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Isolation and Characterization of a Temperature-Sensitive *dnaK* Mutant of *Escherichia coli* B

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A temperature-sensitive *dnaK* mutant (strain MT112) was isolated from *Escherichia coli* B strain H/r30RT by thymineless death selection at 43°C. By genetic mapping, the mutation [*dnaK*7(Ts)] was located near the *thr* gene (approximately 0.2 min on the map). *E. coli* K-12 transductants of the mutation to temperature sensitivity were assayed for their susceptibility to transducing phage λ carrying the *dnaK* and/or the *dnaJ* gene. All of the transductants were able to propagate phage λ carrying the *dnaK* gene. When macromolecular synthesis of the mutant was assayed at 43°C, it was observed that both deoxyribonucleic acid and ribonucleic acid syntheses were severely inhibited. Thus, it was suggested that the conditionally defective *dnaK* mutation affects both cellular deoxyribonucleic acid and ribonucleic acid syntheses at the nonpermissive temperature in addition to inability to propagate phage λ at permissive temperature.

It is known that phage lambda replication requires some host DNA synthesis function since the phage is unable to grow in certain *Escherichia coli* K-12 mutants containing temperature-sensitive (Ts) defects in DNA synthesis (4, 5, 8, 9; J. R. Walker, J. M. Henson, and C. S. Lee, Abstr. Annu. Meet. Genet. Soc. Am., 1976, **83**:s80–81). In *groP* mutants, lambdoid phages were unable to multiply because the gene P product of the phages does not function (7). These *groP* bacteria were usually themselves conditionally defective in DNA synthesis and were identified as *dnaB* mutants (3). Saito and Uchida (12) isolated various *grp* mutants (*grpA*, -*C*, -*D*, and -*E*), in which the initiation of λ DNA replication is specifically affected. Furthermore, it was noted that *grpC* (or *groPC*) mutations were in the *dnaK* gene (12), which is located between *thr* and *leu* on the *E. coli* K-12 map (6, 12, 14). Recently, it was found that this class of mutation separates into two closely linked cistrons, *dnaJ* and *dnaK*, and the gene order is *thr-dnaK-dnaJ-leu* (15). Organization and expression of the *dnaK* and *dnaJ* genes were also characterized extensively in *E. coli* K-12 (13).

To study the mechanism of regulation of macromolecular synthesis, we used a temperature-sensitive mutant which was isolated in our laboratory from *E. coli* B stain H/r30RT from among the survivors of thymineless death at 43°C after mutagenic treatment (J. Ryu and H. Itikawa, Abstr. Annu. Meet. Genet. Soc. Jpn., 1975, **51**:490). Genetic mapping data show that the mutation is located between the *thr* and *leu* genes.

Since *E. coli* B bacteria are unable to adsorb phage lambda, the temperature-sensitive mutation was transduced to *E. coli* K-12 strains for testing phage lambda propagation. The transductants were unable to propagate phage λ*vh* but were able to propagate transducing phage λ carrying the bacterial *dnaK* gene. Thus, the temperature-sensitive mutation of *E. coli* B is a mutation in the *dnaK* gene. It was noted that the *dnaK* mutant was defective in DNA synthesis and that RNA synthesis was also severely inhibited at 43°C.

MATERIALS AND METHODS

Bacteria and bacteriophages. The bacterial and phage strains used in the present study are listed in Table 1.

Media. The following media were used. M9 minimal medium contained the following, in 1 liter of distilled water: Na_2HPO_4, 6g; KH_2PO_4, 3 g; NaCl, 0.5 g; NH_4Cl, 1 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2$, 11 mg; glucose, 2 g. M9CA contained 0.8 g of Casamino Acids (Difco; vitamin-free) per liter of M9 medium. Minimal medium (Davis) contained, per liter: K_2HPO_4, 7 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2SO_4$, 1 g; sodium citrate, 0.5 g; glucose, 2 g. L-broth medium contained 1% Polypepton (Daigo Eiyo Kagaku), 0.5% yeast extract (Difco), 0.5% NaCl, 0.1% glucose, and 2.5 mM $CaCl_2$, (pH 7.0). λ-Broth contained 1% Polypepton and 0.25% NaCl (pH 7.0). Nutrient broth contained 1% meat extract (Kyokuto Seiyaku), 1% Polypepton, and 0.2% NaCl (pH 7.0). Brain heart infusion was obtained from Eiken Chemical Co. Solid medium contained 1.5% (minimal medium or nutrient broth) or 1.2% (L-

TABLE 1. *Bacterial and phage strains*

Strain	Genotype[a]	Origin/source
E. coli B		
H/r30RT	*thy argF*	S. Kondo
MT112	*thy argF dnaK7*	H/r30RT, MNNG[b]
MT114	*thy argF ilv dnaK7*	MT112, MNNG
MT116	*thy ilv leu dnaK7*	ACB1 × MT114 → Leu$^-$
MT117	*thy ilv thr*	Pl*vir*(W3111) × MT116 → Thr$^-$
MT118	*ilv thr serB*	RB29 × MT117 → SerB$^-$
B1	Hfr, wild type	S. Kondo
AC2519	*thr leu met arg his pro trp gal lac rpsL*	S. Kondo
ACB1	Hfr *thr leu met arg pro trp gal lac rpsL*	B1 × AC2519 → Hfr
E. coli K-12		
RB29	Hfr *serB thi cet sup* λ$^-$	R. S. Buxton
RB85	*thr leu thi lacY rpsL supE* λ$^-$	F$^-$ C600, R. S. Buxton
RB851	*leu thi lacY rpsL tonA supE dnaK7* λ$^-$	P1 (MT114) × RB85 → Ts (*dnaK7*)
RB851R1, RB851R2	*leu thi lacY rpsL tonA supE* λ$^-$	Spontaneous revertants from RB851
AB1157T	*thr leu pro his arg thi lac gal xyl mtl tsx rpsL thy* λ$^-$	AB1157, MNNG[b]
AB11571	*leu pro his arg thi lac gal xyl mtl tsx rpsL thy dnaK7* λ$^-$	P1 (MT114) × AB1157T → Ts (*dnaK7*)
K1702	*grpC17* (*dnaK170*) *leu pro his argE ara mtl xyl gal lac thi supE*	AB1133, H. Uchida (13)
K2710	*grpC2* (*dnaJ2*) *gal lac*	HR4, H. Uchida (13)
K7561	*groPAB756* [*dnaK*(Ts)] *thr lac tonA thyA thi supE44*	HR12, H. Uchida (12)
W3111	*thr*	W3110
Phage		
P1	For *E. coli* B	S. Kondo
Pl*vir*	For *E. coli* K-12	M. Abe
λ*vh*	Virulent	M. Abe
λcI857*dnaJK*	Transducing phage for *dnaJ* and *dnaK*	H. Uchida (13)
λcI857*dnaJ*Δ145	Transducing phage for *dnaJ*	H. Uchida (13)
λcI50*dnaK*	Transducing phage for *dnaK*	H. Uchida (13)

[a] Genotypes according to reference 1.
[b] MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

broth or λ-broth) agar. Supplements were added when needed, as follows: 20 μg of of thymine, 40 μg of amino acids, or 2 μg of thiamine per ml.

Growth conditions. Cells were incubated overnight in brain heart infusion broth at 37°C. Cells were washed with saline (0.85% NaCl) by centrifugation and then suspended in the fresh liquid medium and incubated with shaking at 37°C. Cell growth was monitored by measuring the turbidity of the cultures at 660 nm with a spectrophotometer (Hitachi FPO-3). Temperature-sensitive mutant cells were incubated at 30°C unless otherwise noted.

Radioisotopes. [6-^{3}H]thymine, [6-^{3}H]thymidine, [5-^{3}H]uracil, and [*U*-^{14}C]leucine were purchased from Daiichi Pure Chemicals Co. [2-^{14}C]uridine was obtained from Amersham Products Inc.

Determination of DNA, RNA, and protein syntheses. For continuous labeling, cells grown in M9CA were divided into three portions. [^{3}H]thymine (1.0 μCi/ml, 26.0 Ci/mmol), [^{3}H]uracil (0.5 μCi/ml, 14.9 Ci/mmol) with 20 μg of uracil per ml, or [^{14}C]-leucine (0.1 μCi/ml, 262 mCi/mmol) with 40 μg of leucine per ml, respectively, was added to each of them. After five generation times of labeling, 0.4-ml samples were added to 0.4 ml of 10% cold trichloroacetic acid and chilled. After 1 h or more of chilling, they were washed with cold 5% trichloroacetic acid and then with cold 5% acetic acid on glass fiber filters (Whatman GF/C) and dried. Radioactivity in the acid-insoluble fraction was counted by using a Beckman liquid scintillation counter (LS-III) in glass vials containing 10 ml of 0.4% 2,5-diphenyloxazole in toluene.

For pulse-labeling, 0.5-ml portions were removed from the culture and incubated further with the addition of 0.5 ml of M9CA medium containing [^{3}H]-thymidine (1 μCi/ml, 10 Ci/mmol), [^{14}C]uridine (50 μCi/ml, 62 mCi/mmol), or [^{14}C]leucine (0.2 μCi/ml, 262 mCi/mmol) plus 4 μg of leucine per ml. After 2 ([^{3}H]thymidine or [^{14}C]uridine) or 5 ([^{14}C]leucine) min of labeling, acid-insoluble fractions were collected and assayed as described above.

Isolation of temperature-sensitive mutants. Parental cells (H/r30RT), grown exponentially in M9CA medium, were collected by centrifugation and suspended in 1 ml of acetate buffer (pH 5.0). *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was added (final concen-

tration, 1 mg/ml), and cells were allowed to stand for 40 min at 37°C. They were washed twice with acetate buffer and once with M9CA medium, resuspended in M9CA medium, and incubated overnight at 30°C. The culture was diluted in the same medium, and, at midlog phase, cells were shifted from 30 to 43°C. After 1 h, they were washed on a membrane filter (Millipore HAWP, 0.45-μm pore size) and incubated further for 2.5 h at 43°C in M9CA medium without thymine. Thus, growing cells under these conditions were subjected to thymineless death and viable cells were decreased to 0.1% of the intial cell number. Mutants defective in DNA synthesis at 43°C were resistant to thymineless death and were enriched by this treatment. One portion of the culture was then plated on nutrient broth plates and incubated at 30°C. Nine mutant strains unable to grow at 43°C but growing at 30°C on nutrient broth plates were obtained at a frequency of 1% among 10^4 cell survivors. One mutant [*dnaK7*(Ts)] was mainly studied in the present work.

RESULTS

Genetic mapping of *dnaK*(Ts). Since preliminary mating experiments between Hfr CS101 and MT114 indicated that the mutation was located near *leu* on the *E. coli* K-12 map (data not shown), generalized transducing phage P1 was used with strain W3111 (*thr*) as the donor and MT116 (*thy ilv leu dnaK7*(Ts)) was used as the recipient. Resulting Leu^+ transductants were selected on a minimal medium agar plate supplemented with thymine, threonine, isoleucine, and valine. Among 578 Leu^+ clones, temperature resistance and Thr^- were found at a frequency of 0.52% (3 of 578) and temperature resistance and Thr^+ were found at a frequency of 0.69% (4 of 578). The remaining 571 Leu^+ transductants were temperature sensitive and Thr^+, indicating that *dnaK7*(Ts) is located near the *thr* gene. To determine the location of the mutation more precisely, further P1 transduction tests were carried out as follows.

(i) Transduction 1 was MT116 (temperature-sensitive Thr^+ donor) × MT117 (temperature-resistant Thr^- recipient) on minimal medium agar plate supplemented with thymine, isoleucine, valine, and leucine. Among 117 Thr^+ transductants, 108 (92.3%) were temperature sensitive, suggesting a very close linkage between the *thr* and *dnaK7*(Ts) genes.

(ii) Transduction 2 was MT114 (temperature-sensitive donor) × MT118 (temperature-resistant recipient) on minimal medium agar supplemented with threonine, isoleucine, and valine. Among 85 $SerB^+$ transductants, 74.1% were Thr^+ and 61.2% were temperature sensitive (Table 2).

These results suggest that the gene order might be *serB-thr-dnaK7*(Ts). To confirm this order, Thr^+ transductants were selected from the same cross, and $SerB^+$ and temperature-sensitive clones were scored as shown in Table 2. Again the results suggested that the gene order was *serB-thr-dnaK7*(Ts). The map position of the *dnaK* locus, therefore, may be estimated as follows. By using Wu's formula (1), the map distance of *thr-serB* corresponds approximately to 0.1 min (from 87.1% of the cotransduction frequency) and that of *thr-dnaK* corresponds to 0.06 min (91.7% cotransduction). Since the map distance of *thr-serB* is listed as 0.4 min on the *E. coli* K-12 map (1), a reasonable map distance for *thr-dnaK* might be proportionally calculated as around 0.24 min.

TABLE 2. *P1 transduction between MT114 (donor) and MT118 (recipient)*[a]

Cross	Genotype of transductant			No. scored	%
	serB	*thr*	*dnaK7*(Ts)		
A	1	1	1	52	61.2
	1	1	0	11	12.9
	1	0	1	0	0
	1	0	0	22	25.9
B	1	1	1	140	81.9
	1	1	0	10	5.8
	0	1	1	16	9.4
	0	1	0	5	2.9

[a] P1 transduction was done by the method of Lennox (10). In cross A, $SerB^+$ colonies on the selective agar plate were subjected to single-colony isolation and assayed for their genotypes including threonine requirement and temperature sensitivity. In the table, all donor markers [$serB^+ thr^+$ *dnaK7*(Ts)] are designated as 1, and recipient markers (*serB thr* $dnaK^+$) are designated as 0. In cross B, Thr^+ colonies on the selective agar plate were assayed for their requirement for serine and temperature sensitivity after single-colony isolation. Procedures and designation of the markers are the same as in cross A.

Identification of a temperature-sensitivity mutation in the *dnaK* gene. It is known that the *dnaJ* and *dnaK* genes are located near the *thr* gene and that conditionally defective mutations of these genes affect cellular DNA synthesis at nonpermissive temperatures, in addition to affecting the inability to propagate phage λ at permissive temperature (12, 13). Since the temperature sensitivity mutation in strain MT112 was cotransducible with the *thr* gene, the mutation was transferred to *E. coli* K-12 strains AB1157T and RB85 by P1 transduction. The transductants (AB11571 and RB851) were unable to propagate phage λ*vh*, in contrast to the parental cells, which grow λ*vh*. Next, an experiment was done to see whether the mutation resided in the *dnaJ* or the *dnaK* gene, using strains AB11571 and RB851 and transducing phage λ carrying the *dnaJ* and/or the *dnaK* chromosomal gene. Both of the strains are able

to propagate phage λ carrying the *dnaK* gene but not the *dnaJ* gene (Table 3). This finding indicates that the mutation must be in *dnaK*. We designated the mutation *dnaK7*(Ts). Furthermore, it is suggested that the original mutant strain, MT112, carries the single mutation (*dnaK7*) related to its temperature sensitivity, because temperature-resistant revertants were obtained spontaneously in brain heart infusion broth culture at a frequency of about 10^{-9}. This possibility is supported by another finding that the temperature sensitivity mutation and inability to propagate phage λ were not separable by P1 transduction as seen in the transductants, AB11571 and RB851 (see Table 3).

Effect of temperature shift on growth of the mutant. The doubling time of the parental strain (H/r30RT) in M9 medium at 30°C was 45 min, and that of the mutant (MT112) carrying *dnaK7*(Ts) was 60 min. To determine the effect of high temperature on the growth of the mutant, the cells were incubated at 30°C in M9CA medium and shifted to 43°C (nonpermissive temperature; Fig. 1). After a 30-min lag, the increase in optical density was severely inhibited. The residual increase after the temperature shift was about 80%. The viable cell number increased about 30% after 1 h at 43°C, and no decrease in colony-forming units was observed until 4 h at 43°C. Phase-contrast microscopy revealed that a small portion of the cells became very elongated (e.g., snake form).

Effect of the temperature shift on macromolecular synthesis. The effect of the temperature shift on DNA, RNA, and protein syntheses in mutant strain MT112 was examined by continuous or pulse-labeling of radioisotopes. Figure 2 shows the pattern of macromolecular synthesis after temperature shift by pulse-labeling. RNA synthesis decreased rapidly 5 to 10 min after the shift; DNA synthesis was first stimulated and then decreased rapidly. Protein synthesis decreased at a slower rate, and, surprisingly, it continued at more than 30% of the initial value even at 120 min. Thus, RNA synthesis seemed to be initially influenced most drastically by the temperature shift. We also examined the *E. coli* K-12 *dnaK*(Ts) mutant K7561 (carrying *groP*AB756 mutation) by the same procedure. Cellular RNA synthesis as well as DNA synthesis was inhibited after the temperature shift (Fig. 3). Two temperature-resistant revertants, RB851R1 and RB851R2, isolated spontaneously from strain RB851 seemed to regain the ability to synthesize DNA, RNA, and protein at 43°C, since these revertants showed similar patterns of growth at this temperature when compared with strain RB85. Thus, it is suggested that the *dnaK7*(Ts) mutation simultaneously affects cellular nucleic acid synthesis and propagation of phage lambda (see Table 3). It is possible that a second mutation coexisting with the temperature-sensitivite mutation [*dnaK7*(Ts)] might affect RNA polymerase. Therefore, the activity of RNA polymerase extracted from strains MT112 and H/r30RT by the method of Burgess (2), with minor modifications, was tested.

Enzyme activity was examined by incorporation of [5-^{3}H]UTP (1.0 Ci/mmol; Amersham Products Inc.) into cold acid-insoluble material (11). The results showed no alteration of activity of the enzyme from MT112 at 43°C compared with that from the parental strain, H/r30RT (data not shown).

DISCUSSION

In the present study, a *dnaK7*(Ts) mutant was isolated from an *E. coli* B strain by thymineless death screening after mutagenic treat-

TABLE 3. *Efficiency of plating of phage λ carrying the dnaK and/or the dnaJ gene on E. coli K-12 strains*[a]

Bacterial strain	Efficiency of plating of phage:		
	λcI857*dnaJdnaK*	λcI857*dnaJ*Δ*145*	λcI50*dnaK*
AB1157T (temperature resistant)	1	1	1
RB85 (temperature resistant)	1	1	1
RB851R1 (temperature resistant)	0.77	1	1
RB851R2 (temperature resistant)	0.90	1	1
AB11571 [*dnaK7*(Ts)]	0.76	$<2.0 \times 10^{-3}$	0.48
RB851 [*dnaK7*(Ts)]	0.64	$<2.6 \times 10^{-3}$	0.91
K1702 (*dnaK170*)	0.80	$<2.0 \times 10^{-3}$	1
K2710 (*dnaJ2*)	1	1	$<2.6 \times 10^{-3}$

[a] Efficiency of plating denotes the number of plaques produced by a phage strain on a given bacterial host at 37°C relative to the number produced on RB85 cells. For the test, cells were grown in 3 ml of brain heart infusion broth at 37°C. After that, cells were collected by centrifugation and suspended in 1 ml of λ broth. Then 0.25 ml of the cell suspension (4×10^8 cells) and 0.1 ml of phage lysate were added to 2.5 ml of molten λ soft agar (0.5% agar). The mixture was overlaid onto a λ agar plate containing 10 μg of thymine per ml. After overnight incubation at 37°C, the number of plaques was counted.

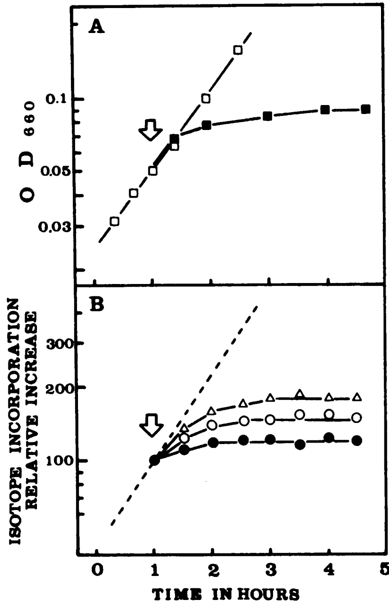

FIG. 1. *Cellular growth of strain MT112 (dnaK7). Growth in M9CA was monitored by measuring the optical density (OD). (A) Initially cells were grown at 30°C (□); then one portion of the culture (■) was transferred to 43°C as indicated by the arrow. (B) Syntheses of DNA, RNA, and protein in cells of MT112. For continuous labeling, cells were grown in M9CA medium containing radioisotopes at 30°C for 5 h. One portion was shifted to 43°C at midlog phase, as indicated by an arrow. The radioisotope incorporation at the time of temperature shift ([^{3}H]thymine, [^{3}H]uracil, and [^{14}C]leucine: 1,400, 3,800, and 2,100 cpm, respectively) was designated as 100%, and relative residual incorporation is shown. Symbols: (○) [^{3}H]thymine; (●) [^{3}H]uracil; (△) [^{14}C]leucine; (- - -) relative incorporation of radioisotopes at 30°C.*

ment. The mutant is conditionally defective in DNA, RNA, and protein syntheses. The temperature sensitivity phenotype is considered to be due to a single mutation, judging from the following findings. (i) Spontaneous revertants were obtained from brain heart infusion broth cultures. (ii) Temperature sensitivity of the macromolecular synthesis and inability to propagate phage lambda were not separable by P1 transduction.

Another temperature-sensitive *dnaK* mutant (*groP*AB756) from *E. coli* K-12 was originally selected for inability to propagate phage lambda (7). It was confirmed in the present study that these mutant cells are conditionally defective in DNA and RNA syntheses as seen in our mutant (MT112). When *E. coli* K-12 *dnaK7* transductants were tested, they were unable to propagate phage λ. Therefore, two different selective procedures are available for the isolation of *dnaK* mutants.

Since it is known in general that other *dna*(Ts) mutants are specifically defective in DNA synthesis at nonpermissive temperature, the block of RNA synthesis expressed by the *dnaK*(Ts) mutants seems to be very characteristic. The activity of RNA polymerase extracted from strain MT112 was normal at 43°C, excluding the loss of enzyme activity as responsible for the block of RNA synthesis in the *dnaK* mu-

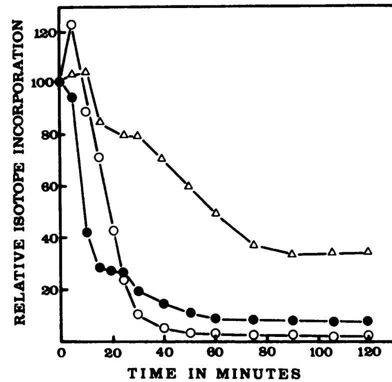

FIG. 2. *Rate of DNA, RNA, and protein syntheses of MT112 by pulse-labeling. Cells were grown in M9CA medium, and at midlog phase they were shifted up to 43°C (zero time). Aliquots were withdrawn and further incubated at 43°C in the medium containing radioisotopes. The incorporation at zero time ([^{3}H]-thymidine, [^{14}C]uridine, [^{14}C]leucine: 112,200, 33,800, and 560 cpm, respectively) was designated as 100%, and the relative incorporation is shown. Symbols: (○) [^{3}H]thymidine; (●) [^{14}C]uridine; (△) [^{14}C]leucine.*

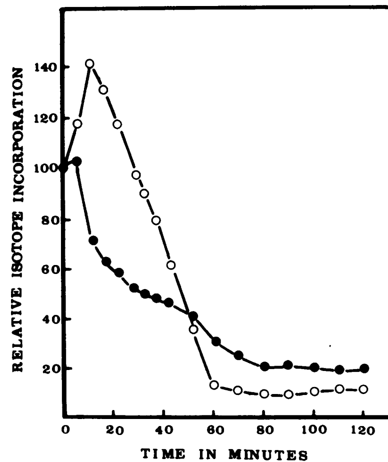

FIG. 3. *Rate of DNA and RNA syntheses of strain K7561 by pulse-labeling. Assays were carried out at 43.5°C as described in the legend to Fig. 2. The incorporation at zero time ([^{3}H]thymidine, 56,800; [^{14}C]uridine, 20,500 cpm) was designated as 100%, and the relative incorporation is shown. Symbols: (○) [^{3}H]thymidine; (●) [^{14}C]uridine.*

tants. Saito and Uchida (13) estimated the size of the *dnaK* cistron as 2.1 ± 0.4 kilobases, corresponding to a protein molecule as large as 77,000 daltons.

What is the function of the *dnaK* gene product? Elucidation of the function might lead us to an understanding of the mechanism of cellular nucleic acid synthesis and of the relationship between host function and multiplication of the lambdoid phages. Though various *dna* mutants have been isolated so far, the gene products still remain unknown, except in a few cases. DNA replication is currently thought of as a complex series of events in which many proteins operate together functionally and perhaps structurally. Thus, the *dnaK* gene product may operate as a part of such a complex for DNA synthesis of the cells and the lambdoid phages, in addition to cellular RNA synthesis.

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