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Participation of *Escherichia coli* K-12 *groE* Gene Products in the Synthesis of Cellular DNA and RNA

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Cells having the temperature-sensitive mutation *groES131*(Ts) were isolated from *Escherichia coli* K-12 strain C600T by thymineless death selection at 44°C. This conditionally expressed mutation affected both cellular DNA and RNA syntheses at nonpermissive temperature, in addition to rendering cells unable to propagate phage λ at permissive temperature.

In the *groE* mutants of *Escherichia coli*, maturation of phage λ or T4 does not occur owing to defective assembly of virion heads (2, 5, 6, 16, 18). *groE* mutants also prevent the assembly of phage T5 tails (25). Because of this, the *groE* gene, whose product is a 65,000-molecular-weight protein (7, 10), has also been designated as *mop* (morphogenesis of phage) on the chromosomal map of *E. coli* K-12 (1). Nevertheless, its product also plays an important role in the growth of host cells (4, 5, 15). The *groE* locus has recently been shown to consist of two closely linked genes, *groES* and *groEL*, the former coding for a 15,000-*M*r polypeptide and the latter for a 65,000-*M*r polypeptide (20); mutations in both of these genes exert similar effects on the λ phage head assembly and bacterial growth at nonpermissive temperatures (19, 20). Another class of mutants defective in supporting the growth of phage λ are *dna* mutants, which are known to prevent the replication of phage λ at 37°C (3, 8, 14, 17). A mutant of this class, *dnaK7*(Ts), was isolated in this laboratory by thymineless death screening at high temperature after mutagenesis and shown to possess an additional characteristic; i.e., its cellular DNA and RNA syntheses are strongly inhibited at nonpermissive temperature (12). Furthermore, a *dnaJ* mutant, *dnaJ259*(Ts) (17), has also been shown to be defective in macromolecular synthesis (22).

To study the mechanisms of cellular regulation of macromolecular synthesis, we further attempted to isolate mutants with characteristics similar to those of *dnaK* and *dnaJ* mutants. In this report, we describe cells having a temperature-sensitive *groES* mutation that were newly isolated by a two-step screening procedure (thymineless death and phage infection) after mutagenesis. The mutant strain MT131, carrying a *groES131*(Ts) mutation, was found to be defective in cellular DNA and RNA syntheses at nonpermissive temperature and unable to propagate phage λ at permissive temperature. Similar characteristics were also observed for other *groES* and *groEL* mutants isolated by Tilly et al. (20).

Parental cells (*E. coli* K-12 strain C600T [F⁻ *thr leu thi thy lacY rpsL supE*]) grown exponentially in M9CA medium (containing 0.8 g of Casamino Acids [Difco Laboratories] per liter of M9 minimal medium) were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and then subjected to thymineless death selection by incubation for 2.5 h at 44°C in thymine-deprived M9CA medium, as previously described (12). After this treatment, the survivors were infected with phage P1 *vir* (at a multiplicity of infection of 100) and then with λ *hv* (at a multiplicity of infection of 10) for further selection of mutants by the method of Sunshine et al. (17). The infected cells were plated onto λ agar medium and incubated at 30°C for 24 h. Seven mutants unable to grow at 44°C or to propagate phage λ at 37°C were obtained from 3,400 colonies screened. One of these mutants, MT131, was mainly analyzed in the present work. Since preliminary mating experiments between Hfr CS101 (*metB*) and MT131 (F⁻ *thr leu thi thy groE lacY rpsL supE*) indicated that the *groE* mutation in MT131 is located near the *metB* gene, the precise map location was determined by phage P1 transduction with mutant strains carrying either the *mel* (92 min), *purA* (94 min), or *ampA* (94 min) mutation as recipients. The results showed that the mutation was located between the *mel* and *purA* genes and is closely linked to the latter, suggesting that it is a mutation in the *groE* gene (data not shown). To determine whether the mutation resides in the *groES* gene or in the *groEL* gene, we measured the efficiencies of plating on MT131 of transducing phage λ carrying the *groES* or the *groEL* chromosomal gene (20). Cells were grown in 3 ml of brain heart infusion broth (12) at 30°C, collected by centrifugation, and suspended in 1 ml of λ broth. Samples of the cell suspension (0.25 ml; 4×10^8 cells) and phage lysate (0.1 ml) were mixed with 2.5 ml of molten λ soft agar (0.5% agar). The mixture was overlaid onto the λ agar plate containing 10 μg of thymine per ml. The number of plaques produced was counted after overnight incubation at 33°C. The mutant MT131 was able to propagate phage λ carrying the *groES* gene but not the *groEL* gene (Table 1), suggesting that the mutation is in the *groES* gene. Thus, the mutation was given the designation *groES131*(Ts). The effects of *groE* mutations on macromolecular biosynthesis were examined next. Before this, the *groES131*(Ts) and other *groE* mutations (20), *groES30*(Ts) and *groEL100*(Ts), were independently transduced into bacterial strains JRG823 (F⁺ *mel gltC ampA supE*) (9) and AB1157T (F⁻ *thr leu pro his arg thi thy lacY galK ara xyl mtl rpsL supE*) (12) to confer isogenic backgrounds. The effects of these three mutations on the biosynthesis of DNA, RNA, and protein at nonpermissive temperature were then examined by pulse-labeling with radioisotopes. The procedures were the same as those described in previous reports (12, 22). Samples of 0.5 ml were withdrawn from the culture at 30, 60, and 90 min and labeled with radioisotopes by adding 0.5 ml of M9CA medium containing either [^{3}H]thymidine (0.5 μCi/ml; 16.2 Ci/mmol), [^{14}C]uridine (0.1 μCi/ml; 58 mCi/mmol) or [^{14}C]leucine (0.1 μCi/ml; 342 mCi/mmol) plus 4 μg of leucine per ml. After a 2-min labeling, acid-insoluble fractions were

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TABLE 1. Efficiency of plating of phage λ carrying the *groEL* or the *groES* gene on *E. coli* K-12 strains

Bacterial strain	Efficiency of plating of following phage[a]:			
	λ *hv*	λ *groES groEL*	λ *groES*	λ *groEL*
C600T	1.0	1.0	1.0	1.0
MT131 [*groES131*(Ts)]	3.6×10^{-6}	1.3	0.8	1.5×10^{-5}
B178	1.0	1.0	1.0	1.0
B178 [*groEL100*(Ts)]	6.1×10^{-7}	1.1	$<1.1 \times 10^{-5}$	1.0
B178 [*groES30*(Ts)]	0.13	0.8	0.8	0.24

[a] Efficiency of plating is expressed by the relative number of plaques produced by a given phage strain on a given bacterial host at 33°C, calculated by setting at 1.0 the numbers on C600T for MT131 and on B178 (*galE*) for the remaining strains. See text for experimental details.

collected on glass fiber filters and dried. Radioactivities of the acid-insoluble fractions were counted with a model LS 7500 Beckman scintillation counter as previously described (12). The results obtained with the strains of JRG823 background are shown in Fig. 1. In all three mutant strains, the rates of cellular DNA and RNA syntheses decreased within 10 to 30 min after the shift-up to 42°C. However, the rate of protein synthesis was not significantly affected by the temperature shift, with the exception of the *groES131* mutant, in which the rate began to decrease after 30 min, though it remained above 100% even at 90 min. Similar effects were observed with a strain of AB1157T background by continuous labeling (data not shown). It was thus concluded that the *groES*(Ts) and *groEL*(Ts) mutations not only render cells unable to propagate phage λ at permissive temperature (see Table 1), but also affect cellular DNA and RNA biosyntheses at nonpermissive temperature.

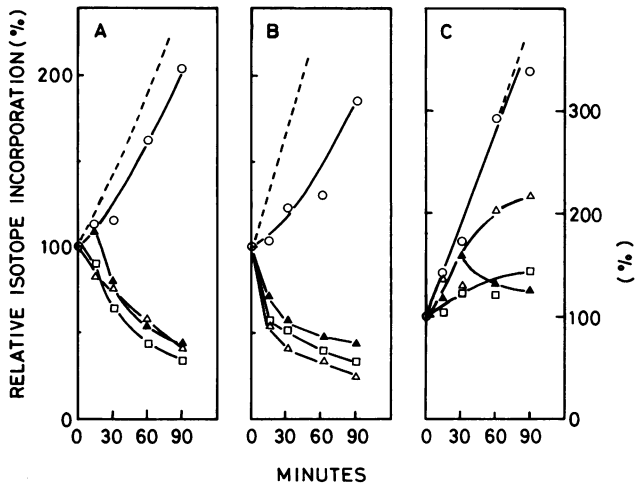

FIG. 1. Rates of DNA, RNA, and protein syntheses of *groE*(Ts) mutant strains. Cells were grown in M9CA medium and subjected to a temperature shift to 42°C at midlog phase (zero time). See text for details of experimental procedures. (A) Rate of DNA synthesis. Relative values of isotope incorporation were calculated by designating as 100% the values of [^{3}H]thymidine incorporation at zero time (52,851, 25,867, 42,339, and 37,015 cpm for JRG823, *groES131*, *groES30*, and *groEL100*, respectively). (B) Rate of RNA synthesis. The values of [^{14}C]uridine incorporation at zero time (27,827, 10,101, 20,480, and 19,670 cpm for JRG823, *groES131*, *groES30*, and *groEL100*, respectively) were designated as 100%. (C) Rate of protein synthesis. The values of [^{14}C]leucine incorporation at zero time (262, 243, 359, and 315 cpm for JRG823, *groES131*, *groES30*, and *groEL100*, respectively) were designated as 100%. ○, JRG823; ▲, JRG823*groES131*; △, JRG823*groES30*; □, JRG823*groEL100*; -----, relative incorporation at 30°C.

In the present study, a mutant, *groES131*(Ts), was isolated from *E. coli* K-12 C600T by a two-step screening procedure (thymineless death and phage infection) after mutagenic treatment. In this mutant, as well as in other previously reported *groE*(Ts) mutants, the synthesis of DNA and RNA were simultaneously inhibited at nonpermissive temperature (Fig. 1). The characteristics of mutants observed here closely resemble those of *dnaK* and *dnaJ* mutants (12, 22). Cellular growth of these *groE* mutants was inhibited by temperature shift, but not to the same extent. The mutants of JRG823 background (*groES30* and *groEL 100*) became elongated two or three times more than did wild type after incubation for 2 h at 42°C, then decreased their optical densities as a result of cellular lysis; the mutant (*groES131* of JRG background) cells became more elongated (three or four times) but not lysed even after a prolonged incubation at 42°C. Our results suggest the possibility that the temperature-sensitive growth of *groE* mutants is due to their defective synthesis of cellular DNA and RNA at nonpermissive temperature. Recently the products of the *groES* and *groEL* genes have been identified as the heat-inducible proteins C15.4 (21) and B56.5 (13), respectively. Although the product of the *groEL* gene has ATPase activity (11, 23, 24), the cellular function, as well as the physiological role of the *groES* gene product, is not understood exactly.

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