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Novel *dnaG* Mutation in a *dnaP* Mutant of *Escherichia coli*

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Reexamination of the *dnaP18* mutant strain of *Escherichia coli* revealed that the mutation responsible for the arrest of DNA replication and cell growth at high temperatures resides in the *dnaG* gene rather than in the *dnaP* locus as previously thought; this mutation has been designated *dnaG2903*.

The *dnaP* gene locus has been assigned, on the standard linkage map of *Escherichia coli* (1), at about 85 min between *ilv* and *metE* (16). Recently we attempted to clone the *dnaP* gene without success, and as a consequence we reexamined the map position of the mutant allele *dnaP18*. The mutant strain used in this study was KY2903 (Table 1), which is, as originally reported (16), a conditionally lethal mutant; at 30°C its growth is normal, but at 42°C DNA synthesis is arrested and the number of viable cells decreases. A lysate of phage P1 *vir* was prepared by infecting a pool of wild-type *E. coli* cells which had been infected with λNK55 (7) and grown on nutrient agar with tetracycline, selecting for those which acquired the transposon Tn*10*. When strain KY2903 was infected with the P1 lysate, plated on nutrient agar with tetracycline, and incubated at 42°C, a few colonies grew up. They were picked, purified by repeated single-colony isolations, and used as donor strains for the next transductional crosses with phage P1. For instance, a P1 lysate of one of the donor strains, KN641, upon infection converted the temperature-sensitive phenotype (Ts) of KY2903 to the wild type (Ts$^+$, temperature insensitive); the selected marker was tetracycline resistance (Tc^r) due to the presence of Tn*10* in the vicinity of the unselected marker (Ts$^+$). The cotransduction frequency between Tc^r and Ts$^+$ was approximately 70%. A recipient-type transductant (Tc^r Ts::KN644) was next used as the donor for a P1-mediated transduction with KN250 (a wild-type strain derived from W3110) as the recipient. The cotransduction frequency between the selected marker (Tc^r) and the unselected marker (Ts) was again 70%. The Tc^r Ts transductant of KN250, named KN654, was characterized and confirmed to be identical to KY2903 with respect to DNA arrest and lethality at high temperatures.

The map position of the Ts allele in KN654 was determined by crosses of the strain with a set of male bacteria, each of which harbored an F′ plasmid with a segment of the *E. coli* chromosome (9; obtained from the *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.). Among 19 F′ strains tested, strains F122 and F140 yielded Ts$^+$ exconjugants, and the remaining 17 F′ strains did not. The overlap of the chromosomal segments carried by F122 and F140 spans the region from 65 to 70 min on the linkage map, where such genes as *tolC*, *dnaG*, and *rpoD* are known to reside. P1-mediated transductional mapping revealed that Tc^r was due to *zgh-641*::Tn*10*, which resided halfway between the *tolC* and the *dnaG-rpoD* cluster (Fig. 1). Additional mapping experiments with transposons, e.g., *zgh-*

TABLE 1. Bacterial strains

Strain	Relevant genotype[a]	Derivation, source, or reference[b]
KY1411	*rpoD40*	14
KY2750	*dnaG2903 sdg-2750*, and a temperature-sensitive mutation	16; this work
KY2903	*dnaG2903 thyA*	Equivalent to KY2901 (16); this work
KN250	*thyA*	6
KN641	KY2903 *dna*$^+$ *zgh-641*::Tn*10*	This work: P1 transduction, a pool of *dna*$^+$ cells with Tn*10*→KY2903
KN644	KY2903 *zgh-641*::Tn*10*	This work: P1 transduction, KN641→KY2903
KN654	KN250 *dnaG2903 zgh-641*::Tn*10*	This work: P1 transduction, KN644→KN250
KN663	KN250 *tol C663*	This work: spontaneous mutant
KN673	KN663 *dnaG2903 zgh-641*::Tn*10*	This work: P1 transduction, KN654→KN663
KN716	KN663 *tol*$^+$ *dnaG3*	This work: P1 transduction, PC3→KN663
KN732	KN673 *tol*$^+$ free of Tn*10*	This work: P1 transduction, KN250→KN673
KN823	KN732 Rif^r *thy*$^+$ *recA1* (*dnaG2903*)	This work: conjugation, KL16-99→KN732 Rif^r
KN825	KN716 Rif^r *thy*$^+$ *recA1* (*dnaG3*)	This work: conjugation, KL16-99→KN716 Rif^r
KN1013	KN663 *tol*$^+$ *dnaG308*	This work: P1 transduction, a derivative of CR34/308→KN663
PC3	*dnaG3 thyA47*	2, 17; CGSC 5932
CR34/308	*dnaG308 thyA6*	4, 10; CGSC 3640
KL16-99	*recA1* (Hfr)	8; CGSC 4206
WZ57	*rpoD2*	15
285c	*rpoD285*	5
YN543	285c *rpsL recA1*	11

[a] For gene symbols, see reference 1. *sdg*, Suppressor of *dnaG*. Rif^r, rifampin resistant. All strains except KL16-99 are F$^-$.

[b] "P1 transduction, A→B" and "Conjugation, A→B" designate phage P1-mediated transduction and conjugational mating, respectively; A is the donor and B is the recipient. CGSC strains were from B. J. Bachmann, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

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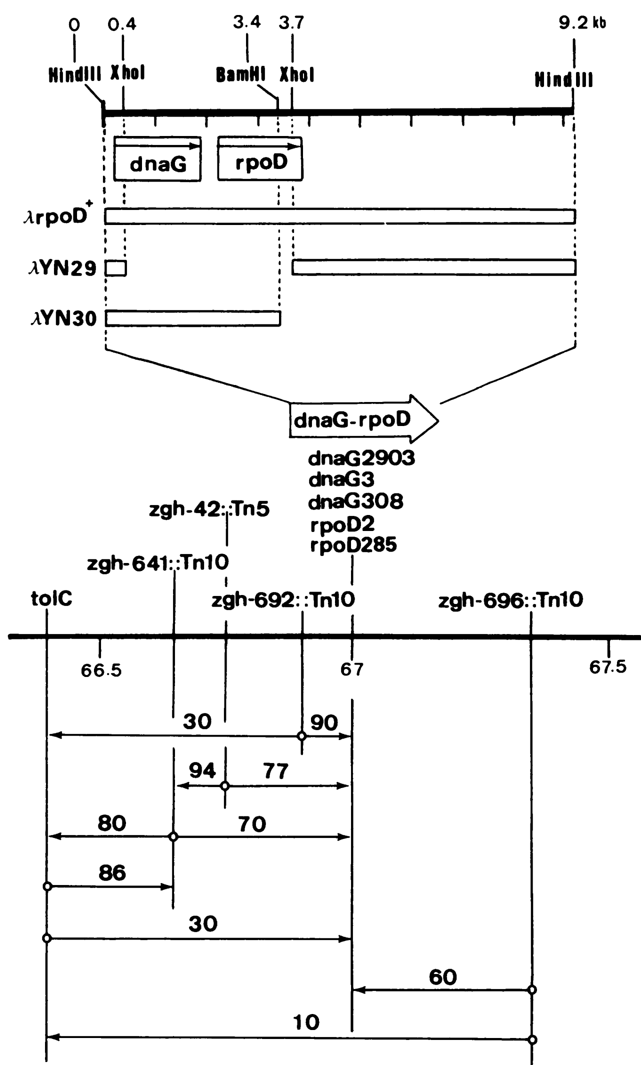

FIG. 1. (Top) The region of the *E. coli* chromosome carried by λ *rpoD*+ and its deletion derivatives λYN29 and λYN30. Redrawn after Nakamura (11) and Wold and McMacken (20). (Bottom) Transductional mapping of *dnaG2903* with phage P1 *vir*. Numbers above arrows represent the cotransduction frequency (percent) between the two markers. Selected markers are shown at the tails of arrows, and unselected markers are shown at the head. Each frequency is the average of results of more than two experiments. The typical strains used for *dnaG2903* were KY2750, KY2903, KN654, and KN732. In addition, such strains as KN641, KN663, KN1013, PC3, WZ57, and 285c were used.

692::Tn*10*, *zgh-696*::Tn*10*, and *zgh-42*::Tn*5*, confirmed that the Ts allele was located at 67 min, as were such mutations as *dnaG3*, *dnaG308*, *rpoD2*, and *rpoD285* (Fig. 1). Since this region has been cloned, determination of the mutant locus was facilitated by such transducing phages as λ *rpoD*+, λYN29, and λYN30, as well as some plasmids which contain within pBR322 the 9.2-kilobase *Hin*dIII fragment with the *dnaG* and *rpoD* cistrons (Fig. 1); pYN48 and pYN51 carry the *rpoD*+ gene and the mutations *dnaG9* and *dnaG24*, respectively (12), and pYN62 and pYN68 carry the *dnaG*+ gene and the mutations *rpoD32* and *rpoD40*, respectively (13). The results are summarized in Table 2, showing the following. (i) The Ts mutant was transduced to Ts+ only by those phages that also effectively transduced *dnaG3*. (ii) The Ts mutation (in the presence of *recA1*) was complemented by those plasmids that carried the *dnaG*+ *rpoD* genes, but not by those plasmids that carried the *dnaG rpoD*+ genes. This pattern was reproduced with *dnaG3* (*recA1*) and was reversed with *rpoD285* (*recA1*), indicating that the Ts allele resided in *dnaG*; thus it was named *dnaG2903*. (iii) The *dnaG2903* mutation could be considered recessive to the wild type.

Since strain KY2903 can be transduced by phage P1 to Ts+ by using such linked markers as *tolC* or *zgh*::Tn*10*(Tn*5*) elements as the selected markers (Fig. 1), KY2903 does not seem to carry any Ts mutation other than *dnaG2903*. The ancestral strain of KY2903 is KY2750, which is the one originally isolated as temperature sensitive and characterized as bearing the *dnaP18* mutation (16). Reexamination of KY2750 revealed that it contains, besides *dnaG2903*, a second Ts mutation and a mutation which suppresses the phenotype of *dnaG2903*. The relationship between the suppressor and the second Ts mutation is not known. There is a possibility that the second Ts mutation represents *dnaP18*, and if so it should be linked to the *ilv* gene at 84.5 min. P1-mediated transductional analysis, however, produced no evidence for the linkage. P1 grown on KY2750 was used as donor to change a number of *ilv* Ts+ recipient strains to *ilv*+; among these no temperature-sensitive transductant was detected. The *dnaP18*(Ts) mutation recognized to be linked to *ilv* in the original strain has probably reverted to the wild type. There is no mutant allele other than *dnaP18* reported so far which provides evidence for the presence of the *dnaP* gene. Our extensive search, using localized mutagensis techniques, for new alleles of the *dnaP* gene in the *ilv-metE* region has not been successful.

Other than the phenotype of *dnaG2903* already mentioned, the mutant has a number of additional characteristics. We observed that phage λ could not multiply at 42°C on a strain with the mutation. It was reported that the temperature-sensitive phenotype of the typical *dnaG* mutants (such as *dnaG3* and *dnaG308*) could be partially suppressed by a plasmid-coded primase if a plasmid such as ColI *drd-1* or R64 *drd-11* was introduced into *dnaG* mutants (18, 19). This was found also to be the case with our *dnaG2903* phenotype. A *dnaG2903* strain spontaneously produced Ts+ revertants at a rather high frequency (ca. 10^{-5}); the majority of these

TABLE 2. Complementation analysis of *dnaG2903*[a]

Mutation	Complemented by:						
	λ *rpoD*+	λYN29	λYN30	pYN48 (*dnaG9*)	pYN51 (*dnaG24*)	pYN62 (*rpoD32*)	pYN68 (*rpoD40*)
dnaG2903	+	−	+				
dnaG3	+	−	+				
rpoD40	+	−	−				
dnaG2903 (*recA1*)				−	−	+	+
dnaG3 (*recA1*)				−	−	+	+
rpoD285 (*recA1*)				+	+	−	−

[a] Bacterial strains used were KY1411, KN654, KN716, KN732, KN823, KN825, and YN543. Complementation with λ phages was determined by cross-streak tests: a loopful of each cell suspension (10^8 to 10^9 cells per ml) was cross-streaked against each phage suspension (10^8 to 10^9 PFU/ml) on nutrient agar plates, which were incubated overnight at 42°C. +, Growth occurred at the cross area; −, no growth occurred. Complementation by plasmids was analyzed by transforming the bacterial strains with the plasmids (3), and ability (+) or inability (−) to grow at 42°C was determined for each transformant.

are not true revertants but are mostly due to extragenic suppressors. This property of *dnaG2903* may be useful in elucidating the role of the primase in DNA replication by means of genetic analysis of the factors that functionally interact with the *dnaG* gene.

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