that regulation of *groE* and *dnaK* expression in the λ Q^- infection is indistinguishable from that in a wild-type infection (data not shown). This is not surprising, since the regulation of host proteins is strongly manifested in the first 10 min of

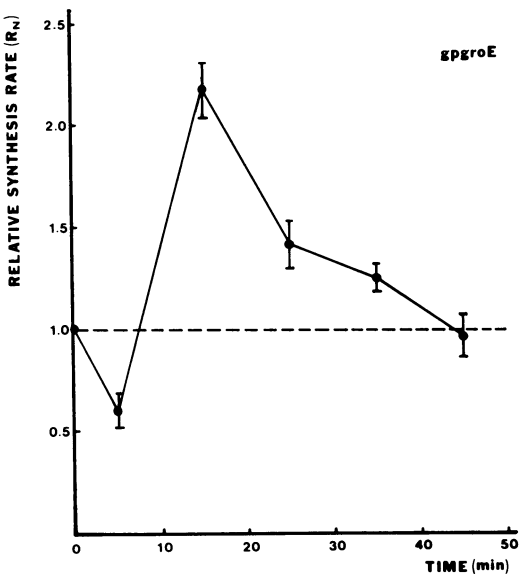

FIG. 5. Normalized rates of synthesis (R_N) for *groE* protein after λ *c*I857 *S*am7 infection. The *groE* protein spots from gels such as those shown in Fig. 5 were cut out, eluted, and counted for ^3H and ^{35}S. The R_N values were calculated from the $^3H/^{35}S$ ratios as described in the text; an R_N value of 1.0 represents the rate of synthesis before infection. The error bars are the standard deviations from three independent experiments.

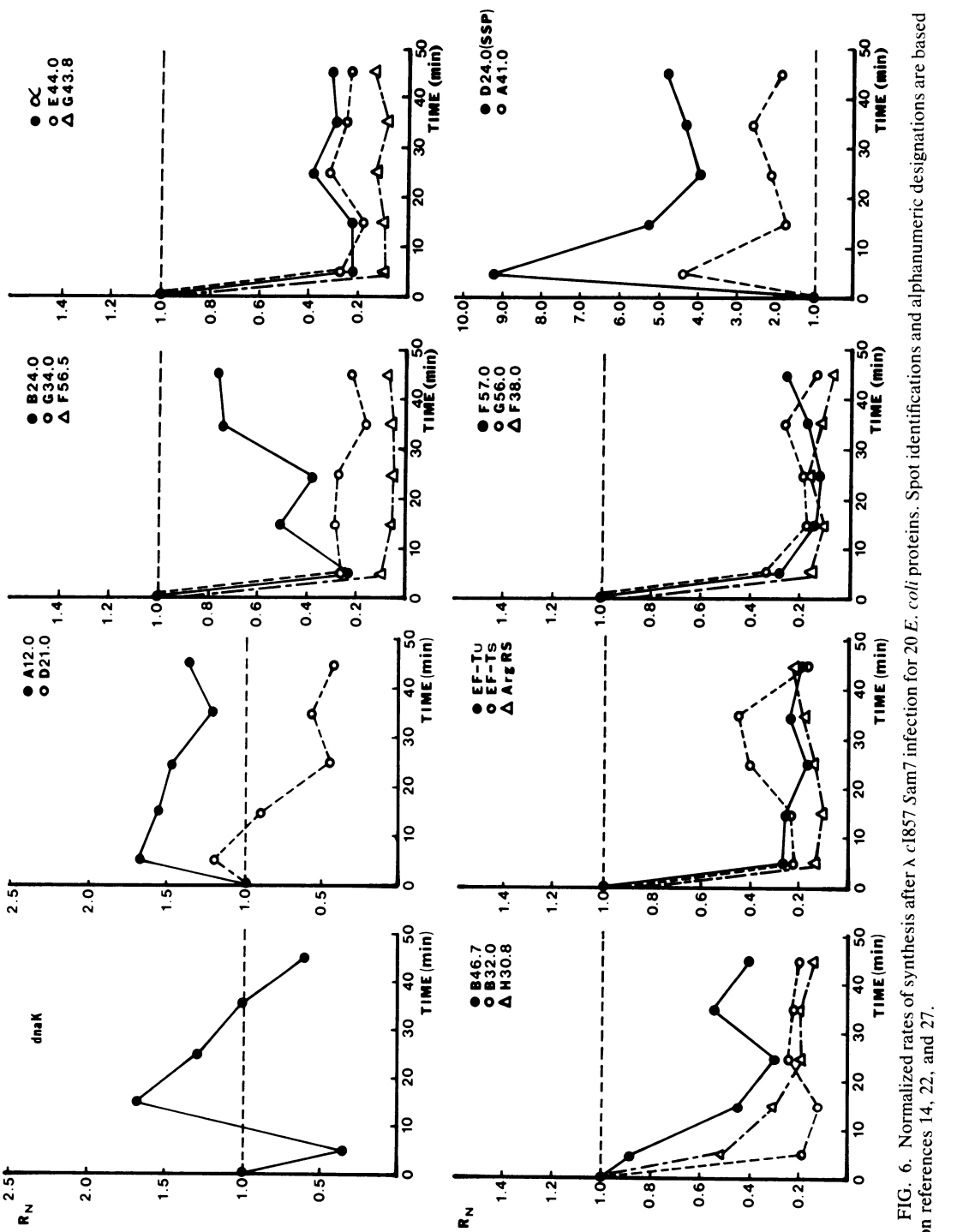

FIG. 6. Normalized rates of synthesis after λ cI857 Sam7 infection for 20 *E. coli* proteins. Spot identifications and alphanumeric designations are based on references 14, 22, and 27.

infection, but the first late proteins to be expressed do not begin to be made until 9 min after infection (29).

Gene *N* codes for a positive regulator of all other early genes except *cro*; thus the only phage proteins made in the λ N^- infection should be the *cro* protein and an amber fragment of gp*N*. When λ N^--infected cells were pulse-labeled and the extracts were fractionated on gels, we could detect no changes in the rates of synthesis of *groE* protein or any other host proteins (data not shown). Furthermore, the protein synthesis in λ N^--infected cells was indistinguishable from that in uninfected cells. Specific examination of *groE* protein synthesis by immunoprecipitation of *groE* protein from the extracts confirmed that there is no regulation of *groE* protein synthesis after λ N^- infection. We conclude that the regulation that we saw in a wild-type or λ Q^- infection requires expression of early λ genes.

In their investigation of the reduction of β-galactosidase synthesis after λ infection, Cohen and Chang (5) found that the turnoff of the enzyme could be eliminated by deletion of the early region between *att* and *N*. Intermediate-sized deletions gave intermediate effects. We repeated these experiments with double-labeled two-dimensional gels as described above. Figure 7 shows the extents of the deletions in the λ p*bio* deletion-substitution mutants we used. Table 1 lists the synthesis rates (R_N) for the different mutants at 0 to 10 and 10 to 20 min after infection for *groE* protein, *dnaK* protein, and three other host proteins. These R_N values for the 10- to 20-min interval are plotted above the map in Fig. 7. In addition, we included in Fig. 7 some of the data of Cohen and Chang (5) for β-galactosidase.

With λ *bio*10, in which the entire region of the λ genome between *att* and *N* is deleted, the effect of infection on synthesis was essentially abolished, both for the proteins that are stimulated by wild-type infection (*groE* and *dnaK* proteins) and for the proteins that are normally depressed. With deletions shorter than *bio*10, the wild-type effect was progressively restored. With the apparent exception of protein G43.8, there was no significant difference in the effects of the wild type and λ *bio*7-20; this argues that the gene(s) responsible for the regulation of these host proteins lies between the endpoints of the *bio*7-20 and *bio*10 deletions (however, see below).

The pattern for *dnaK* protein is the simplest of the six represented. Stimulation of *dnaK* protein was nearly the same for wild type and all deletions up to *bio*252; then it decreased suddenly when the deletion was extended to the length of *bio*10. This argues that all, or nearly all, of the stimulation of *dnaK* protein synthesis by λ^+ is due to a gene in the region between the endpoints of *bio*252 and *bio*10. This region includes two known genes, *Eal0* (15) and *ral* (43), and it is unlikely that there is room in this interval for more than these two genes (34).

The pattern of *groE* protein stimulation was similar to that for *dnaK* protein, but with some indication that additional genes may be involved. Roughly 50% of the difference between *groE* protein synthesis by λ^+ and by λ*bio*10 was accounted for by the *bio*252-*bio*10 region, suggesting that a gene in this region also plays a major role in *groE* protein stimulation. Most of the remaining stimulation of *groE* protein can be assigned to the *bio*7-20-*bio*11 and *bio*11-*bio*252 regions.

The R_N values for *groE* protein and *dnaK* protein synthesis in the 0- to 10-min interval (Table 1) mirror the results in the 10- to 20-min interval plotted in Fig. 7. Depression of synthesis for both proteins was essentially constant for wild type and for all deletions up to *bio*252, and with *bio*10 the depression was abolished. Thus the initial depression of *groE* protein and *dnaK* protein synthesis may well be due to the same phage genes as is their subsequent stimulation.

Cohen and Chang (5) concluded that depression of β-galactosidase synthesis is mediated primarily by genes in the *bio*7-20-*bio*72 and the *bio*11-*bio*10 regions. The three proteins we present data for that are depressed after infection generally followed a pattern similar to Cohen and Chang's β-galactosidase data, although there were some quantitative differences among them. One rather surprising result for these proteins is that the R_N values for the 0- to 10-min interval were significantly different between the λ^+ and λ *bio*7-20 infections. This suggests that the rapidity of the onset of depression may depend on something in the region of the phage genome under the *bio*7-20 deletion.

Effects of temperature shift and amino acid starvation. The effects on the rates of synthesis of various *E. coli* proteins caused by shifts in growth temperature or by starvation for an amino acid have been investigated by Lemaux et al. (22), Yamamori et al. (40), and Reeh et al. (28). These authors report striking changes in synthesis rates for several proteins, including *groE* and *dnaK* proteins. To compare the effects of these perturbations with the effect of λ infection, we repeated some of the temperature shift and amino acid starvation experiments, using our strain of *E. coli* and collecting data for the same proteins that we measured after λ infection. The format of these experiments was the same as for those reported above: cells were labeled with ^{35}S for successive 10-min periods after temperature shift-up or commencement of starvation, the resulting extracts were mixed with samples

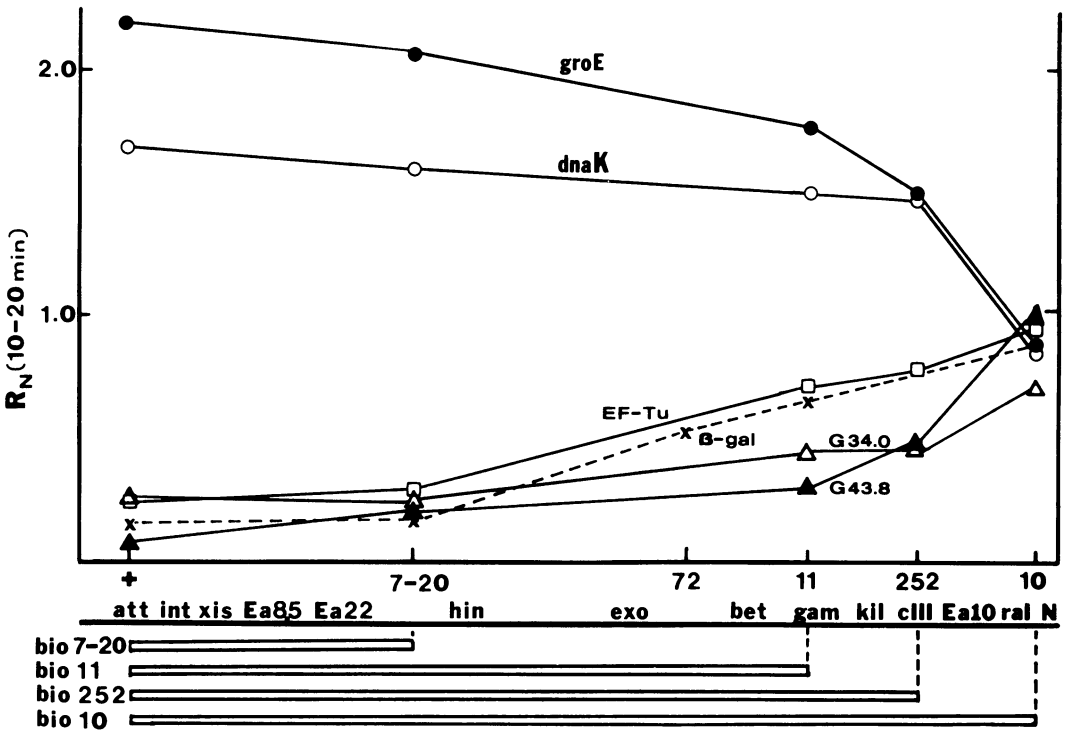

FIG. 7. Effect of deletion of phage genes on the regulation of host protein synthesis. The partial map of the λ genome at the bottom of the figure shows the extents of the deletions in the λ p*bio* phages used. Cells were infected with these phages and pulse-labeled, and the R_N values for five selected proteins were determined. The values for a given phage mutant are plotted above the right endpoint of the deletion carried by that mutant; the values for a wild-type infection are plotted above the left endpoint of the deletions. Only the R_N values for the 10- to 20-min pulse are shown. The values for β-galactosidase are taken from data in reference 5 for 15 min postinfection.

of a ^{3}H-labeled extract, the mixtures were separated on two-dimensional gels, and the $^{35}S/^3H$ ratio was determined for each spot.

Figure 8 shows the rates of synthesis of these proteins after a temperature shift from 30 to 42°C. Some of the proteins, including F56.5, F57.0, G56.0, G43.8, and F38.0, continued to be synthesized at the same rate after shift-up as before or increased slightly in parallel with the increase in total rate of protein synthesis, whereas other proteins showed substantial increases in their rates. *groE* protein synthesis increased and peaked in the 10- to 20-min interval, as it did after infection, but it did not show the initial

TABLE 1. Rates of synthesis after infection by phage deletion mutants[a]

Protein	Rates of synthesis at min after infection by:									
	λ wild type		λ *bio*7-20		λ *bio*11		λ *bio*252		λ *bio*10	
	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20
groE	0.56	2.18	0.59	2.07	0.61	1.74	0.65	1.50	0.98	0.87
dnaK	0.36	1.68	0.40	1.60	0.45	1.51	0.45	1.49	0.99	0.84
EF-Tu	0.27	0.26	0.60	0.29	0.79	0.72	0.86	0.80	0.99	0.95
G43.2	0.06	0.06	0.44	0.18	0.44	0.30	0.51	0.46	1.00	0.98
G34.0	0.23	0.25	0.66	0.24	0.69	0.42	0.89	0.46	0.97	0.71

[a] Exponentially growing *E. coli* 594 was infected with phage carrying varying lengths of *bio* deletion-substitutions. Normalized synthesis rates (R_N) for the indicated host proteins are listed for the 0- to 10-min and 10- to 20-min labeling periods.

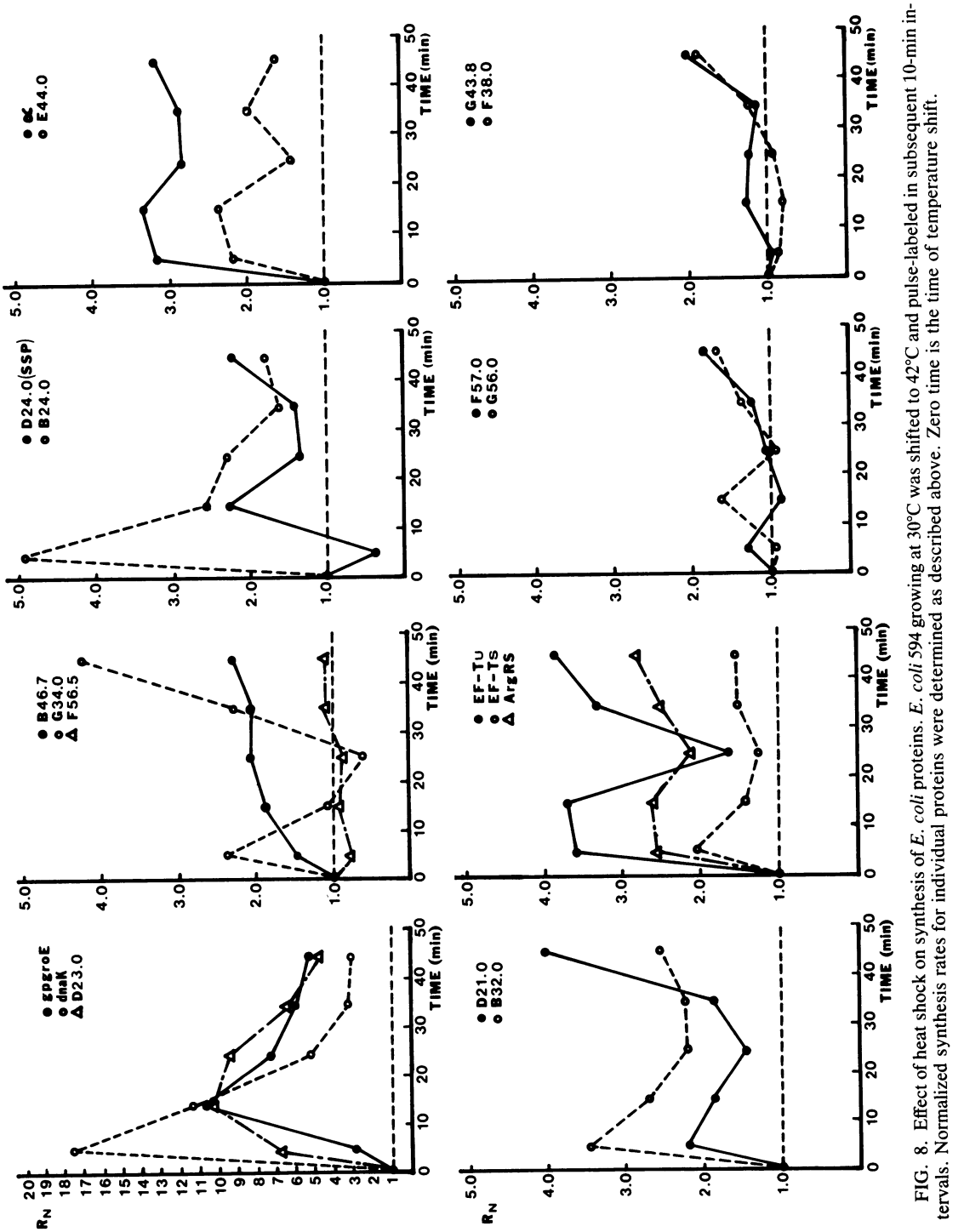

FIG. 8. Effect of heat shock on synthesis of *E. coli* proteins. *E. coli* 594 growing at 30°C was shifted to 42°C and pulse-labeled in subsequent 10-min intervals. Normalized synthesis rates for individual proteins were determined as described above. Zero time is the time of temperature shift.

decline, and the magnitude of the increase was fivefold greater for temperature shift than for infection. The increase of *dnaK* protein synthesis was even more pronounced, reaching 17-fold above the preshift rate in the 0- to 10-min interval and declining thereafter. Comparison of the patterns for the other proteins between infection and temperature shift also shows significant differences in most cases. EF-Tu, EF-Ts, αRNAP, ArgRS, B46.7, and B32.0 all increased two- to threefold on temperature shift but decreased fivefold after infection. SSP, which increased nearly 10-fold on infection, initially decreased after temperature shift and then rose to about twofold above the preshift rate. B24.0 decreased fivefold after infection and increased fivefold after temperature shift. Beyond the simple fact that infection and temperature shift both produced numerous changes in individual protein synthesis rates, it appears that the two processes are quite different from each other.

Figure 9 shows the results of a similar experiment in which we investigated the effect of partial amino acid starvation on the synthesis of these proteins. The starvation was accomplished by adding the phenylalanine analog β-2-thienylalanine to the culture (9). The inhibition of protein synthesis, which is due to false feedback inhibition of aromatic amino acid synthesis, was not complete but allowed synthesis to continue at a substantial fraction of the normal rate. This method has the advantage that starvation is effected without subjecting the cells to a temperature shift or other potentially confusing perturbation. In contrast to what we saw in the temperature shift case, the spectrum of changes that occurred with starvation is rather similar to the spectrum of changes we saw after infection. For example, the *groE* protein and *dnaK* protein patterns were qualitatively the same for starvation as for infection, and the quantitative differences were for the most part, less than a factor of 2. The same can be said of 14 more of the 19 proteins that the infection and starvation experiments have in common. The three proteins that showed greater differences were SSP, B24.0, and D21.0. Even for these three proteins the differences were more of a quantitative than a qualitative nature.

The similarities of protein synthesis patterns after λ infection and during starvation prompted us to investigate the levels of ppGpp after infection. ppGpp is known to increase during starvation (3), and it is thought to be involved in the biochemical manifestations of the so-called stringent response. Figure 10 shows the levels of ppGpp after starvation and infection. As expected, starvation produced a large increase in ppGpp levels. However, we were unable to detect any increase in ppGpp during λ infection.

DISCUSSION

In an earlier study of the effects of λ infection on host synthesis, Cohen and Chang (5) reported that host DNA, RNA, and protein syntheses all decrease after λ infection. Their investigations of host protein synthesis were confined to synthesis of β-galactosidase, which they showed decreases about fivefold after infection, in a way that depends on λ genes in the *att-N* region. We extended these results to include 21 other *E. coli* proteins. Our results show that there are several different responses to infection exhibited by different proteins, including increases in rates of synthesis to above the preinfection rates, as seen with *groE* protein, *dnaK* protein, SSP, and others. It appears that λ infection triggers a complex response in the cell, in which the rates of synthesis of most, if not all, host proteins are affected.

These changes are under the control of early phage genes in the *att-N* region. With the probable exception of the *dnaK* protein, the changes in synthesis rate cannot be assigned to a single λ gene. However, the DNA region that includes genes *Ea10* and *ral* plays an important role in the effect, both for those proteins that are stimulated and those that are depressed. Information on the functions of these two genes is sparse. *Ea10* protein is a DNA-binding protein (R. W. Hendrix, unpublished data) and the most abundant early protein made by the phage (15); it is required for expression of the Tro phenotype, a complex phenotype that follows induction of a λ *cro*$^-$ prophage and that includes an exaggerated form of the changes in protein synthesis rates reported here for λ wild-type infection (11; D. J. Drahos, unpublished data). The *ral* product appears partially to block the host restriction system and may antagonize the transcription termination factor, rho (7, 8, 42).

Although we were able to map the effect of λ infection on host protein synthesis to a particular region of the λ genome, our experiments did not address the question of how that effect is exerted. One possibility is that the responsible λ proteins intervene directly in the synthesis of individual host proteins, for example, by interacting with promoters or ribosome-binding sites. Alternatively, the phage proteins may act less directly, either specifically triggering regulatory circuits that are available in the cell or causing a more general alteration in cellular metabolism that in some way causes the observed changes in cellular protein synthesis. Our observation of the similarity of the effects of infection and amino acid starvation suggests a common underlying mechanism in these two cases. For this reason we favor the view that the phage proteins act indirectly, causing a change in the cell to a

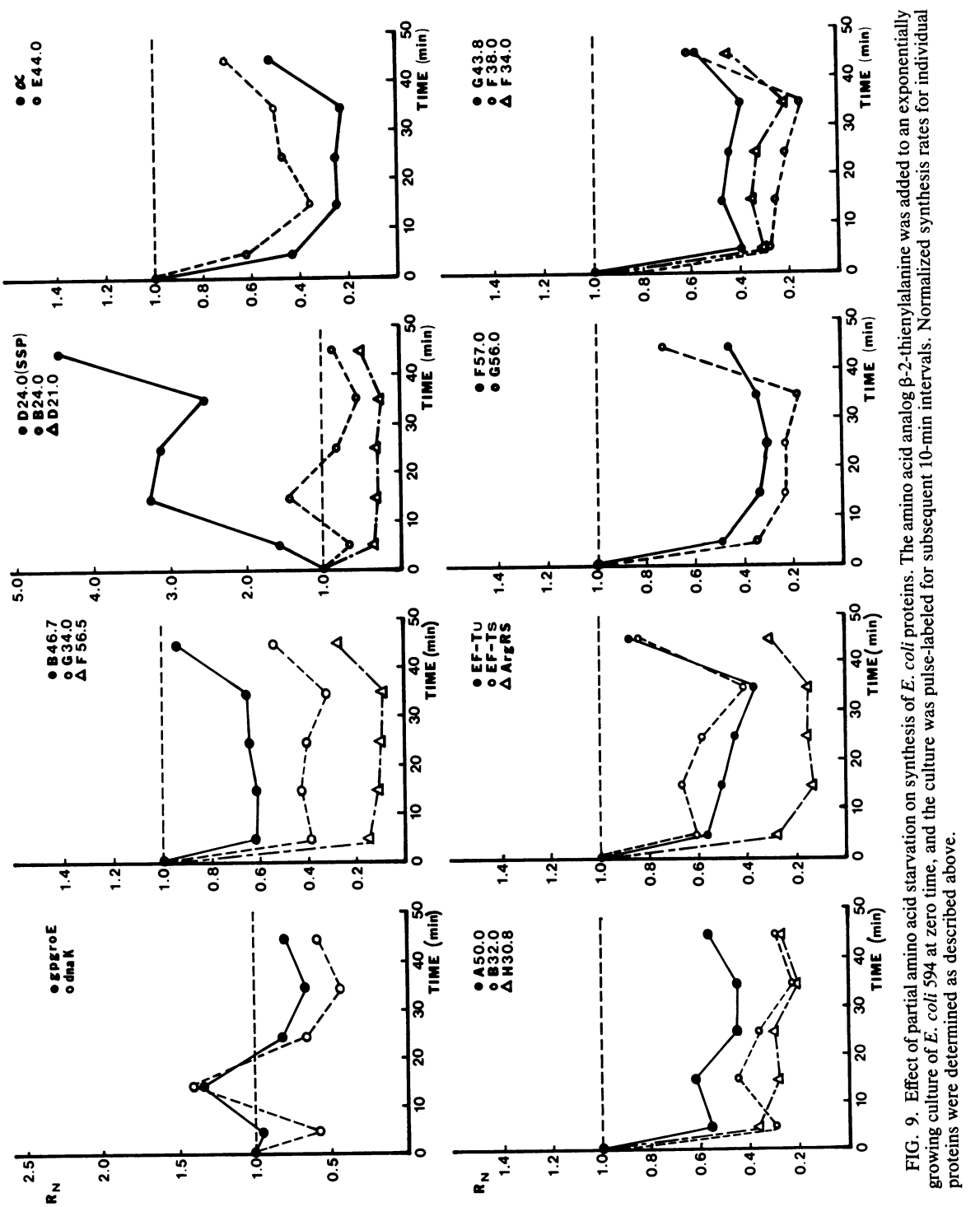

FIG. 9. Effect of partial amino acid starvation on synthesis of *E. coli* proteins. The amino acid analog β-2-thienylalanine was added to an exponentially growing culture of *E. coli* 594 at zero time, and the culture was pulse-labeled for subsequent 10-min intervals. Normalized synthesis rates for individual proteins were determined as described above.

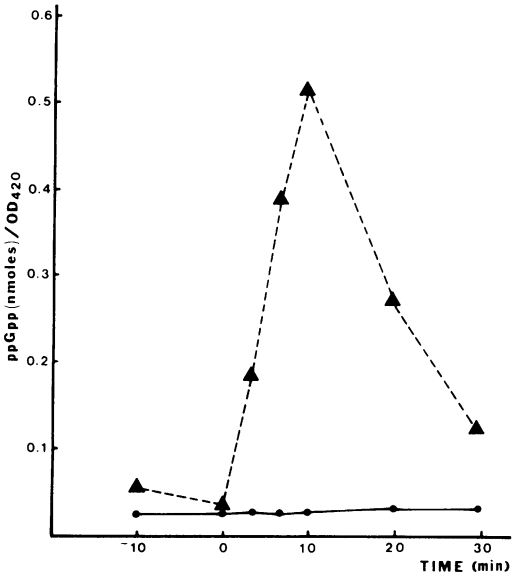

FIG. 10. ppGpp synthesis after infection and amino acid starvation. ppGpp pools were determined as described in the text after infecting with λ *c*I857 *S*am7 (●) and after inducing partial amino acid starvation by the addition of β-2-thienylalanine (▲). OD_{420}, Optical density at 420 nm.

state similar to that in a starved cell and that it is something about this altered metabolic state rather than the direct action of the phage proteins that modulates individual protein synthetic rates.

If it is true, as we suggest, that the similar changes in protein synthesis seen in amino acid starvation and λ infection are the result of similar underlying biochemical changes in the cells, why do we see no increase in ppGpp after infection? Possibly the phage proteins activate the (hypothetical) sequence of regulatory events leading to these changes at some point beyond the level of ppGpp action. Indeed, there is recent evidence that under some conditions the stringent response to amino acid starvation (as measured by decrease in stable RNA synthesis) need not be accompanied by an increase in ppGpp (26). Furthermore, it is not clear to what extent the changes in protein synthesis seen during amino acid starvation should be regarded as part of the stringent response and therefore be expected to be correlated with ppGpp synthesis. When Reeh et al. (28) compared the effects of starvation on protein synthesis in *rel*$^+$ and *relA* strains, they found that the changes were still seen in the *relA* strain, although they were less pronounced than in the *rel*$^+$ strain and different proteins showed different levels of response.

There are now several examples of pronounced changes in the synthesis of *groE* and *dnaK* proteins after perturbation of *E. coli*. According to the data of Pedersen et al. (27), these proteins are present in 10,500 and 6,200 copies per cell during balanced growth, which makes them the 6th and 17th most abundant proteins in the cell. It seems likely that major changes in the levels of *groE* and *dnaK* proteins play important roles in adaptation of the cells to new conditions, but the biochemical functions of these changes are still obscure.

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