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Genetic Analysis of Two Genes, *dnaJ* and *dnaK*, Necessary for *Escherichia coli* and Bacteriophage Lambda DNA Replication*

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Summary. We show that a collection of 93 *E. coli* mutations which map between *thr* and *leu* and which block phage lambda DNA replication define two closely linked cistrons. Work published in the accompanying paper shows that these mutations also affect host DNA replication, so we designate them *dnaJ* and *dnaK*; the gene order is *thr–dnaK–dnaJ–leu*. Demonstration of two cistrons was possible with the isolation of lambda transducing phages carrying one or the other or both of the *dna* genes. These phages were employed in phage *vs* bacterial complementation studies which unambiguously show that *dnaK* and *dnaJ* are different cistrons.

Introduction

Several groups have recently described mutations of *E. coli* which interfere with replication of bacteriophage lambda and which map between *thr* and *leu*. Because lambda mutants with an altered *P* gene product ($\lambda\pi$'s) are able to grow on these strains, the bacterial genetic designations given were: *groPC756* for the mutant studied by Georgopoulos, 1977 (formerly called *groPAB756*, Georgopoulos and Herskowitz, 1971); *groPC259* for the mutant studied by Sunshine, et al. 1977; and 91 mutants isolated by Saito and Uchida, 1977, were designated *grpC*. We now report experimental evidence that these mutations fall into two cistrons. These new findings necessitate some changes in the genetic designations.

Designations used in an analogous situation provide a rationale for renaming these mutants. Two previously studied classes of host mutations, called *groPA* and *groPB* were later found to map in the *dnaB* gene (Georgopoulos and Herskowitz, 1971). The primary genetic designation of the host gene is *dnaB*, and GroPA and GroPB remain as phenotypic symbols for those *dnaB* mutations that confer the GroP$^-$ phenotype. The Gro designations are useful when one characterizes strains for ability to support lambda growth.

Saito and Uchida (1977) have already presented experiments indicating that the *groPC756* mutation affects host DNA metabolism and accordingly they renamed the gene *dnaK*.

Evidence that mutations corresponding to *groPC259* are also in a gene affecting host DNA metabolism will be presented in the accompanying paper (Saito and Uchida, 1978), and thus this gene is renamed *dnaJ*. The designation *groPC* should not be retained for mutations mapping in *dnaJ*. We suggest using GroPF or GrpF as alternative phenotypic designations for *dnaJ* mutations. To simplify presentation of results, we adopt these designations for all the studies presented.

Materials and Methods

Strains. Principal strains are listed in Table 1. Several derivatives of MF634 and GR756 were employed: *thr*$^+$ derivatives were obtained by selecting spontaneous *thr*$^+$ revertants; *rec*Al derivatives were obtained by first selecting spontaneous streptomycin resistant (*rps*L) mutants, which were then mated with KL16–99, selecting for *lac*$^+$ streptomycin resistant recombinants. The recombinants were screened to identify those containing *rec*Al by testing for sensitivity to ultraviolet light (Feiss, et al., 1972).

Georgopoulos (1977) described a phage capable of transducing GR756 to Gro$^+$. The complete genotype of the phage is λ *Δsr*Iλ1-2 *shin*IIIλ3$^+$ *att*$^+$ *imm*21 *nin*R5 *dnaK* (see Murray and Murray, 1975, and below for details). This strain, which we will call *λdnaK*, was the starting strain from which two additional transducing phages were derived as described below. Derivatives of *λdnaK* (and *λdnaJ dnaK*) which were *imm*λ and *nin*$^+$ were recovered from standard crosses with λ*c*I857 or λ*c*I60. λ*imm*434 *c*I IS contains an unidentified insertion segment 62.4% (±0.1%) from the left (*A* gene) chromosome end. Additional phage strains mentioned were from our collections.

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Table 1. Bacterial strains

Strain	Remarks	Source/Reference
C600	*thr leu thi sup*E *lac*Y	Bachmann (1972)
MF634	C600 *dnaJ259*	Sunshine et al., (1977)
GR756	C600 *dna*K756	Georgopoulos (1977)
HR1 series	91 independent *dnaK* and *dnaJ* mutant isolates	Saito and Uchida (1977)
MF184	C600 *Δ (gal attλ bio)*	Iowa Collection
W3805	*gal*E22	E. Lederberg
KL16-99	Hfr, *thi*, *rec*A1, transfers counterclockwise with *rec*Al an early marker	Low (1968)
MF228	*sup*E, *gal*Tl, *rec*A13	Franklin (1971)
MF687	*mut*D *thr leu thi his arg lac xyl mtl ara rpsL*	Degnen et al., (1974)
MF237	*sup*°, *gal*Tl, *rec*A13	Franklin (1971)

Media. Tryptone broth (TB), TB soft agar and TB agar were as described by Campbell (1961) except each was supplemented with 0.01 M $MgSO_4$. The synthetic medium was that of Davis and Mingioli (1950), appropriately supplemented. Eosin methylene blue agar lacking sugar (EMBO) was as described by Gottesman and Yarmolinsky (1968).

P1 Transduction. We repeated the crosses described earlier using exactly the same strains and procedure (Sunshine, et al. 1977).

Lysogens. Lysogens of MF184 and *rec*A1 derivatives of MF634 and GR756 were identified by plating infected cells on EMBO agar with 10^9 *λc*I60 *dnaJ dnaK* phages and incubated at 33C. Lysogens form healthy pink colonies under these conditions (Gottesman and Yarmolinsky, 1968).

Heat-Pulse Curing. Lysogens of *c*I857 phages were grown at 30 C, shifted to 40C for 6 minutes, then grown for several hours at 30 C in the presence of anti-λ serum, K = 1, to allow segregation (Weisberg and Gallant, 1967). The cultures were then plated in the presence of anti-λ serum and colonies were tested for the presence or absence of a prophage.

Phage Deletions. Deletions were isolated as described in the accompanying report (Saito and Uchida, 1978).

Electron Microscopy. Heteroduplex analysis was carried out as described by Davis, et al. (1971); the accompanying report (Saito and Uchida, 1978) contains additional details.

Results and Discussion

1. Transducing Phages Provide Evidence for Two Cistrons

Georgopoulos (1977) described a transducing phage, here called *λdnaK*[1], which was able to grow on (and transduce to Gro$^+$) GR756 but not MF634. This observation raised the possibility that the mutations in the two bacterial strains were not allelic. We have accumulated additional information by further study of *λdnaK* and by the isolation of two derivatives, *λdnaJ dnaK* and *λdnaJ*. The isolation and structure of the three phages is discussed below. The phages divide the bacterial mutants MF634, GR756 and the 91 mutants of Saito and Uchida into two classes. *λdnaJ dnaK* is able to grow on all the strains. *λdnaK* is able to grow on GR756 and three of the isolates of Saito and Uchida, *dnaK165*, *dnaK170* and *dnaK515*. *λdnaJ* is able to grow on MF634 and the remaining 88 isolates of Saito and Uchida, including *dnaJ2*, *dnaJ70* and *dnaJ285*. These results are consistent with the existence of two cistrons into which the 93 mutations fall; additional genetic experiments are presented below.

2. The Order of Genes is thr dnaK dnaJ leu

To order the *dnaJ259* and *dnaK756* mutations with respect to *thr*, we performed the reciprocal P1 crosses between the mutants that are diagrammed in Figure 1. In cross I, the donor was *thr*$^+$ *dnaJ259* and the recipient (GR756) was *thr*$^-$ *dnaK756*. *thr*$^+$ recombinants were selected and scored as Gro$^+$ or Gro$^-$: of 283 *thr*$^+$ recombinants, 13 were Gro$^+$ (these results include 50 *thr*$^+$ recombinants scored in a previous study by Sunshine, et al., 1977). For the reciprocal cross the donor was *thr*$^+$ *dnaK756* and the recipient was *thr*$^-$ *dnaJ259*: of 471 *thr*$^+$ recombinants (including 122 described in Sunshine, et al., 1977) only 1 was Gro$^+$. The results are consistent with the marker order *thr dnaK dnaJ leu*.

3. Origin of the Transducing Phages

Phage *λdnaK* was isolated from a pool of transducing phages that had been generated by in vitro insertion of *E. coli* DNA segments into the chromosome of the cloning vector strain of lambda called phage 540 (Murray and Murray, 1975). Insertion was accomplished by the annealing and ligation of *E. coli* and phage 540 DNA preparations that had been digested with the *hind*III restriction enzyme. Phage 540 contains one *hind*III site, at 56.8 on the lambda chromosome (where 0 is at the left, or *A* gene, chromosome end; and 100 is at the right, or *R* gene, end of the wild type lambda chromosome). Thus the bacterial DNA segment is expected to be inserted at 56.8.

In attempting to isolate *λdnaJ dnaK*, we reasoned that, since the mutations in GR756 and MF634 were closely linked, integration of *λdnaK* into the bacterial

[1] Phage *λdnaK* contains a number of genetic alterations including *imm*21 and *nin*R5, as described in Materials and Methods and in section 4, below. For simplicity we will omit references to these alterations except when necessary

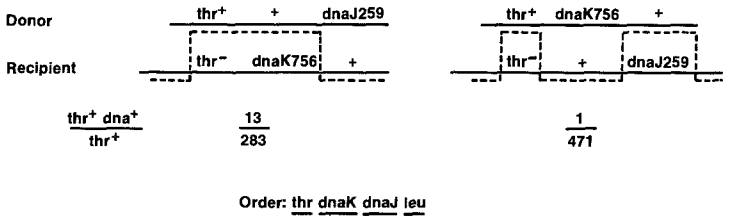

Fig. 1. Ordering of *dnaJ259* and *dnaK756* by phage P1-mediated general transduction

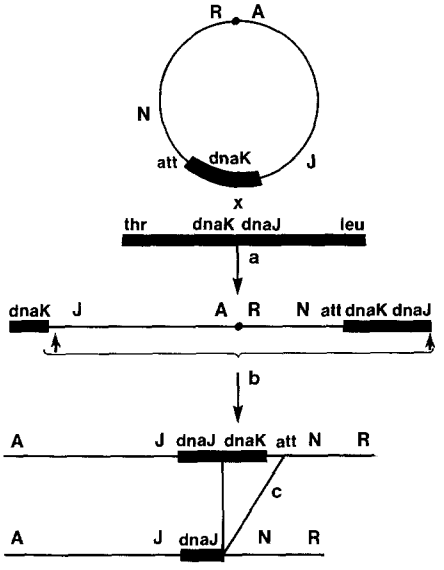

Fig. 2a–c. Structure of *λdnaK* and the origin of derivatives *λdnaJ dnaK* and *λdnaJ*. Step (a) shows the integration by general recombination of *λdnaK* into the *dnaK* region of MF184, a strain lacking the normal lambda attachment site (att*λ*). Step (b) shows the illegitimate recombination event giving rise to *λdnaJ dnaK*. Step (c) was the isolation of *λdnaJ* by pyrophosphate selection from a stock of *λdnaJ dnaK*. The arrangement of markers (see text) is derived from (a) the order of *dnaK* and *dnaJ* on the bacterial chromosome and the structure of *λdnaJ* which contains a deletion removing *dnaK* and part of *attλ*

chromosome by homologous recombination would place the resulting prophage in close proximity to the bacterial gene(s) altered in MF634 and GR756 (this is diagrammed in Figure 2).

We lysogenized MF184, which is *thr⁻ leu⁻ Δ (gal attλ bio)* with *λdnaK*. The prophage was shown to be in the correct position by a P1 cross: P1 grown on W3805, a prototrophic non-lysogen, was used to transduce MF184 (*λdnaK*) to *thr⁺ leu⁺*. Of 50 such transductants 38 no longer carried the *λdnaK* prophage. Control recipient cells were mock-infected and plated on a complete medium; all 50 tested retained the *λdnaK* prophage.

MF184 (*λdnaK*) was induced with ultraviolet light and the resulting lysate was plated on MF634. Plaques were obtained at a frequency of 2×10^{-5} (relative to the titer on C600); one of these was purified and designated *λdnaJ dnaK*, as it retained the ability to grow on GR756. A control plating of a plate lysis stock of *λdnaK* contained $< 10^{-7}$ phage able to plate on MF634.

Phage *λdnaJ* is a deletion mutant derivative of *λdnaJ dnaK*. Deletion mutants were selected from a lysate of *λdnaJ dnaK* (an *immλ nin⁺* strain of *λdnaJ dnaK* was used) by repeated cycles of pyrophosphate selection (Parkinson and Huskey, 1971). Among deletion mutants, *λdnaJ* strains, able to grow on MF634 but not on GR756, were a large class.

4. Structure of the Transducing Phages

The structures of the transducing phages were determined by electron microscopic examination of heteroduplex DNA molecules formed by annealing strands of the transducing phage chromosomes and *λimm*434 *c*I IS. The transducing phage strains employed were *nin⁺* and either *immλ* or *imm*21. Reference lengths used were the position on the lambda chromosome of the non-homology with *imm*434 (73.6 to 79.1) and *imm*21 (71.1 to 79.8; Davidson and Szybalski, 1971). Position figures denote position on the wild type lambda chromosome, with 0 at the left (*A* gene) end and 100 at the right (*R* gene) end. The results are diagrammed in Figure 3 and summarized in Figure 4.

(i) *λdnaK*. An *immλ nin⁺* derivative of this phage should contain two alterations that affect the DNA content. These are the insertion of bacterial DNA at 56.8 and the deletion mutation *Δsr*1*λ*1-2, which extends from 44.5 to 54.3 (Gottesman and Adhya, 1977). Our measurements confirm this expectation (Fig. 3a, b). The *Δsr*I*λ*1-2 deletion is calculated to extend from 44.3 to 54.1, the latter position is calculated by subtracting the distance between the *immλ*–*imm*434 non-homology and the deletion loop (2.6+5.7+11.2) from the non-homology position 73.6. The position of the bacterial insertion is calculated to be at 56.7 (73.6−11.2−5.7). The size of the bacterial insertion is calculated from the information of Figure 3e. The distance from the left end of *dnaK*/*dnaJdnaK* homology to the *immλ*−*imm*21 non-homology is 25.3, which should equal the distance between hindIII site and *imm*21 (14.3) plus the size of the insertion, which is thus estimated to be 11.0.

(ii) *λdnaJ dnaK*. The measurements for this phage are shown in Figure 3c, d and e. The illegitimate

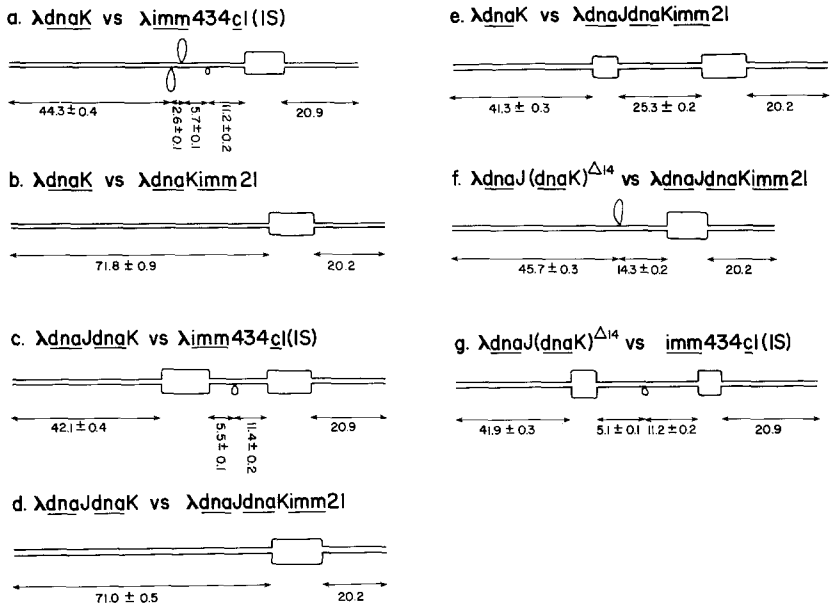

Fig. 3. Structures of heteroduplex molecules constructed by annealing of transducing phage DNA and standard phage DNA

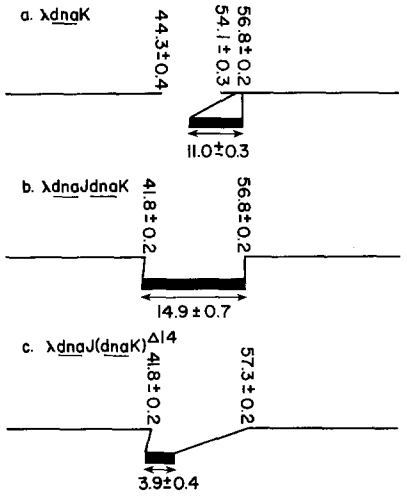

Fig. 4. Structures of the transducing phages, derived from the measurements shown in Figure 3 and described in the text

recombination event generating this phage has resulted in the loss of phage DNA so that formally, the resulting phage contains a substitution of bacterial DNA for phage DNA. The phage sequence missing extends from 41.8 (average of results in c, e, and g) to 56.8. The bacterial substitution has a length of 14.9, calculated as the difference between the duplex DNA length from 0 to the *imm*λ−*imm*21 nonhomology (71.0) minus the phage DNA length in that segment (56.1 or 71.1 minus the 15.0 deleted phage segment). This information is summarized in Figure 4b.

(iii) λ*dnaJ*. One of a number of isolates has been examined. This strain (*Δ*14) is a deletion derivative of λ*dnaJdnaK*; the measurements are shown in Figure 3f and g. The deletion has removed an 11.5 unit segment from the right part of the bacterial DNA segment. The right deletion endpoint is calculated to be at 57.3 on the chromosome. This would coincide with the *att*λ site: *Δ*14 phage has intact *int* and *xis* genes but it is unable to form a stable lysogen (Saito and Uchida, 1978). Moreover, many deletion mutations isolated in *int*$^+$ strains of lambda do originate at *att*λ (Parkinson and Huskey, 1971). At any rate, the bacterial segment remaining in this λ*dnaJ* strain is calculated to be 3.9% the length of the wild type lambda chromosome. The deletion serves to order the *dna* mutations on the λ*dnaJ dnaK* chromosome as *A*−*dnaJ*−*dnaK*−*R*, this information was used in the drawing of Figure 2.

5. Mutant Derivatives of λdnaJ dnaK Give Further Evidence for Two Cistrons

We isolated mutant derivatives of λ*dnaJ dnaK* to see if loss of ability to grow on one of the two Gro bacterial mutants would be accompanied by loss of ability to grow on the other, as expected if there were a single gene. Phage λ*dnaJ dnaK* was mutagenized by growth on MF687, a strain carrying the mutator mutation *mut*D. The *mut*D strain introduces point mutations (Fowler, et al., 1974). Phages mutated in the *dnaJ dnaK* segment were identified by a mixed indica-

Table 2. Complementation properties of bacterial heterozygotes

Markers		λ*imm*21	λ*imm*21 *dnaJ dnaK*	Number cured in heat pulse curing
Chromosome	Prophage			
dnaJ$^+$ *dnaK*$^+$	*dnaJ*$^+$ *dnaK*$^+$	+	+	4/10
dnaJ$^+$ *dnaK*$^+$	*dnaJ*$^+$ *dnaK*$^-$	+	+	3/10
dnaJ$^+$ *dnaK*$^+$	*dnaJ*$^-$ *dnaK*$^+$	+	+	2/10
dnaJ$^-$ *dnaK*$^+$	*dnaJ*$^+$ *dnaK*$^+$	+	+	2/10
dnaJ$^-$ *dnaK*$^+$	*dnaJ*$^+$ *dnaK*$^-$	+	+	3/10
dnaJ$^-$ *dnaK*$^+$	*dnaJ*$^-$ *dnaK*$^+$	−	+	4/10
dnaJ$^+$ *dnaK*$^-$	*dnaJ*$^+$ *dnaK*$^+$	+	+	3/10
dnaJ$^+$ *dnaK*$^-$	*dnaJ*$^+$ *dnaK*$^-$	−	+	2/10
dnaJ$^+$ *dnaK*$^-$	*dnaJ*$^-$ *dnaK*$^+$	+	+	3/10

Bacterial strains were MF237 (*dna*$^+$ *recA*$^-$) and *recA*$^-$ derivatives of MF634 and GR756. Prophages were λ*c*I857 *dnaJ*$^+$*dnaK*$^+$ and the point mutant derivatives (see text). Duplicates of each lysogen were tested. Structures of lysogens were confirmed by (a) testing genotype of phages released upon induction and (b) testing bacterial genotype of heat-pulse cured derivatives

tor technique: phage particles were adsorbed to a Gro$^+$ host (C600 or MF228), mixed with one of the Gro$^-$ hosts (MF634 or GR756), and plated in a soft agar layer. Mutants were identified as those phages forming turbid plaques; these were purified and characterized. All mutant phages unable to grow on one of the Gro$^-$ strains were still able to grow on the other. We isolated 10 independent λ*dnaJ*$^+$ *dnaK*$^-$ mutants, and 5 independent λ*dnaJ*$^-$ *dnaK*$^+$ mutants. Each of the mutants is presumed to contain a point mutation, and to examine this one mutant from each class was banded in CsCl and the DNA content of each was found to be the same as the parent phage λ*dnaJ*$^+$ *dnaK*$^+$.

These results represent strong evidence for two cistrons in that loss of ability to grow on one Gro$^-$ host was not accompanied by loss of the ability to grow on the other host. The principal alternative hypothesis, interallelic complementation, is unlikely because one does not expect that each of the 15 mutations described above would have left intact the ability of the altered polypeptide to complement the polypeptide specified by the other Gro$^-$ host[2].

The complementation behavior of the phage mutants was verified in bacterial diploids by constructing lysogens of *rec*A derivatives of MF634 and GR756. This was done to verify that the complementation behavior observed in the previous study was not influenced by the high dosage ratio of the bacterial genes carried on the replicating phage chromosome to those on the host chromosome.

The results of this study, presented in Table 2, are completely consistent with the phage *versus* bacterial complementations presented above.

[2] Recently, we have identified several nonsense mutant derivatives of λ*dnaJ dnaK*. Each such mutant has lost the ability to complement one of the two *dna* functions while retaining the ability to complement the other (Yochem, Rudolph and Feiss, unpublished)

Concluding Remarks

Division of the mutations in the *thr-dap*B interval into two cistrons increases to five the number of host genes which mutate to GroP (Grp). For extensive discussion of these mutants the reader may consult earlier publications (Georgopoulos and Herskowitz, 1971; Georgopoulos, 1977; Saito and Uchida, 1977; Sunshine, et al., 1977) and the accompanying report (Saito and Uchida, 1978). Three of the genes, so far, have been shown to play a role in host DNA synthesis (*dnaB, dnaJ* and *dnaK*) and it seems reasonable to expect the remaining two, *grpD* and *grpE,* will also be shown to be involved in host DNA synthesis. Study of the molecular basis for the interaction of the products of these five genes with the product of the lambda *P* gene should prove a fascinating endeavor.

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