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BACTERIAL MUTANTS WHICH BLOCK PHAGE ASSEMBLY

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groE bacterial mutants of *E. coli* have been isolated on the basis of their inability to propagate bacteriophage λ. The block exerted on λ growth has been shown to operate at the level of head assembly. Some groE mutations express pleiotropic effects, such as inability to propagate T4 and T5 or inability to form colonies at high temperature. P1 transduction experiments show that these groE mutations map at 83 min on the genetic map of *E. coli* and that a single mutation is responsible for the pleiotropic effects observed. At 43°C, some of the groE strains are temperature sensitive for growth and form long filamentous structures. Examination of the proteins synthesized at 43° by one of the temperature-sensitive groE strains, groEA44, by SDS gel electrophoresis reveals a pattern of synthesis somewhat different from that exhibited by the gro^+ parent strain: some new bands appear, while others disappear.

INTRODUCTION

In an effort to understand the role that the bacterial host plays in phage development, we isolated *Escherichia coli* mutants that do not propagate bacteriophage λ (1). These gro mutants were selected as bacteria that can form large colonies in the presence of phage λ, under conditions where sensitive bacteria cells form very small colonies. All of the gro mutants adsorb λ, and λ DNA injection is normal. One of these classes, called groE, does not support the growth of phages T4 and T5 which are unrelated to λ, although no selective pressure was exerted during their isolation (2, 3). Other investigators have independently isolated bacterial strains analogous to groE (4–6, 17; H. Revel, personal communication).

When bacteriophage λ infects groE bacteria, phage adsorption, DNA injection, DNA replication, and cell lysis are all normal (2). In vitro complementation studies reveal that full active phage tails are produced but no head activity is detectable. Electron microscopic examination of extracts of λ-infected groE cells revealed the absence of λ heads and the

presence of petit λ, abnormal headlike structures devoid of DNA and very long tubular structures, up to 100 times the length of a normal head (2). In this respect the phenotype of λ-infected groE bacteria is similar to the phenotype of λB^- or λC^- infected gro^+ bacteria (7). Mutants of λ which can overcome the block in groE hosts have been isolated and shown to map in the E gene (hence the name groE) or in the B gene (2). During λ head morphogenesis a series of proteolytic cleavages takes place giving rise to protein products h3, x1, and x2 (2; Hendrix and Casjens, this issue). None of these cleavages are detected in λ-infected groE bacteria.

When bacteriophage T4 infects groEA44 bacteria all aspects of phage development appear to be normal except head formation (3). The block that the groEA44 mutation exerts on T4 head morphogenesis has been shown to be related to the functioning of the gene 31 product as judged by the following observations: a) mutants of T4 that can overcome the block map in gene 31 (3). b) in the absence of p31, the product of gene 23 (the main structural protein capsid of the head) sediments with the bacterial membrane (8). This also happens in the groE strains which do not propagate T4 (3). c) no morphogenetic proteolytic cleavages, such as P23 to P23*, are observed in T4-infected groEA44 bacterial extracts, a phenotype shared by many head gene mutants including gene 31 (9).

Zweig and Cummings (10) have recently shown that two groE bacterial mutants block T5 development primarily at the level of morphogenesis of the tail. A morphogenetic protein cleavage required for proper tail assembly is specifically blocked in T5-infected groE bacteria. Head morphogenesis is not critically affected by the production of completed heads, although some abnormal head structures are observed.

In addition to the apparent role that the groE mutation plays in phage morphogenesis, what, if any, is its role in bacterial cell growth? In this report we begin to analyze the physiological effects of the groE locus on bacterial growth and development.

METHODS AND MATERIALS

In addition to the apparent role that the groE mutation plays in phage morphogenesis, what, if any, is its role in bacterial cell growth? In bacterial cell growth? In this report we begin to analyse the physiological effects of the groE locus on bacterial growth and development.

Tryptone agar is 1% Tryptone broth, 0.5% NaCl and 1% BactoAgar. M9S medium (11), which contains 0.2% casamino acids, was used for bacterial growth and ^{14}C-amino acid labeling. For labeling experiments bacteria was grown in M9S at 30°C to a density of 1 to 2×10^8/ml and pulsed at various temperatures for 10 min in the presence of a mixture of ^{14}C-labeled amino acids (New England Nuclear, NEC 445) at 50 μCi/ml. The culture was quickly chilled to 0°C by the addition of crushed ice, centrifuged at 15,000 g for 30 sec resuspended in 1/20 volumes of electrophoresis sample buffer (12), and stored at −30°C. The basic procedure followed for discontinous sodium dodecyl sulfate-acrylamide gel electrophoresis is the one described by Russel (12). For chase experiments, a tenfold excess of cold amino acids was added at the end of the 10 min pulsing period at 43.5°C, followed by a 3 min additional incubation. An aliquot was collected on a millipore filter, washed with unlabeled M9S medium, and the collected cells were resuspended in

the same medium and grown at 30°C for an additional 45 min.

Bacterial particle counts were estimated using a Petroff-Hausser bacteria counter.

Temperature-resistant bacterial revertants were isolated by simply spreading approximately 10^8 bacteria on the surface of a Tryptone agar plate and incubating at 43°C for 20 hr.

The conditions for P1L4 growth and P1 transductions were the ones described by Caro and Berg (13).

Bacterial membranes were prepared according to the lysozyme method described by Kaback (14).

RESULTS

The groE Phenotype

Although the groE bacterial strains were isolated at 30°C or 37°C many of them appeared unable to form colonies at 43°C. Is this inability to grow at high temperature due to the same mutation that blocks λ and T4 head development or is it due to another mutation induced by the mutagenesis? Phage P1 was propagated on groE $purA^+$ strains and used to transduce strain H882 gro^+ purA to $purA^+$. The $purA^+$ transductants were tested for possession of both the groE and temperature-sensitive phenotypes. In the case of groEA44, groEA7, and groEA97 mutations, 9 to 10% of the $purA^+$ transductants simultaneously received the groE phenotype. The temperature-sensitive phenotype and the groE mutation always cotransduced together, regardless of whether the primary selection was for $purA^+$groE (and subsequently tested for temperature sensitivity) or whether the primary selection was for $purA^+$ t.s. In no case was the groE phenotype separated from temperature sensitivity for growth. These results strongly suggest that the groE phenotype and bacterial temperature sensitivity are both caused by the groE mutation. Another strain, groEB764, was found to map closer to purA, with 18% of the $purA^+$ transductants simultaneously receiving the groE phenotype. Assuming that there are no artifacts in the isolation procedure of $purA^+$ transductants (e.g., the $purA^+$ $groE^+$ transductants having a selective advantage over the $purA^+$groE transductants), it appears that there are at least two groE loci in *E. coli*. In this respect it is interesting to note that groEB764 belongs to the groEB bacterial type, which is defined by the ability of λεB to grow specifically on it (2).

In a preliminary experiment we found that groEA44 is closely linked to ampA, placing the groE mutation at about 83 min of the genetic map of *E. coli* (Fig. 1). The exact order of groE, ampA and purA has not yet been determined.

groE Temperature-Resistant Revertants

All the groE bacterial strains which are simultaneously temperature sensitive for growth at 43° give rise to temperature-resistant revertants at a frequency of 10^{-6}–10^{-7}. Many of these temperature-resistant revertants were tested for their ability to grow various phages. The results with the temperature-resistant revertants of strain groEA44 are summarized in Table I. A high percentage of these revertants (e.g., t.s.$^+$2) were able to plate both λ and T4, supporting the conclusion that the groEA44 mutation is solely responsible for a) blocking λ development, b) blocking T4 development, and c) inability

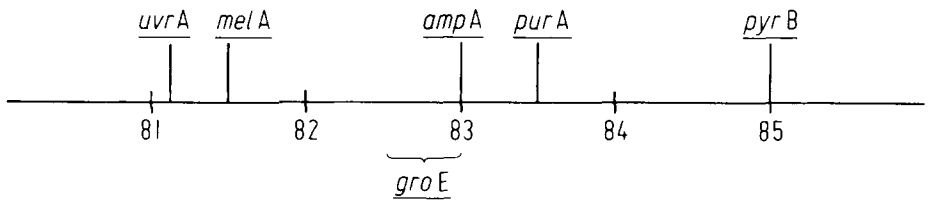

Fig. 1. Map position of the groE mutations on the genetic map of E. coli. The relative order of the groE and ampA mutations has not been definitely established.

TABLE I. Efficiency of Plating on groEA44 t.s.$^+$ Revertants

Bacterial Strain	λcI	T4	T4ϵ1
gro$^+$	1.0	1.0	1.0
groEA44 t.s.	$<10^{-5}$	$<10^{-5}$	1.0
groEA44 t.s.$^+$2	1.0	1.0	1.0
groEA44 t.s.$^+$28	1.0	$<10^{-5}$	$<10^{-5}$
groEA44 t.s.$^+$47	$< 10^{-5}$	$< 10^{-5}$	$<10^{-5}$

Efficiency of plating is measured as the number of plaques produced by a phage strain on a given bacterial host at 37°C relative to the number produced on strain gro$^+$. T4ϵ1 is a T4 spontaneous mutant isolated as able to form plaques on groEA44 t.s.

of the bacterial cell to form colonies at 43°. Other temperature-resistant revertants showed varying abilities to plate λ and/or T4 phage suggesting that they are pseudorevertants. The presumed second site mutations that cause the t.s.$^+$ phenotype in these pseudorevertants were shown by P1 transduction experiments to map very close to the original groEA44 mutation, suggesting that they represent a class of intragenic suppressors. In many instances it was impossible to separate the groEA44 mutation from the partial suppressor mutation that caused the temperature-resistant phenotype. These intragenic-suppressor type mutants grow slower at 43° than the gro$^+$ revertants.

Analysis of the t.s.$^+$ revertants of more groE strains, such as groEA7, groEA42, and groEA97, revealed an unexpected phenomenon. As expected, some of the t.s.$^+$ revertants (e.g., t.s.$^+$2, Table II) plated λ normally and were shown to map at or very near the original groE site. Other t.s.$^+$ revertants (e.g.,t.s.$^+$1,t.s.$^+$3, Table II), although they regained the ability to plate λ, acquired simultaneously the new property of not plating phage T4. These groE-like t.s.$^+$ revertants block the normal functioning of the T4 gene 31 protein as does groEA44. This was verified by the isolation of T4 mutants (e.g., T4ϵ711, Table II) that allow the phage to bypass the bacterial block. These phage mutations map in at least four distinct sites in gene 31. One of these sites is at or very near the site of T4ϵ1 that allows gorwth on groEA44 (3). The maximum recombination frequency between these sites is less than 1%.

Some groEA7 t.s.$^+$ revertants showed varying ability to plate λ and/or T4 depending on the temperature. Revertant t.s.$^+$3 (Table II), for example, plated λ at 37°C and 42°C but exerted a groE-like effect on λ development at 30°C, as judged by both the phenotype of infection and λ mutants that can plate.

TABLE II. Efficiency of Plating on groEA7 t.s.$^+$ Revertants

Bacterial Strain	λ	T4	T4ϵ1	T4ϵ711
gro$^+$	1.0	1.0	1.0	1.0
groEA44 t.s.	$<10^{-5}$	$<10^{-5}$	1.0	$<10^{-5}$
groEA7 t.s.	$<10^{-5}$	1.0	1.0	1.0
groEA7 t.s.$^+$2	1.0	1.0	1.0	1.0
groEA7 t.s.$^+$1	1.0	$<10^{-5}$	$<10^{-5}$	1.0
groEA7 t.s.$^+$3	1.0 (10^{-4} at 30°)	$<10^{-5}$	$<10^{-5}$	1.0

Efficiency of plating is measured as the number of plaques produced by a phage strain on a given bacterial host at 37°C relative to the number produced on strain gro$^+$. T4ϵ711 is a T4 spontaneous mutant isolated as able to form plaques on groEA7 t.s.$^+$3.

Phenotype of Bacterial Growth at 43°C

groEA44 bacteria have the same growth rate as their gro$^+$ parent strain at 30° and 37°C (Fig. 2 A and B). When groEA44 bacteria growing exponentially at 30° are shifted to 43.5°C, bacterial growth mimics that of the gro$^+$ parental strain for the first 30–45 min (Fig. 2C). After this time, the growth rate of groEA44 slows down considerably compared to the parent strain. The bacteria stop dividing at this time, forming long filamentous structures without septa. The results of Fig. 2C are consistent with measurements of colony forming ability, which remains constant after 45 min at 43° (unpublished observations).

The groE phenotype is completely reversible upon shifting at 30°C. After shift-down, the filamentous structures acquire septa and "break up" into individual, viable cells. Most of the t.s.$^+$ revertants examined do not form filaments at 43°C, suggesting that their formation is a consequence of the groE mutation. A few t.s.$^+$ revertants, however, tend to form some short filaments at 43°C, which in contrast to the filaments formed by groE t.s. bacteria, are clearly separated into individual cells by the presence of septa. The formation of these filamentous structures at 43° is not unique to the groEA44 mutation. Other groE strains, such as groEA7, groEA97, and groEA42, exhibit a similar phenotype at high temperature, the extent of which varies according to the particular mutation and the temperature employed.

Because a common feature of the phenotype of phage-infected groE bacteria was the lack of morphogenetic protein cleavages, we considered the possibility that proteolytic cleavages might be required during normal *E. coli* growth and morphogenesis and that these cleavages might be blocked at high temperature in groE mutants. To test this, we labeled bacterial cultures with a mixture of ^{14}C-labeled amino acids for 10 min at various temperatures and examined total cell proteins made during that time interval on SDS polyacrylamide gels. No difference in the protein patterns could be detected between groEA44 and gro$^+$ bacteria at 30° or 37°. Figure 3 shows the gel pattern obtained with groEA44 bacteria at various times after the shift-up to 43.5°. There appears a class of protein bands, called class A, exemplified by bands 1 and 4, whose synthesis ceases about 60 min after the shift-up. Another class, class B, exemplified by bands 2 and 3, appear to be specifically made at accelerated rates between 50 and 100 min after the shift-up to 43°C. No such changes were observed with the gro$^+$ parental strain. For com-

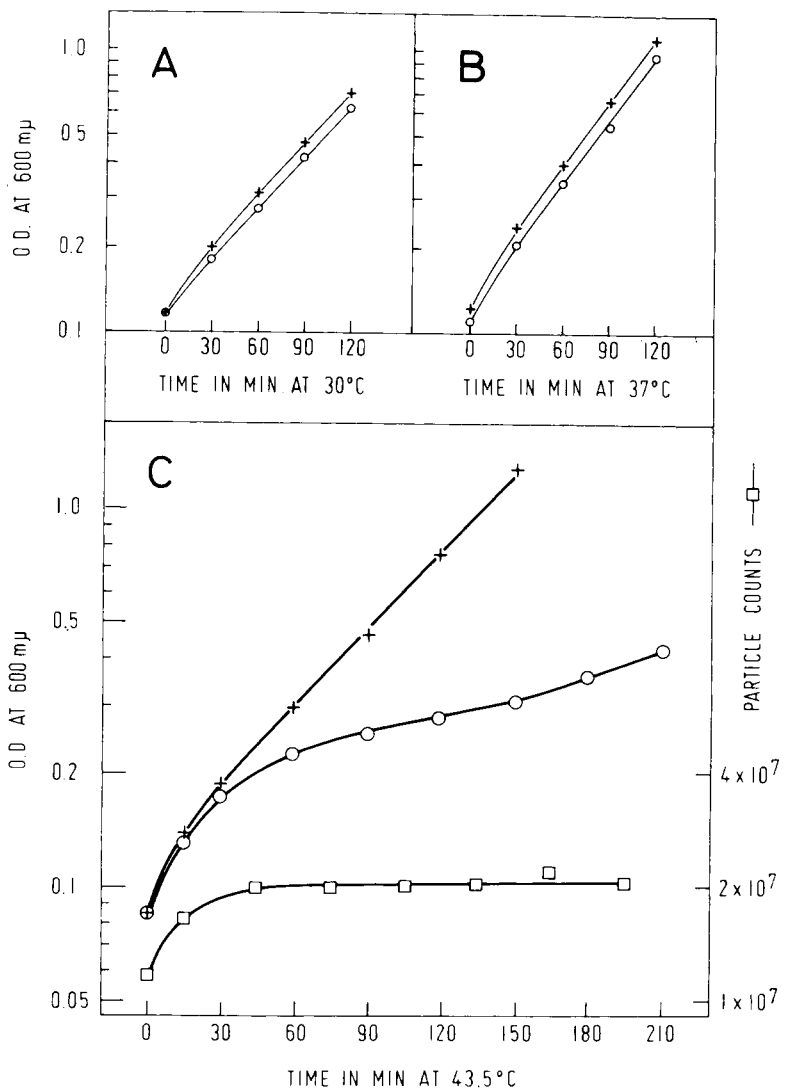

Fig. 2. Growth of groEA44 (—— O ——) and gro$^+$ (——X ——X——) bacteria at various temperatures. A) at 30°C; B) at 37°C; and C) at 43°C. For the experiment at 43°C the bacteria were pregrown at 30°C and shifted to the higher temperature at t = 0. (——□——□——) denotes bacterial particle counts of groEA44.

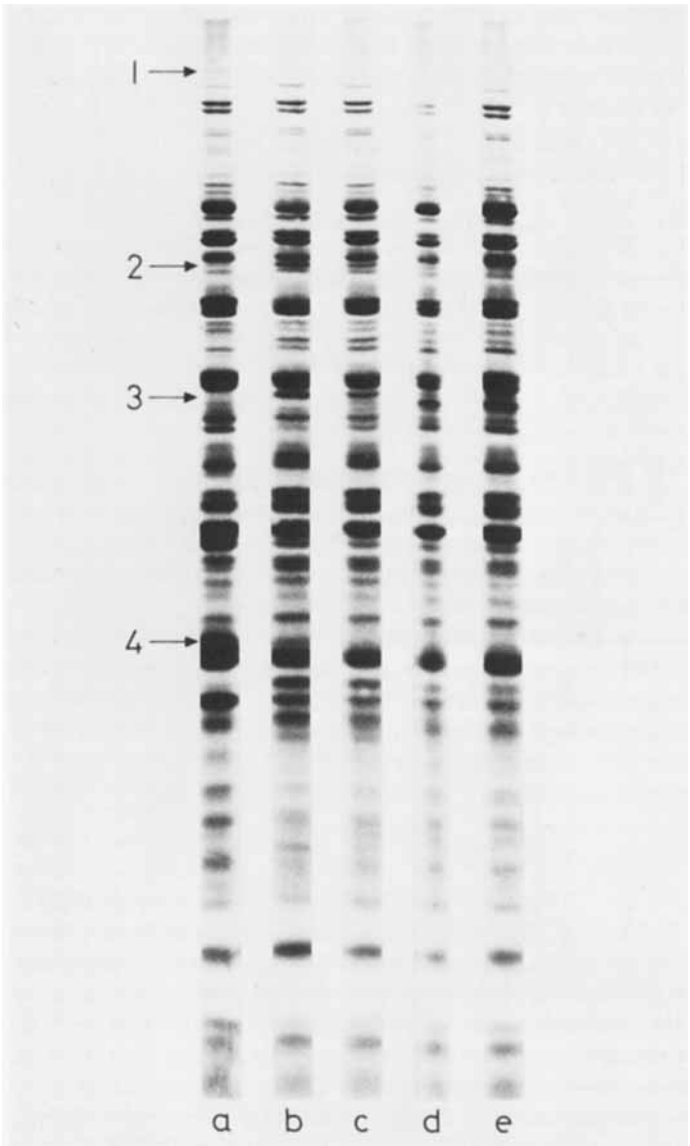

Fig. 3. Autoradiograms of 10% sodium dodecyl sulfate-acrylamide gels of groEA44 cells pulse-labeled with ^{14}C-amino acids at various times after shifting to 43.5°C. a) 30 –40 min pulse; b) 60–70 min; c) 90–100 min; d) 120–130 min; and e) 150–160 min.

parison Fig. 4 shows the protein pattern of gro^+ and groEA44 synthesized between 75 and 85 min after the shift-up (gels a, b). Protein bands 1 and 4 are made in gro^+ but not groEA44 bacteria, whereas bands 2 and 3 exhibit the opposite behavior. The gro EA44 cells labeled between 75 and 85 min after the shift-up were chased at 30°C for 45 min. The protein band pattern did not change after the chase (Fig. 4c and d), suggesting that if bands 1, 2, 3, and 4 are part of a precursor-product protein processing sequence, the process cannot be reversed at 30°C for proteins synthesized at 43°C. All of the temperature-resistant revertants shown in Fig. 5 exhibit a protein pattern similar to that shown by the parental gro^+ strain, suggesting that the protein band changes observed with groEA44 are direct or indirect consequences of the groEA44 mutation.

Preliminary experiments with membrane preparations prepared by the method of Kaback (14) indicate that band 4 is found in bacterial membranes. It is not yet known if bands 1, 2, and 3 are also associated with the membrane fractions.

DISCUSSION

The experimental work reported here, as well as previously published experiments (2, 3), convincingly show that a single mutation is responsible for all the phenotypes expressed in groE bacteria, namely a) a block in head morphogenesis of λ and T4, and b) a temperature-sensitive bacterial growth, accompanied by filament formation and an abnormal pattern of protein synthesis.

What is the mechanism by which the groE mutation causes a block in phage morphogenesis ? We have considered two types of generalized explanations: a) the absence of a functional groE product results in an alteration of an *E. coli* "site" which is essential for the phage morphogenetic protein cleavages observed. The groE product could be an integral part of this site or necessary for its formation. Alternatively, in the absence of the groE function the conditions in the bacterial cell may be so altered that the correct head structures cannot be properly assembled. A likely location for this site is the bacterial membrane. The elegant electron microscopic pictures of Simon suggest that both T4 head and base plate assembly occur near the bacterial membrane (15, 16). In this respect, it is interesting that groEA44 bacterial growth is more sensitive to the detergent deoxycholate than the parent strain (unpublished observations). b) Since a common feature of phage growth on groE bacteria is the absence of morphogenetic protein cleavages, another possibility is that the groE gene product is part of a protease activity system, which alone or in conjunction with a phage gene product performs the specific protein cleavages necessary for phage assembly. This possibility was tested indirectly by looking at the protein synthesis pattern of groE bacteria growing under nonpermissive conditions. We thought that proteolytic protein cleavages might be important during some stages of the cell cycle, and that these changes might be blocked in groE bacteria which do not divide at high temperature. We do not have any direct evidence that the behavior of the class A and class B protein species observed in groE bacteria at high temperature have any precursor–product relationship to any other proteins found in *E. coli.* The fact that neither class is chased after shifting to permissive conditions might argue against this possibility. Another fact that argues against the possibility of band 4 representing the product of a proteolytic cleavage is that there does not seem to exist a larger molecular weight precursor protein in large enough quantity to account for the missing radioactivity at the position of band 4. This of course would not happen if the

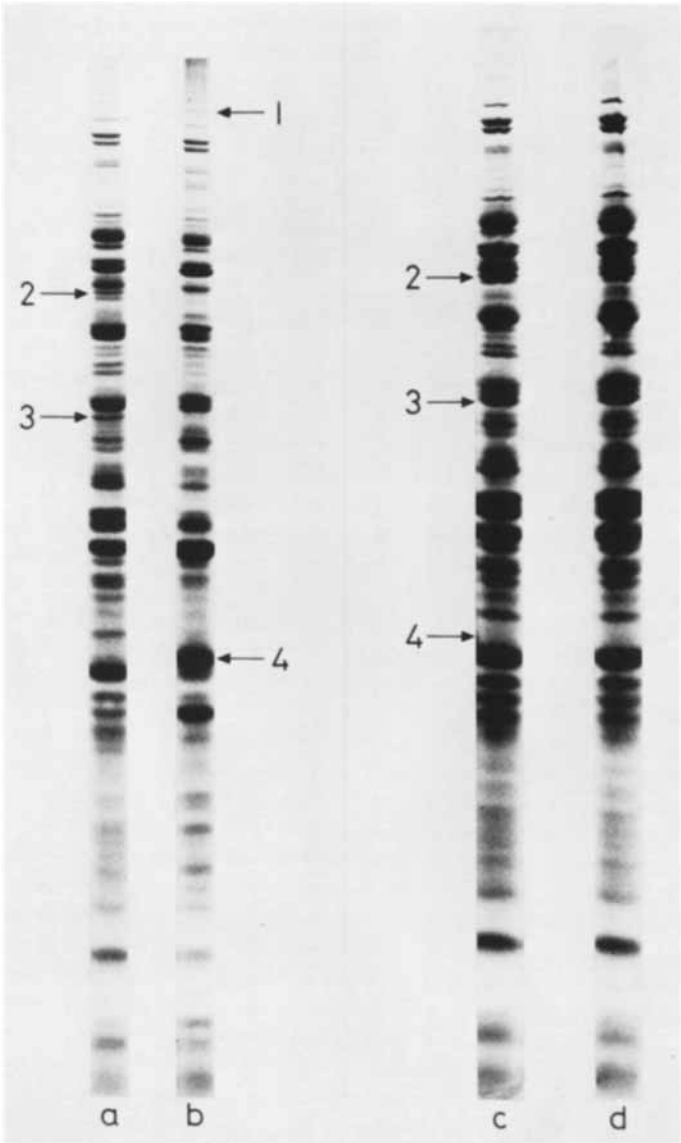

Fig. 4. Autoradiograms of 10% sodium dodecyl sulfate-acrylamide gels of a) groEA44 cells pulsed between 75 and 85 min after shifting to 43.5°C; b) gro^{+} cells pulsed between 75 and 80 min after shifting to 43.5°C; c) groEA44 cells pulsed between 75 and 85 min after shifting to 43.5°C; and d) similar to c), but chased at 30°C for an additional 45 min.

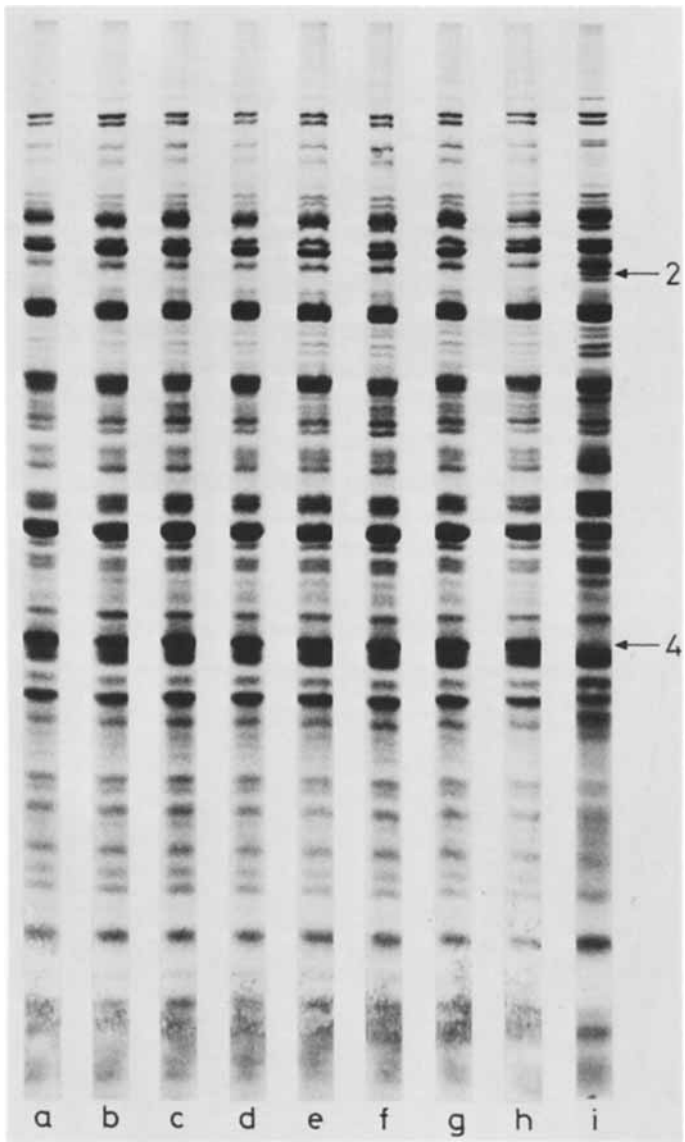

Fig. 5. Autoradiograms of 10% sodium dodecyl sulfate-acrylamide gels of (a–h) various groEA44 t.s.$^+$ revertants independently isolated, and i) groEA44. All samples represent pulses between 75 and 85 min after shifting to 43.5°C.

postulated precursor species were destroyed during the 10 min pulse. Another interpretation of the existence of class A and B protein patterns is that they are due to cellular regulatory phenomena and hence are indirect consequences of the groE mutation. For example, class B proteins could be produced between 50 and 100 min after the shift-up in response to changed conditions inside the bacterial cell that lead to a specific derepression in the synthesis of these protein species. The lack of synthesis of class A proteins could be due to a regulatory system that specifically represses their synthesis under unfavorable conditions inside the bacterial cell. In this respect, it would be interesting to know whether the class A and B protein band changes are specific for the groE mutations or are also found in other *E. coli* temperature-sensitive mutants with similar phenotypes, e.g., filament formation at high temperature, slow growth rate, etc. We are now in the process of analyzing the protein synthesis pattern of more groE temperature-sensitive mutants at high temperature to see whether results analogous to groEA44 can be obtained, or whether the altered protein bands observed with groEA44 are specifically associated with this mutation.

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