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Stimulation of *groE* Synthesis in *Escherichia coli* by Bacteriophage Lambda Infection

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We found that infection of *Escherichia coli* by λ results in at least a twofold stimulation in the rate of synthesis of one of the products of *groE*. To determine what λ-coded factors were responsible for this stimulation, numerous phage λ mutants carrying *bio* substitutions were analyzed for their ability to stimulate *groE* synthesis. Our results revealed that the main factor(s) which is responsible for stimulating *groE* synthesis is located between the endpoints of the λ *bio*69 and λ *bio*252 substitutions, a region of DNA coding for *bet*, *gam*, *kil*, and *c*III.

In one of the earliest studies concerning λ-mediated effects on host metabolism, Cohen and Chang showed that λ infection or prophage induction results in depression of host DNA, RNA, and protein synthesis (2). These workers attributed this effect to the *hin* function (for "host inhibition") of λ and demonstrated that at least two genetic loci near the phage genes *exo*, *bet*, and *gam* are involved in this process. Court et al. (3) have recently reexamined the phenomenon of inhibition of host metabolism by phage lambda and have concluded that the region of DNA between 62.5 and 66.3 fractional lengths defines a new gene(s) *hin*.

In addition to the *hin* phenotype, bacteriophage lambda induces two other functions which alter the metabolism of *Escherichia coli*. One of them, the *tro* phenotype, is also concerned with the shut-off of host macromolecular synthesis. This effect, however, can only be observed in induced lysogens carrying a *cro*-prophage and is due to the overproduction of the *Ea10* and *N* gene products (4). The other function involves the expression of the lambda *kil* gene, located between *gam* and *c*III, and results in the ultimate death of the bacterial host (6). However, gross RNA and protein synthesis alterations do not seem to be caused by the *kil* gene product.

In contrast to these observations, we report here that the infection of *E. coli* by phage λ resulted in an increase in the rate of synthesis of a few host proteins, among them the product of one of the *groE* genes (designated gpgroE). The *groE* genes have been shown to be required for the assembly of λ heads (5, 14, 16). Although the *groEL* protein has recently been purified (7, 9), its mechanism of action remains obscure. Here we describe the alterations in the rate of gp groE synthesis and the preliminary mapping of phage genetic determinant(s) responsible for such alterations.

To see whether the kinetics of host protein synthesis was altered upon λ infection, radioactively labeled extracts of uninfected and λ *c*I857-infected cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The extracts were prepared from cells pulse-labeled at different times after the beginning of incubation at 37°C (Fig. 1). Besides the differences due to the presence of the λ-coded proteins in the infected cells (12), we found a number of bands which increased more markedly in their intensity with time in infected cells than in uninfected cells. The most prominent band was a doublet in the 65,000-molecular-weight range. This band does not correspond to any previously identified λ-coded protein, but because of the apparent molecular weight and its migration in two-dimensional O'Farrell gels (13), we concluded that the 65,000-molecular-weight protein corresponds to the previously identified product of the host gene *groEL* (1, 7, 9, 11).

Figure 1 shows the rate of gpgroE synthesis in λ-infected cells compared with uninfected cells. Although there was a small increase in the rate of synthesis of gpgroE with time in uninfected cells, it is clear that the increase in the rate of synthesis in λ-infected cells was more pronounced. We do not know the reason for the small increase observed in uninfected cells, but it could be due to the metabolic recovery of the cells upon shift to 37°C after the 20-min preincubation period at 0°C. In contrast, the λ-infected cells showed a much greater increase in gpgroE levels. The maximum rate of gpgroE synthesis was achieved at 14 min after the shift to 37°C (Fig. 1).

To determine the origin of the λ-coded factor(s) responsible for mediating the stimulation

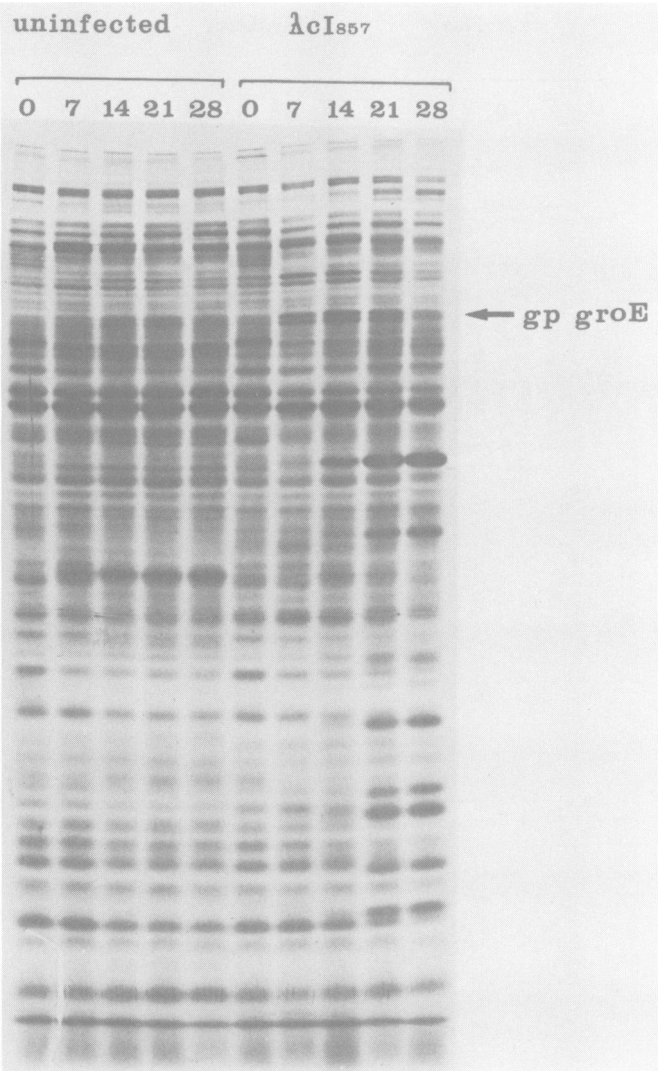

FIG. 1. The levels of gpgroE in uninfected and λ-infected *E. coli*. Samples (1 ml) of uninfected and λ *c*I857-infected *E. coli* 159 cells were pulse-labeled for 3 min with 20 μCi of a ^{35}S-labeled hydrolysate of *E. coli* at 7-min intervals, beginning at time zero, which was the start of the 37°C incubation. The pulses were terminated by the addition of 10 mM NaN_3 and chilling on ice. The cells were pelleted, suspended in 100 μl of sodium dodecyl sulfate sample buffer (10), and boiled. The extracts were then loaded and run on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. The dried gels were autoradiographed for 12 h. The time points at which samples were started to be pulse-labeled are indicated on top of the gels and are in minutes.

of gpgroE synthesis, we infected cells with a series of mutant bacteriophages and analyzed the levels of gpgroE synthesis at different times postinfection in one-dimensional sodium dodecyl sulfate-polyacrylamide gels. To determine whether the factor(s) is the product of an early or a late gene, cells were infected with λ N^- or λ Q^- phages, and the levels of gp groE were measured. Infection of *E. coli* by λ Q^- phage resulted in the stimulation of *groE* synthesis, whereas the λ N^- infection did not (Fig. 2). Therefore, the factor(s) responsible for *groE* stimulation is either *N* or a factor under *N*-mediated control, and it is not mediated by *Q* or a protein under *Q* control.

To localize the factor(s) which is responsible for the induction phenomenon, several *bio* substitution mutants were examined for their ability to stimulate gpgroE synthesis. The phage λ *bio*10 has all of the known early leftward genes

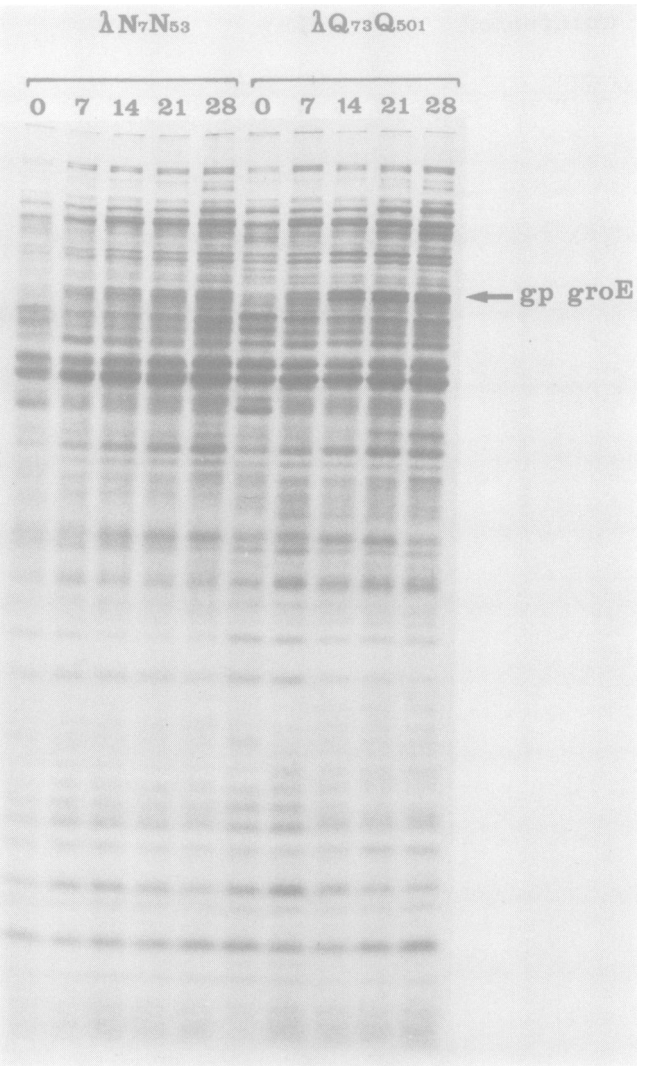

FIG. 2. *groE* stimulation is an early phage function under *N*-mediated control. *E. coli* 159 cells were infected with λ *N*am7 *N*am53 *c*I857 or λ *Q*am73 *Q*am501 *c*It1 (8). Extract preparation and conditions are described in the legend to Fig. 1.

deleted, with the exception of *N*. However, the presence of the *N* gene itself is not sufficient for the stimulation of gpgroE synthesis, as cells infected with λ *bio*10 (Fig. 3) showed the same levels of gpgroE found in N^- infections. When we examined shorter *bio* substitutions, such as λ *bio*252 (Fig. 3), we found that this phage was still unable to stimulate the synthesis of gpgroE. This would suggest that *N*, *ral*, and *Ea10* are not directly responsible for stimulating gpgroE synthesis.

On the other hand, cells infected with λ *bio*69 (Fig. 3) (or λ *bio*72) and other shorter *bio* substitutions (data not shown) showed levels of gp groE similar to those found in λ wild-type and λ Q^- infections. These results suggest that all of the determinants controlling the stimulation of gpgroE synthesis in λ-infected cells are encoded by the region of λ DNA between the endpoints of the *bio*69 and *bio*252 substitutions. The early lambda genes which are known to lie between these two endpoints are *c*III, *kil*, *gam*, and *bet* (15). Therefore, the stimulation of gpgroE synthesis could be controlled by any one (or more) of these genes or by an unknown factor which is encoded within this region of DNA. We are presently trying to identify the factor(s) responsible for stimulating gpgroE synthesis.

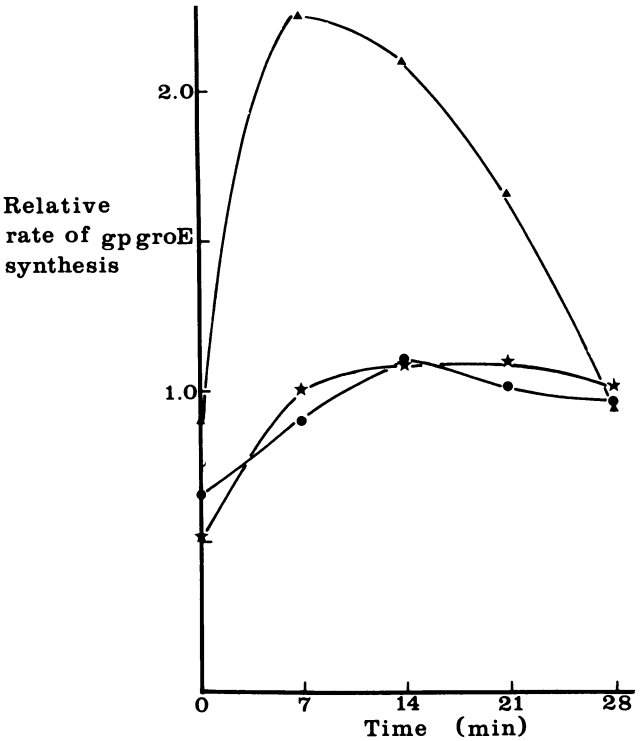

FIG. 3. The relative rate of gpgroE synthesis in *E. coli* infected with different λ *bio* deletion-substitution mutants. Symbols: ▲, λ *bio*69; ★, λ *bio*252; ●, λ *b2bio*10. Conditions are identical to those described in the legend to Fig. 1, except that the proteins were fractionated on 7.5% sodium dodecyl sulfate-polyacrylamide gels. Densitometric scans of the autoradiograms were obtained, and the area of the gpgroE peak, as well as the area of several reference peaks, was calculated. To obtain the relative rate of gpgroE synthesis, the area of the gpgroE peak was divided by the area of a single reference peak, and this value was plotted against the various time points. The results were similar for several reference peaks chosen; therefore the results for only one reference peak are presented.

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