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Physiological Properties of Cold-Sensitive Suppressor Mutations of a Temperature-Sensitive *dnaZ* Mutant of *Escherichia coli*

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Suppressors of a temperature-sensitive *dnaZ* polymerization mutant of *Escherichia coli* have been identified by selecting temperature-insensitive revertants. Those suppressed strains which concomitantly became cold sensitive were chosen for further study. Intragenic suppressor mutations, which caused cold-sensitive defects in DNA polymerization, were located in *dnaZ* by transduction with λ *dnaZ*$^+$ phages. Extragenic suppressor mutations were mapped within the initiation gene *dnaA*. These suppressor-containing strains were defective in initiation at low temperature as determined by measurements of DNA synthesis in vivo and in toluene-treated cells. The occurrence of suppressor mutations of *dnaZ*(Ts) within the *dnaA* gene is considered evidence that the *dnaA* and *dnaZ* products interact in vivo. A second indication of a *dnaA-dnaZ* protein-protein interaction was provided by the observation that the introduction of additional copies of the *dnaZ*$^+$ gene into a strain carrying the *dnaA* suppressor mutation was lethal [whether the strain was *dnaZ*$^+$ or *dnaZ*(Ts)].

Components of the *Escherichia coli* DNA replication system have been defined by genetic and biochemical studies. The DNA synthesis genes can be classified as initiation (*dnaA*, *-C*, *-I*, and *-P*) (3, 9, 11, 17, 23, 41) and polymerization (*dnaB*, *-C*, *-E*, *-G*, *-N*, *-X*, *-Y*, and *-Z* and *ssb*) (9, 12, 14, 16, 17, 31, 34, 38, 43, 45) specific. Biochemical studies with phage DNAs as templates in vitro have identified protein products of several *dna* genes as well as replication proteins for which genes have not yet been identified. DNA polymerase III holoenzyme, the enzyme which catalyzes chromosome replication, can be prepared as a complex of at least eight factors (α, ε, θ, β, γ, δ, τ, and ζ) (7, 21, 29). This implies that replication in vivo is catalyzed by a multiprotein complex.

It is possible to identify in vivo protein-protein interactions by isolating extragenic suppressor mutations which correct temperature-sensitive (Ts) defects (20, 44). Thus, interactions of replication components were investigated by characterizing suppressor mutants of a *dnaZ*(Ts) polymerization mutant. The *dnaZ* protein is the γ subunit of DNA polymerase III (19). To facilitate study of the suppressor mutations, only those which caused a cold-sensitive (Cs) phenotype, in addition to their temperature insensitivity (Ts$^+$), were analyzed. A total of 54 independent Cs mutants were found among about 6,000 Ts$^+$ revertants (42).

The *dnaZ*(Ts) suppressors have been divided into two classes by physiological and genetic analyses. About half of them map very near the original *dnaZ*(Ts) mutation and confer a polymerization defect on the cells (42). They probably are intragenic suppressor mutations. About one-third map within the *dnaA* initiation gene (42) and confer phenotypes expected of initiation-defective mutants. Properties of the suppressor-containing strains and additional evidence that *dnaA* protein interacts with other replication factors are reported here and in the accompanying paper (4).

MATERIALS AND METHODS

Strains. The bacterial strains used are listed in Table 1. The suppressor-containing mutants are spontaneous derivatives of strain AX733, a *dnaZ2016*(Ts) mutant (10).

Media. Yeast extract-tryptone (YET) (18) was supplemented with thymine (50 μg/ml) as needed. Minimal medium (18) was supplemented with glucose (10 mg/ml), thiamine-HCl (5 μg/ml), Casamino Acids (50 μg/ml), and thymine (50 μg/ml) as needed. Kanamycin

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TABLE 1. Principal bacterial strains and plasmids used

Strain/plasmid	Relevant genotype	Source or reference
Strain		
AX727	*dnaZ2016*(Ts)	12
AX733	*dnaZ2016*(Ts) *thyA deo*	10
Z1	*dnaZ2017*(SUZ2016, Cs)	Spontaneous mutant of AX733
Z7	*dnaZ2018*(SUZ2016, Cs)	Spontaneous mutant of AX733
S1	*dnaA71*(SUZ2016, Cs)	42; spontaneous mutant of AX733
S2	*dnaA72*(SUZ2016, Cs)	42; spontaneous mutant of AX733
S3	*dnaA73*(SUZ2016, Cs)	42; spontaneous mutant of AX733
JR1	*dnaA*$^+$ *rbs*::Tn*406*	42
JR8	*dnaZ*(Ts) *dnaA*(SUZ, Cs) *rbs*::Tn*406*	Transductant of S1 by P1 grown on JR1
KL198	*ilvD pyrD trp his polA1 thyA deo bgl galK rpsL rpsE*	B. Bachmann
DK3	*dnaA*$^+$ *ilv*$^+$	Transductant of KL198 by P1 grown on S1
DK4	*dnaA71*(SUZ, Cs) *ilv*$^+$	Transductant of KL198 by P1 grown on S1
GM241	*dnaA*$^+$ *dnaZ*$^+$	16
JR10	*dnaA71*(SUZ, Cs) *rbs*::Tn*406*	Transductant of GM241 by P1 grown on JR8
JR11	*dnaA*$^+$ *rbs*::Tn*406*	Derivative of GM241
GM36	*dnaX*(Ts)	16; derivative of GM241
RV-F*lac*	F*lac*$^+$	M. Malamy
W3747	*met*/F13 *lac*$^+$ *dnaZ*$^+$ *dnaX*$^+$ *purE*$^+$	16
AX1001	*thyA* mutant of Hfr AB2528	
KL14	Hfr	B. Bachmann
KL226	Hfr	B. Bachmann
Hfr H	Hfr	B. Bachmann
Plasmid		
pJH16	*dnaZ*$^+$ *dnaX*$^+$	Fragment in pBR322
pJH16::Tn*5*-3	*dnaZ*$^+$ *dnaX*$^+$	Derivative of pJH16
pJH16::Tn*5*-16	*dnaZ dnaX*	Derivative of pJH16
pAL1	pJH16::Tn*2301* [*dnaZ*$^+$*X*$^+$]	
pAL2	pJH16::Tn*5*-16::Tn*2301* [*dnaZ*$^-$*X*$^-$]	

(25 μg/ml), tetracycline (25 μg/ml), carbenicillin (500 μg/ml), and streptomycin (200 μg/ml) were added as needed.

Mass and RNA and DNA syntheses. Absorbance at 540 nm was used as an index of growth (mass). The steady-state synthesis of RNA and DNA were followed by incorporation of [^{3}H]uracil or [^{3}H]thymine into 5% trichloroacetic acid-insoluble material. [5-^{3}H]uracil at 1 μCi/20 μg per ml or [*methyl*-^{3}H]thymine at 1 μCi/ml (0.5 μCi/μg) was added nine generations before experiments began.

DNA synthesis in toluene-treated cells. The procedure of Moses and Richardson (33) was used for DNA synthesis. Cultures were grown in YET to an absorbance of 0.5, harvested, and toluene treated for 3 min at room temperature. Cultures at 34°C which were to be "preincubated" at 20 or 42°C were diluted to such an extent that the final absorbance after preincubation was 0.5.

Conjugation. Donors growing exponentially were mixed with exponential- or stationary-phase recipients and incubated with gentle shaking.

Transformation. The procedure of Lederberg and Cohen (27) was used for transformation.

RESULTS

Suppressor mutation mapping. About half of the suppressor mutations are thought to be intragenic. They mapped between the origins of strains HfrH and KL266 and were complemented by F13 and by a set of λ *dnaZ*$^+$ phages (42; Fig. 1, Table 2). Although a second *dna* gene, *dnaX*, is thought to be adjacent to *dnaZ* (16) (Mullin, Henson, and Walker, unpublished

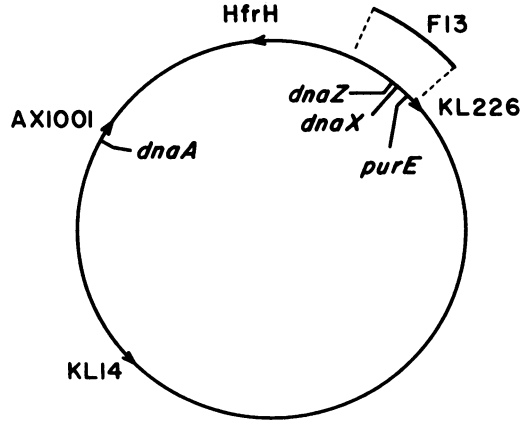

FIG. 1. *E. coli* chromosome map adapted from Bachmann and Low (5).

TABLE 2. Transduction[a] of Cs^+ by λ *dnaZ*$^+$ phages

λ *dnaZ*$^+$ phage	Recipient			
	AX727 [*dnaZ*(Ts)]	Z1 [*dnaZ*(SUZ, Cs)]	Z7 [*dnaZ*(SUZ, Cs)]	GM36 [*dnaX*(Ts)]
14	+	+	+	−
38	+	+	+	−
6	+	+	+	+
11	+	+	+	+
2	+	+	+	+
17	+	+	+	+
18	+	+	+	+
20	+	+	+	+

[a] Lawns of recipients (all λ$^+$ lysogens) were spotted with 0.05 ml of phage lysate (about 10^{10}/ml) and incubated at the nonpermissive temperature. Positive transduction was recorded if several hundred wild-type colonies formed.

data), two phages can be used to separate *dnaX* from *dnaZ*. Phages λ *dnaZ*$^+$14 and λ *dnaZ*$^+$38 carry *dnaZ*$^+$ but not *dnaX*$^+$ (16). The suppressor mutant strains Z1 and Z7 were transduced to Cs^+ by these two phages. We conclude that these suppressors probably are in *dnaZ*, and they are designated *dnaZ2017*(SUZ2016, Cs) and *dnaZ2018*(SUZ2016, Cs). The numbers *2017* and *2018* represent *dnaZ* alleles; the SUZ2016, Cs designation indicates suppression of the *dnaZ2016* allele with a concomitant cold-sensitive defect.

Strains Z1 and Z7 have been studied in detail. From strain Z7, it was possible to separate the original *dnaZ2016*(Ts) and the second suppressor mutation *dnaZ2018*(SUZ2016, Cs). Phage P1 grown on the *purE*$^+$ suppressor strain and used to transduce a *purE* recipient to *purE*$^+$ yielded Ts^+ Cs transductants. Therefore, strain Z7 retained the original Ts mutation and it acquired a second, Cs mutation in *dnaZ*. The cold sensitivity of this suppressor did not require the presence of the original *dnaZ*(Ts) mutation. Similar experiments with strain Z1 did not separate the Ts and Cs mutations. It is possible that the suppressor mutation in strain Z1 is located at the same site as the original *dnaZ2016*(Ts) mutation or that this strain carries two mutations.

About one-third of the Cs suppressors were found by mapping and complementation to lie within *dnaA* (42; Fig. 1) and are designated *dnaA*(SUZ2016, Cs). This will be abbreviated in this paper to *dnaA*(SUZ, Cs). These suppressor-containing mutants also retained the original *dnaZ*(Ts) mutation; transduction by phage P1 recovered the *dnaZ*(Ts) mutation linked to *purE* (42).

Macromolecular synthesis at 19°C. The cold-sensitive defects of both the *dnaZ*(SUZ, Cs) and the *dnaA*(SUZ, Cs) double mutants were due to inhibition of DNA synthesis. This was demonstrated when cultures growing at 34°C were shifted to 19°C for measurements of growth, RNA, and DNA (Fig. 2).

In the *dnaZ*(SUZ, Cs) strain (Fig. 2A), growth and RNA synthesis stopped gradually; the increments were 75% of the initial amounts. DNA synthesis, however, stopped immediately, with no detectable residual increase. This pattern is consistent with a polymerization defect.

In the *dnaA*(SUZ, Cs) strain (Fig. 2B), mass increase and RNA synthesis continued for at least 8 h at 19°C, increasing 250 and 160%, respectively. DNA synthesis stopped gradually and the increment was 55% over about 5 h. This result was observed in three independent *dnaZ*(SUZ, Cs) strains and is consistent with a defect in initiation. Mapping and complementation analysis indicate that these suppressors are within the *dnaA* gene (42), a known initiation gene (9, 17, 43).

Properties of *dnaA*(SUZ, Cs) mutation in *dnaZ*$^+$ genetic background. When the *dnaA*-(SUZ, Cs) mutation was transferred into a *dnaZ*$^+$ genetic background, the strain became cold sensitive. Residual DNA synthesis at 19°C was quantitatively very similar to that obtained with a *dnaZ*(Ts) *dnaA*(SUZ, Cs) strain (Fig. 2) (42).

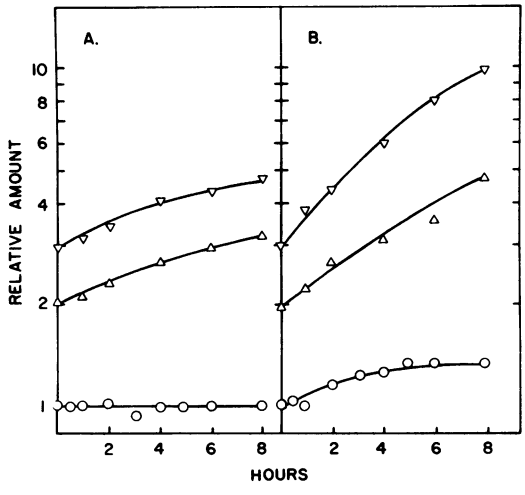

FIG. 2. The *dnaZ*(SUZ, Cs) and *dnaA*(SUZ, Cs) suppressors create defects specifically in DNA synthesis. Strains Z7 [*dnaZ2016*(Ts), *dnaZ2018*(SUZ,Cs)] (A) and S1 [*dnaZ2016*(Ts), *dnaA71*(SUZ2016, Cs)] (B) were grown at 34°C in glucose-Casamino Acids medium and shifted to 19°C at 0 h. Absorbance (▽), RNA (△), and DNA (○) were measured. Minimum absorbance values were 0.11 (A) and 0.06 (B); for trichloroacetic acid-insoluble [^{3}H]uracil, 3,000 (A) and 2,000 (B) cpm/ml; for trichloroacetic acid-insoluble [^{3}H]thymine, 4,300 (A) and 1,800 (B) cpm/ml.

The *dnaA*(SUZ, Cs) mutation in a *dnaZ*$^+$ strain also created sensitivity to high temperature. DNA synthesis and growth were inhibited, but the inhibition occurred slowly. At 42°C, growth rate was stimulated initially, but then gradually declined over an 8-h period during which mass increased 300-fold. The rate of DNA synthesis dropped immediately upon shifting to 42°C, but synthesis continued and the total amount of DNA increased 90-fold. At 44°C, mass increased 150-fold and DNA increased 40-fold (Fig. 3). The temperature sensitivity appears to be specific for DNA replication because DNA was affected more drastically than was mass increase. The mechanism of this gradual inhibition of DNA synthesis, which occurs over a period of about six generations, is unknown; models for initiation or polymerization defects are possible.

An isogenic *dnaA*$^+$ *dnaZ*$^+$ strain continued growth and DNA synthesis at 42 and 44°C for at least 11 generations at rates which were 1.3 times the 34°C rates (i.e., the rates of growth and DNA synthesis were the same at 42 and 44°C) (Fig. 4).

DNA synthesis in toluene-treated *dnaZ*$^+$ *dnaA* (SUZ, Cs) cells. DNA synthesis in toluene-treated cells depends on polymerization at existing replication forks (6). This procedure can be used to distinguish between defects in initiation and polymerization. Replicative synthesis was inhibited at 42°C in a polymerization mutant, but proceeded at 42°C in a Ts initiation mutant until replication cycles in progress were completed (32).

Replicative synthesis continued in toluene-treated *dnaZ*$^+$ *dnaA*$^+$ cells at 20, 34, and 42°C (Fig. 5). An isogenic *dnaZ*$^+$ *dnaA*(SUZ, Cs) strain also supported replicative synthesis when the culture was grown at 34°C and assayed at 20 or 42°C (Fig. 6). The rate of incorporation, compared with the wild type, was reduced at all three temperatures. At the permissive 34°C temperature, the rate of incorporation was 0.35 times the wild-type rate; at 42 and 20°C the rates were reduced to 0.2 and 0.1 times the wild-type rates, respectively.

Growth of *dnaZ*$^+$ *dnaZ*(SUZ, Cs) cells at 20 or 42°C before harvesting and toluene treatment, however, reduced replicative synthesis in subsequent assays at 20 or 42°C. Incubation of *dnaA*(SUZ, Cs) cells at 20°C, which causes gradual inhibition of DNA synthesis (42; cf. Fig. 2), gradually inactivated synthesis in vitro at

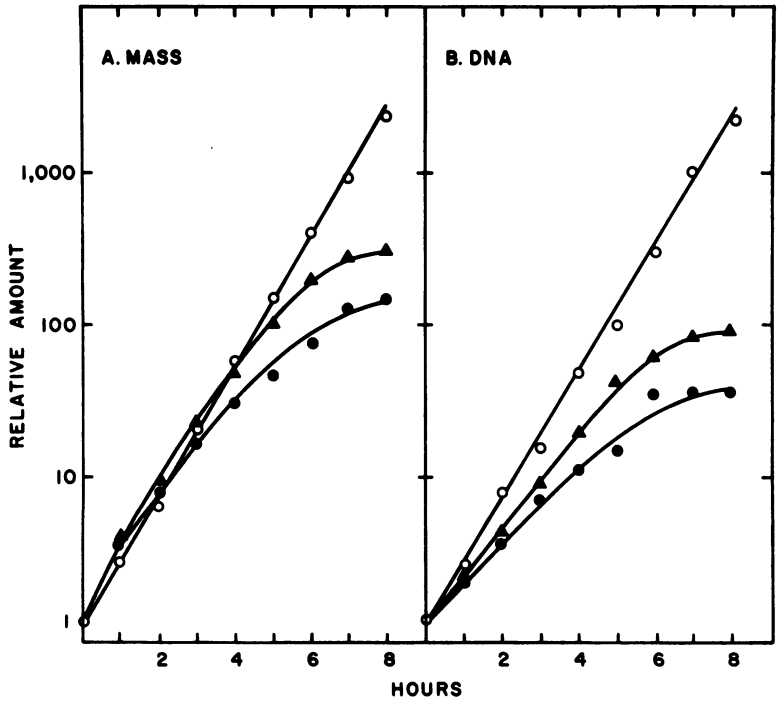

FIG. 3. Mass increase (A) and DNA synthesis (B) in a *dnaZ*$^+$ *dnaA*(SUZ, Cs) mutant at 34 (○), 42 (▲), and 44°C (●). Strain DK4 was grown in YET broth containing [^{3}H]thymine, and portions of the culture were shifted to 42 and 44°C at 0 h. Exponential growth was maintained by dilution into equilibrated media. Relative amount 1 represents 1,400 cpm of trichloroacetic acid-insoluble [^{3}H]thymine per ml and an absorbance of 0.09.

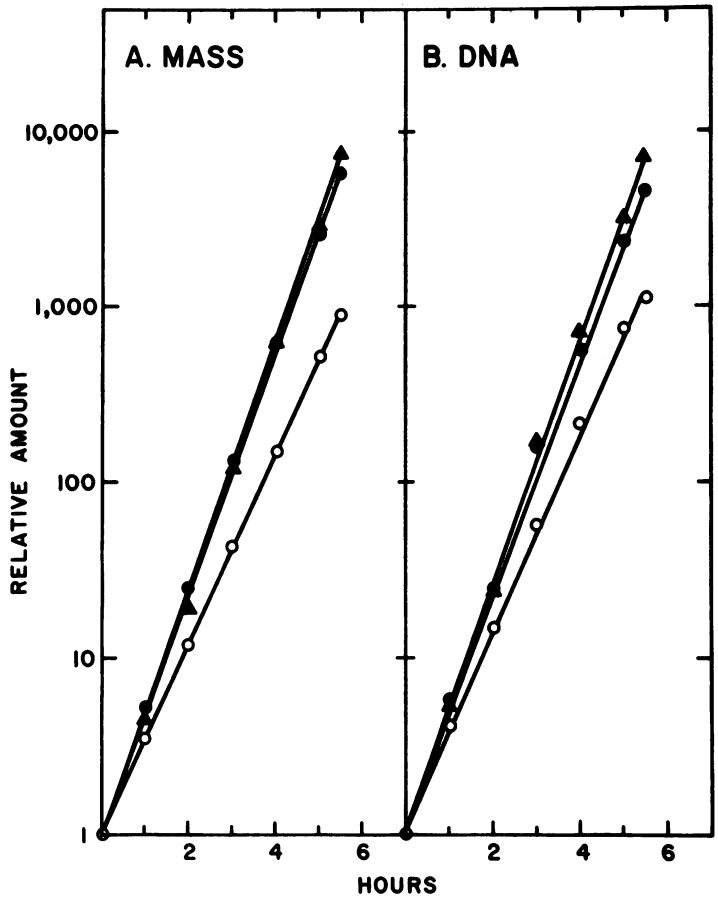

FIG. 4. Mass increase (A) and DNA synthesis (B) in a *dnaZ*$^+$ *dnaA*$^+$ strain at 34 (○), 42 (▲), and 44°C (●). Strain DK3 was grown and shifted as in the legend to Fig. 3. Relative amount 1 represents 1,500 cpm of trichloroacetic acid-insoluble [^{3}H]thymine per ml and an absorbance of 0.1.

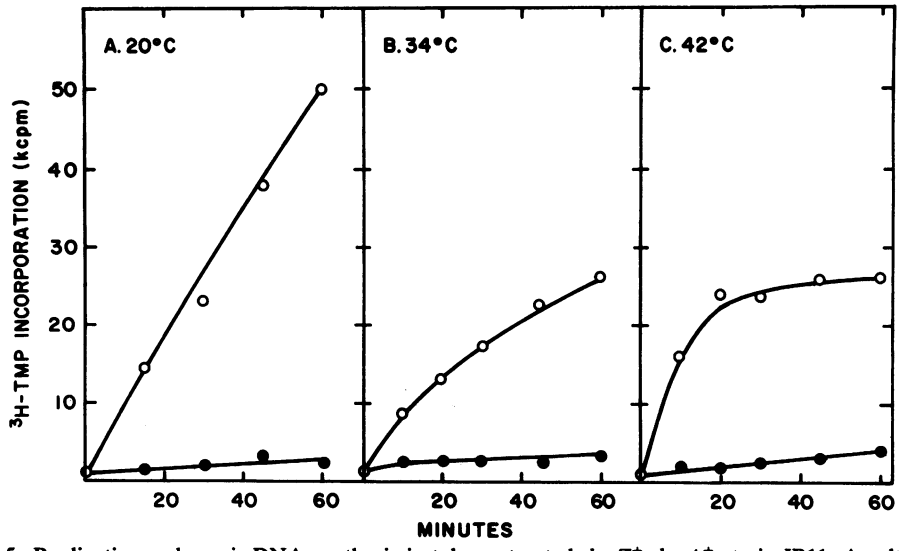

FIG. 5. Replicative and repair DNA synthesis in toluene-treated *dnaZ*$^+$ *dnaA*$^+$ strain JR11. A culture was grown in YET broth at 34°C and toluene treated, and replicative (○) (ATP-dependent) and repair (●) (ATP-independent) DNA syntheses were assayed at 20 (A), 34 (B), and 42°C (C).

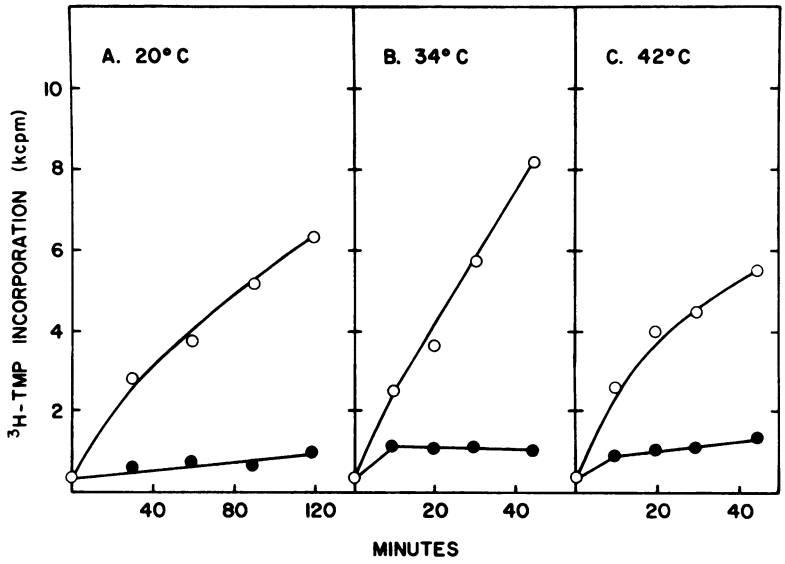

FIG. 6. Replicative and repair DNA synthesis in toluene-treated *dnaZ*$^+$ *dnaA*(SUZ, Cs) strain JR10. A culture was grown in YET broth at 34°C and toluene treated, and replicative (○) and repair (●) DNA syntheses were assayed at 20 (A), 34 (B), and 42°C (C).

20°C (Fig. 7). When the cells were preincubated at 20°C for 80 min, 66% of the in vitro activity at 20°C was eliminated. Growth of wild-type cells at 20°C did not affect DNA synthesis at 20°C in this assay (Fig. 7). Incubation of *dnaA*(SUZ, Cs) cells at 42°C, which also causes gradual inhibition of DNA synthesis (Fig. 3), also gradually inactivated subsequent replicative synthesis at 42°C (data not presented). In summary, the *dnaA*(SUZ, Cs) mutant grown at the permissive temperature was able to sustain replicative synthesis in toluene-permeabilized cells at 20 or 42°C, albeit at reduced rates, but growth of the mutant at either of the nonpermissive temperatures gradually inactivated this replicative synthesis ability. These results are consistent with initiation defects at 20 and 42°C in this mutant. A polymerization defect at 42°C is not excluded.

A *dnaA*(SUZ, Cs) mutant is unable to survive the introduction of *dnaZ*$^+$ on an episome even at the permissive temperature. Strains which are *dnaA*(SUZ, Cs) are killed at the permissive temperature by the introduction of an episome carrying a wild-type *dnaZ* allele. F*lac* (*lac*$^+$) and F13 (*lac*$^+$ *dnaZ*$^+$) could be introduced by conjugation into *dnaZ*(Ts) recipient cells with efficiencies of 37 to 67% (Table 3). The F*lac* episome was transferred into, and formed stable partial diploids with, *dnaA*(SUZ, Cs) *dnaZ*(Ts) strains. The efficiency of mating was 97%. However, when the longer *dnaZ*$^+$ F13 was mated for 2 h (at 34°C) with *dnaA*(SUZ, Cs) *dnaZ*(Ts) recipi-

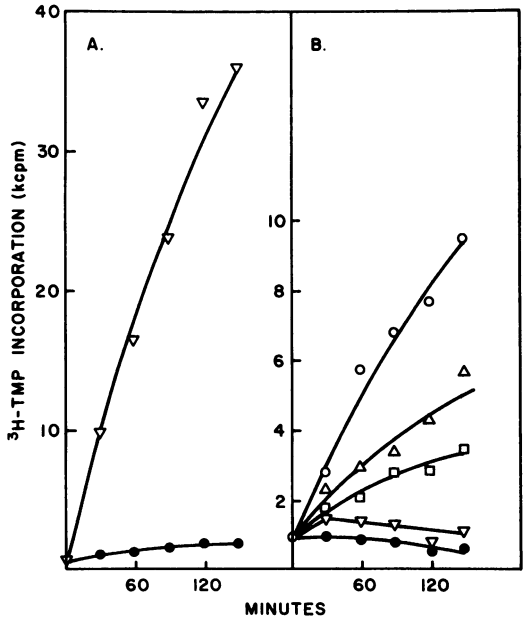

FIG. 7. Replicative and repair syntheses in toluene-treated wild-type strain JR11 (A) and *dnaA*(SUZ, Cs) strain JR10 (B) after growth at 20°C. The cultures were grown in YET broth at 34°C, shifted to 20°C for 0 (○), 40 (△), 80 (□), or 120 (▽) min, and toluene treated at room temperature, and DNA synthesis was assayed at 20°C. The absorbance of all cultures at the time of harvesting was 0.5. Repair synthesis (●) is reported only for those cultures incubated at 20°C for 120 min.

TABLE 3. Lethality of *dnaZ*$^+$ F episome in *dnaA*(SUZ, Cs) host

Recipient strain	Genotype	No. of colony-forming exconjugant and recipient cells per ml after mating[a]			Ratio of total viable cells after mating/ intial no. of cells
		Episome	*lac*	*lac*$^+$	
AX733	*dnaA*$^+$ *dnaZ*(Ts)	F*lac*	3.0×10^7	6.0×10^7	9.0
		F13	3.6×10^7	2.2×10^7	5.8
S1	*dnaA*(SUZ, Cs) *dnaZ*(Ts)	F*lac*	7×10^5	2.6×10^7	2.7
		F13	1×10^6	1.3×10^{6b}	0.2

[a] After 2 h of mating at 34°C.
[b] These *lac*$^+$ strains grew slowly and as very small colonies.

ents, about 90% of the initial cells could not form a colony when plated at the permissive temperature of 34°C. Apparently, those cells to which the F13 episome was transferred were killed even at 34°C because the number of *lac*$^+$ exconjugants after mating with F13 was only 5% of the number after mating with F*lac*. Those rare *lac*$^+$ exconjugants which formed grew very slowly and formed very small colonies. On restreaking, they grew as normal colonies, but 90% of these contained F13 derivatives which no longer carried *dnaZ*$^+$. Quantitatively, very similar results were obtained with a second *dnaA*(SUZ, Cs) strain, S2.

Possibly, this killing results from DNA synthesis inhibition. This was tested by measuring DNA synthesis in a partial diploid population immediately after mating. A low-thymine-requiring *dnaA*(SUZ, Cs) *dnaZ*(Ts) streptomycin-resistant recipient was grown at 34°C to stationary phase in the presence of [^{3}H]thymine. Cultures of streptomycin-sensitive F*lac* and F13 donors were grown to 4×10^8 cells per ml also in [^{3}H]thymine, although they could not incorporate it. Mating mixtures in a ratio of two donors to one recipient were incubated at 34°C for 15 min with [^{3}H]thymine in the medium. The mixtures were diluted into medium containing streptomycin and [^{3}H]thymine, incubated at 34°C, and sampled at intervals for DNA synthesis and colony-forming units by plating at 34°C on streptomycin-containing medium. Streptomycin inhibited growth of the donor during the sampling period and on the selective plates. [^{3}H]thymine incorporation represented DNA synthesis specifically in the newly mated partial diploids and unmated recipients because the donors could not incorporate the exogenous thymine.

Colony-forming ability decreased during the first 2 h after mating with *dnaZ*$^+$ F13, but then increased exponentially (Fig. 8). The killing appears to be caused by introduction of F13 (with its *dnaZ*$^+$ gene); the resumption of the exponential increase in viable cells probably was due to growth and division of unmated recipients. DNA synthesis was inhibited after mating with *dnaZ*$^+$ F13. During the first 40 min there was an increase in DNA of only 10%; synthesis then resumed but gradually decreased in rate during 4 h.

The killing observed after entry of the *dnaZ*$^+$ gene did not require the presence of the *dnaZ*(Ts) gene because it occurred also in strains which were *dnaA*(SUZ, Cs) *dnaZ*$^+$. Use was made of a *dnaZ*$^+$ hybrid plasmid, pJH16, to prove this point. This plasmid consists of a 3-kilobase pair fragment containing the *dnaZ* gene cloned into the vector pBR322, which carries tetracycline resistance (Mullin et al., unpublished data). If the *dnaZ*$^+$ plasmid pJH16 was transformed into wild-type or *dnaZ*(Ts) recipients, tetracycline resistance transformants could be selected at 32°C. However, transformation of this plasmid into *dnaA*(SUZ, Cs) *dnaZ*$^+$ or *dnaZ*(Ts) strains did not yield tetracycline-resistant clones (Table 4) even at the permissive 32°C temperature. Therefore, the increase in *dnaZ*$^+$ gene copies in a strain which contains *dnaA*(SUZ, Cs) protein is lethal, regardless of the *dnaZ* genotype. Killing due to pJH16 also rules out the possibility that F or an F product is the lethal agent.

The *dnaZ* region contains the closely linked *dnaX* gene (16). It is possible, therefore, that the lethal factor might be the *dnaX* (or some other) protein. To determine which gene was responsible for the killing, derivatives of pJH16 which contained Tn*5* in *dnaZ* and *dnaX* were transformed into *dnaA*(SUZ, Cs) recipients (Table 4). If Tn*5* insertion inactivated *dnaZ* and *dnaX*, the plasmid no longer caused killing; if the insertion inactivated *dnaX* and left *dnaZ*$^+$ activity intact, the plasmid retained its ability to kill the recipients. The plasmid which had *dnaZ*$^+$ complementing activity (but not *dnaX*) was lethal when transformed into a strain carrying the *dnaZ*(SUZ, Cs) allele, even when the recipient was *dnaZ*$^+$. Therefore, it is the presence of extra copies of the *dnaZ* gene in the presence of *dnaA*(SUZ, Cs) even at the permissive temperature of 32°C which is the cause of the lethality.

Killing of a *dnaA*(SUZ, Cs) recipient by a *dnaZ*$^+$ hybrid plasmid was demonstrated also by conjugation. The F$^+$ carbenicillin-resistant tran-

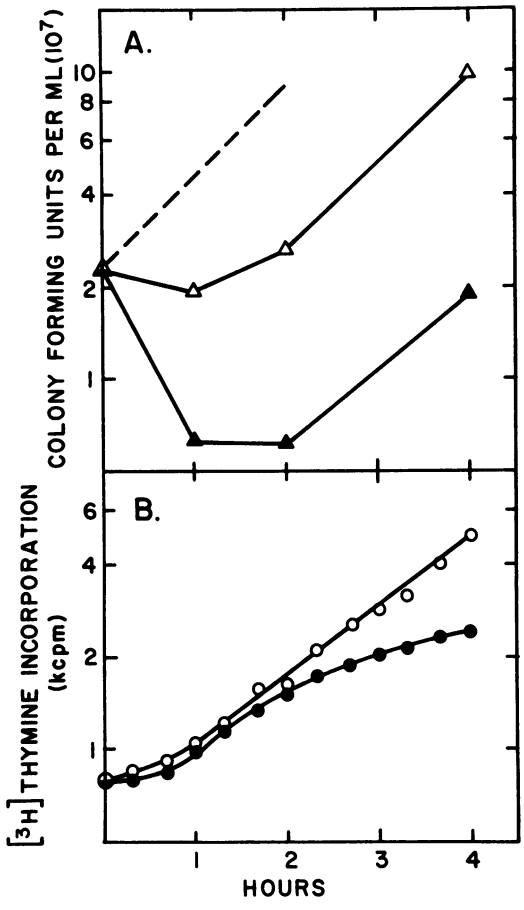

FIG. 8. Colony-forming ability (A) and DNA synthesis (B) in a *dnaA*(SUZ, Cs) recipient after mating with F*lac* or *dnaZ*$^+$ plasmid F13. Donor and recipient (strain S1) cells were grown at 34°C in [^{3}H]thymine, mixed, and allowed to mate at 34°C for 15 min. The mixture was diluted at 0 h into equilibrated medium containing [^{3}H]thymine and streptomycin. Samples were plated at 34°C on MacConkey medium plus lactose and streptomycin to measure colony-forming ability or used for determinations of DNA synthesis. Open symbols, F*lac* donor; closed symbols, F13 donor. The dashed line represents colony-forming ability after mating a *dnaA*$^+$ recipient with an F13 donor.

sposon Tn*2301* (22) was inserted into plasmids containing *dnaZ*$^+$ and *dnaZ*::Tn*5* to form conjugative hybrid plasmids pAL1 and pAL2. Donors of plasmids were mated with *dnaA*$^+$ and *dnaA*(SUZ, Cs) recipients, and the mixtures were plated to select exconjugants at the permissive temperature of 32°C. If the plasmid carried *dnaZ*$^+$, no exconjugants formed.

DISCUSSION

Suppressor mutations of a *dnaZ*(Ts) polymerization mutant have been identified and mapped within *dnaZ* and in the initiation gene *dnaA*. The intragenic suppressors in *dnaZ* caused immediate stops in DNA synthesis at 19°C.

The suppressors within *dnaA* [*dnaA*(SUZ, Cs)] cause a defect in initiation as determined by comparing residual DNA synthesis at 20°C with that observed when rifampin (25, 26, 30) inhibited initiation at 34°C (42). The *dnaA*(SUZ, Cs) mutant also was temperature sensitive. Growth stopped at 42°C, after preferential inhibition of DNA synthesis, but a period of 8 hours, during which mass and DNA increased 300- and 90-fold, respectively, was required for complete inhibition. The existence of *dnaZ* suppressor mutations in *dnaA* has been interpreted as evidence for interaction in vivo between the mutant, and presumably also the wild-type, *dnaA* and *dnaZ* proteins (42).

The *dnaA*(SUZ, Cs) mutations create an in vivo environment such that the host cell is killed by the introduction by conjugation or transformation of a wild-type *dnaZ* gene on a plasmid. This lethality occurs when the recipient is *dnaA*(SUZ, Cs) and the incoming DNA is *dnaZ*$^+$, regardless of the *dnaZ* genotype of the resident chromosome. Presumably, the incoming *dnaZ*$^+$ gene is expressed and excessive amounts of the *dnaZ*$^+$ protein are responsible for killing. Excessive amounts of *dnaZ* protein could result from the presence of several new copies of the *dnaZ* gene or from uncontrolled expression of the incoming *dnaZ* gene. The latter is possible only if expression of *dnaZ* is regulated. Two models to explain the killing are as follows. (i) The *dnaZ* protein competes with

TABLE 4. Lethality of *dnaZ*$^+$ hybrid plasmids in *dnaA*(SUZ, Cs) strains

Plasmid	Phenotype[a]	No. of transformants per ml with the following recipient			
		AX733 [*dnaA*$^+$ *dnaZ*(Ts)]	JR8 [*dnaA*(SUZ, Cs) *dnaZ*(Ts)]	GM241 [*dnaA*$^+$ *dnaZ*$^+$]	JR10 [*dnaA*(SUZ, Cs) *dnaZ*$^+$]
pJH16	DnaZ$^+$X$^+$	2.3×10^3	<10	3.3×10^3	<10
pJH16::Tn*5*-16	DnaZ$^-$X$^-$	8.0×10^2	7.0×10^2	2.8×10^3	1.3×10^3
pJH16::Tn*5*-3	DnaZ$^+$X$^-$	2.3×10^3	<10	3.7×10^3	<10

[a] Phenotype of the plasmid based on complementation tests (Mullin et al., unpublished data).

the mutant *dnaA*(SUZ, Cs) protein for participation in the replication complex. The complex would be inactive without *dnaA* protein. (ii) Perhaps the *dnaZ* protein complexes with and removes *dnaA* protein from the pool of functional *dnaA* product.

Another line of evidence that *dnaA* and *dnaZ* proteins interact in vivo is presented in the accompanying paper (4). The *dnaA*(SUZ, Cs) suppressor mutation could not be transferred by transduction into *dnaC*(Ts) or *dnaG*(Ts) recipients. It was transduced with very low efficiency into *dnaB*(Ts) and *dnaE*(Ts) recipients because such double mutants usually were lethal. Presumably, the lethality results from protein interactions of replication factors.

Synthesis of DNA precursors, channeling of the precursors to the replication point, and DNA replication are thought to occur in complexes in several systems (5, 13, 36, 37, 40). Additional evidence for an *E. coli* complex includes the following observations. McHenry and Kornberg (29) have extracted DNA polymerase III activity as a complex. An extragenic suppressor of a *dnaA*(Ts) mutation has been mapped in *rpoB* (2), the structural gene for RNA polymerase β subunit (8, 15). *dnaA*(Ts) mutations can be suppressed by the introduction of additional copies of uncharacterized, wild-type genes (35) or by mutations in several genes designated *das* (1, 44). Extragenic suppressors of *dnaE* mutants, defective in one of the DNA polymerase III core enzyme subunits (14), occur in the *dnaN* gene (24) which codes for the β subunit of DNA polymerase III holoenzyme (7). Elevated $dnaC^+$ gene dosage suppresses the initiation defect characteristic of *dnaB252*(Ts) mutants (39), which suggests that the *dnaB252* protein has a lowered affinity for *dnaC* protein and that formation of a *dnaB-dnaC* complex is necessary for initiation (39). A suppressor of a *dnaB* mutant maps in, or very near, the *dnaG* gene (28). Thus, although the particular functions of each of the individual gene products within the complex remain to be elucidated, there is ample evidence that such a complex exists and a growing number of mutations which affect complex function. These mutations may provide clues to the roles of the individual components.

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