Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3340–3344, May 1982
Microbiology

Interaction of the *Escherichia coli* dnaA initiation protein with the dnaZ polymerization protein *in vivo*

(extragenic suppressor/DNA synthesis)

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Communicated by Esmond E. Snell, February 10, 1982

ABSTRACT **To define *in vivo* interactions of *Escherichia coli* DNA replication components, extragenic suppressors of a *dnaZ*(TS) mutant were isolated. A temperature-sensitive *dnaZ* mutant, which is defective in polymerization, was plated at 39°C to select temperature-insensitive revertants. Some of these revertants also were cold sensitive, a phenotypic property that facilitated study of the suppressor. Mapping of the cold sensitivity indicated that some of the suppressor mutations are intragenic but others are located within the initiation gene, *dnaA*. The *dnaA* mutations that suppress the *dnaZ*(TS) defect are designated *dnaA*(SUZ, CS). The *dnaA*(SUZ, CS) strains have a defect in DNA synthesis at low temperature that is typical of an initiation defect. These data suggest that the *dnaA* product, an initiation factor, interacts *in vivo* with the dnaZ protein, a polymerization factor.**

A temperature-sensitive (TS) *dnaZ* mutant of *Escherichia coli* is inhibited in DNA polymerization at 42°C. The *dnaZ*$^+$ product is required *in vivo* for synthesis of M13 and ϕX174 parental replicative form, progeny replicative form, and single-strand DNA (1–3). Wickner and Hurwitz (4) isolated the dnaZ protein and showed that it functions *in vitro* in polymerization. Wickner (5) proposed that the dnaZ protein and elongation factor III function to transfer elongation factor I to a primed template. DNA polymerase III then binds to the elongation factor I–primer–template complex.

What components of the replication machinery interact with the dnaZ protein *in vivo*? We sought to answer this question by isolating extragenic mutations that suppress the phenotype of a *dnaZ*(TS) mutant. Jarvik and Botstein (6) have shown that TS or cold-sensitive (CS) missense mutations can be suppressed by extragenic mutations located in genes whose products are known to interact physically with the product of the mutant gene being suppressed. The original mutation presumably destroys an interaction or activity that the extragenic suppressor restores by altering a second protein that is in physical contact with the original protein. Extragenic suppressors sometimes acquire a phenotype in their own right (6). For example, temperature-insensitive (TS$^+$) revertants of a TS mutant are sometimes CS.

We used this approach and isolated, from a *dnaZ*(TS) mutant, TS$^+$ revertants, some of which were cold sensitive in DNA replication (7). Some of these mutants contained an extragenic suppressor that we found by transductional mapping and complementation to lie in *dnaA*, a gene whose product participates in the initiation of rounds of chromosome replication. Thus, the *dnaA* product can be altered to compensate for a mutation in *dnaZ*, possibly by physical interaction between the two gene products.

MATERIALS AND METHODS

Strains. Spontaneous TS$^+$ revertant strains S1, S2, and S3 were selected from strain AX733, which carries the *dnaZ*2016(TS) allele (8). The extragenic suppressors in these strains are designated *dnaA*71, *dnaA*72, and *dnaA*73. To these allele numbers will be added the suffix (SUZ2016, CS) to indicate that, for example, the *dnaA*71(SUZ2016, CS) allele suppresses the *dnaZ*2016(TS) allele but becomes CS; the suffix will be shortened to (SUZ, CS) for this paper.

Strain JR1 contains the carbenicillin-resistance transposon, Tn*406*, in the *rbs* gene; *rbs* is ≈15% cotransducible with *dnaA*. The availability of this strain made it possible to introduce Tn*406* into *rbs* in SUZ, CS strains by P1 and to use cotransduction of SUZ, CS and the carbenicillin resistance of Tn*406* as a convenient means of moving the SUZ, CS mutations. Strain JR1 was derived from strain 1307, constructed by Richard Meyer, which is *dnaA*$^+$ *rbs*$^+$ and contains pRK296, a tetracycline-resistance kanamycin-resistance plasmid that also carries a TS mutation for plasmid maintenance and Tn*406*. Strain JR1 was isolated by selecting carbenicillin-resistant 42°C survivors of strain 1307 and scoring for inactivation of *rbs*.

P1*vir*a was used for generalized transduction. λ*tna* phages (9) were from K. von Meyenburg, λ425 and λ423 (10) were from A. Wright, and λ*imm*^{21} *tna* phages (11) were from Y. Sakakibara.

Media. Yeast extract tryptone medium (12) was supplemented with 0.5% NaCl and thymine (2 or 50 μg/ml) as needed. Minimal medium (12) was supplemented with glucose (10 mg/ml), thiamin·HCl (5 μg/ml), indole (2 μg/ml), DL-5-methyltryptophan (50 μg/ml), thymine (2 or 50 μg/ml), and Casamino acids (5 mg/ml) as desired. Carbenicillin (500 μg/ml), tetracycline (25 μg/ml), kanamycin (25 μg/ml), and rifampicin (150 μg/ml) were added as needed.

Transduction. Spot tests for transduction to TS$^+$ or CS$^+$ were carried out according to the procedure of Walker *et al.* (13) on plates at 42°C or 20°C. *tna*$^+$ transductants were selected as described by Hansen and von Meyenburg (9). Quantitative tests were done by the procedure of Shimada *et al.* (14).

Mass Increase and DNA Synthesis. Culture optical density at 540 nm was used as an index of mass increase. Steady-state synthesis of DNA was followed by incorporation of [^{3}H]thymine

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Abbreviations: TS, temperature sensitive; TS$^+$, temperature insensitive; CS, cold sensitive.

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into 5% trichloroacetic acid-insoluble material. [*methyl*-^{3}H]Thymine at 1 μCi/ml (specific activity, 0.5 μCi/μg; 1 Ci = 3.7×10^{10} becquerels) was added nine generations before experiments began.

RESULTS

Isolation and Preliminary Genetic Mapping of CS Suppressors of a *dnaZ*(TS) Mutant. Cultures of the *dnaZ*2016(TS) strain AX733 were grown at 30°C and plated at 39°C to select TS$^+$ revertants. Of ≈6,000 revertants, 54 independent strains grew at 34°C but could not form colonies at 20°C. Preliminary mapping by Hfr strains showed that about one-half of the CS mutations mapped near *dnaZ*. Six of these have been mapped in or very near the *dnaZ* gene by the use of λ*dnaZ*$^+$ specialized transducing phages (ref. 13; unpublished data). About one-third of the CS mutations were located in the region between minutes 66 and 83, based on early donation by Hfr strains KL14 and AX1001 (Fig. 1). As shown below, these mutations probably are located within the *dnaA* gene; they are designated *dnaA*-(SUZ2016, CS), which will be abbreviated to *dnaA*(SUZ, CS).

These extragenic suppressor strains actually were double mutants—i.e., they retained the original *dnaZ*(TS) mutation. This was demonstrated by growing phage P1 on them and transducing *purE*$^+$ into a *purE dnaZ*$^+$ recipient; 2% of the transductants became *dnaZ*(TS).

Suppression of the Temperature Sensitivity of *dnaZ*(TS) Mutants by a *dnaA*(SUZ, CS) Mutation. In cultures growing at 40.5°C, the *dnaA*(SUZ, CS) mutations partially suppressed the temperature sensitivity of DNA synthesis in the *dnaZ*(TS) mutant (Fig. 2). DNA synthesis was restored to 67% of the wild-type rate. When the cultures were grown at 39°C, the SUZ, CS suppressor restored DNA synthesis to 90% of the wild-type rate (Fig. 2). At 39°C, the *dnaZ*(TS) mutant stopped DNA synthesis gradually; presumably the TS protein was inactivated slowly at this temperature.

The *dnaA*(SUZ, CS) CS defect was specifically in DNA synthesis. A *dnaZ*(TS) *dnaA*(SUZ, CS) culture grown at 37°C and shifted to 19°C stopped DNA synthesis gradually; in 7 hr, DNA increased 55–60% while RNA and mass increased 160% and 250%, respectively. When the *dnaA*(SUZ, CS) mutation was transferred into a *dnaZ*$^+$ genetic background, the strain became CS. Thus, the cold sensitivity of these strains is the result of the SUZ, CS mutation and does not require the presence of the *dnaZ*(TS) allele.

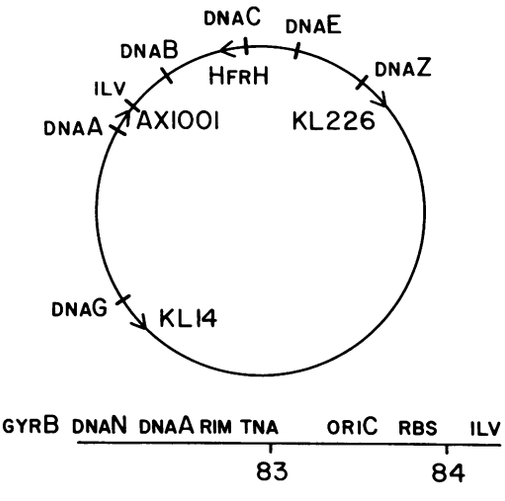

FIG. 1. Chromosome map of *E. coli* from Bachmann and Low (15). Origins of Hfr strains are indicated by arrows. The *dnaN dnaA oriC* region around minute 83 is expanded at the bottom (9, 11).

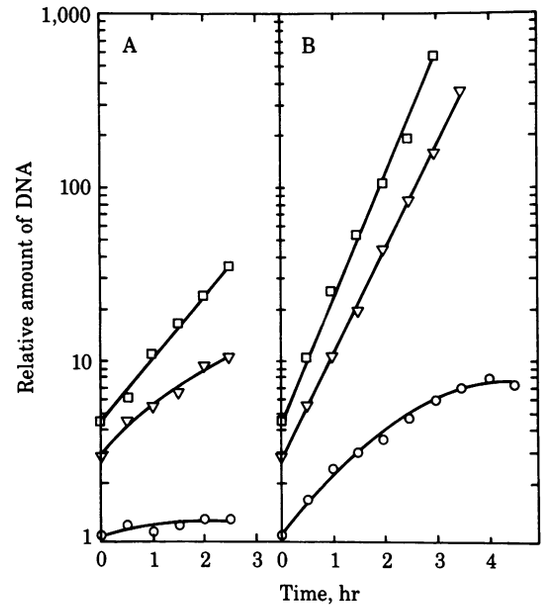

FIG. 2. *In vivo* suppression of the *dnaZ*(TS) defect in DNA synthesis by a *dnaA*(SUZ, CS) mutation. (*A*) Strains were grown at 34°C in glucose/Casamino acids medium and shifted at 0 time to 40.5°C. (*B*) Strains were grown at 34°C in yeast extract/tryptone medium and shifted at 0 time to 39°C. □, *dnaZ*$^+$ wild-type revertant of strain AX733; ▽, *dnaZ*(TS) *dnaA*(SUZ, CS) strain S1; ○, *dnaZ*(TS) strain AX733. Initial values are separated along the ordinate arbitrarily. Minimum trichloroacetic acid-insoluble [^{3}H]thymine incorporation (cpm/ml) values for the strains were as follows: 7,000, wild-type revertant of AX733; 3,700, S1; 5,000, AX733.

The SUZ, CS Mutation Coincides with the *dnaA* Locus. The finding that the SUZ, CS mutations mapped in the region of minute 66–83 suggested that these mutations might be in the *gyrB dnaN dnaA tna oriC ilv* region near minute 83 (Fig. 1). Transduction by P1 showed that the suppressor is located near *dnaN dnaA* by proving the sequence *gyrB* SUZ, CS *ilv* (data not shown).

To determine the location of SUZ, CS more precisely, transduction of SUZ, CS$^+$ into SUZ, CS recipients by λ*dnaA* phages was tested. Hansen and von Meyenburg (9) have prepared a family of λ*cI857tna* transducing phages that carry different amounts of host DNA from the *gyrB dnaA tna* region. The λ*tna* phages that carried *dnaA*$^+$ also donated SUZ, CS$^+$ to three independent SUZ, CS mutants. Those λ*tna* phages that did not

Table 1. Mapping of the SUZ, CS mutation by λ*cI857tna* phages

Phage	Transduction test				
	*dnaN**	*SUZ, CS*†	*dnaA*‡	*rim*§	*tna*¶
λ*tna*11	− (<1 × 10^{-9})	− (<1 × 10^{-9})	− (<1 × 10^{-9})	+	+
7	− (<1 × 10^{-9})	+ (4 × 10^{-5})	+ (1 × 10^{-5})	+	+
30	− (<1 × 10^{-9})	+ (2 × 10^{-6})	+ (2 × 10^{-4})	+	+
406	+ (4 × 10^{-5})	+ (2 × 10^{-6})	+ (2 × 10^{-6})	+	+

+, Positive test; −, negative test. Results in parentheses represent no. of transductants per plaque-forming phage. All recipients were λ$^+$ lysogens.

* Strain HN3(λ$^+$), a *dnaN*59(TS) derivative of BE280.

† Strains DK4(λ$^+$) and JR10(λ$^+$) are SUZ, CS derivatives of KL198 and GM241, respectively.

‡ Strains DC12(λ$^+$) *dnaA*508(TS), E177(λ$^+$) *dnaA*177(TS), and KH691(λ$^+$) *dnaA*46(TS).

§ Data from Hansen and von Meyenburg (9).

¶ Strain DC7(λ$^+$).

carry *dnaA*$^+$, did not transduce SUZ, CS recipients (Table 1). Although the host insertions of five of the independent phages (λ*tna*7, 30, 45, 315, and 624) terminated between *dnaN* and *dnaA* and insertions of two phages (λ*tna*11 and 530) terminated between *dnaA* and *rim*, none of these separated SUZ, CS from *dnaA*. Transduction by these phages depended on recombination, as shown by the fact that TS recipients were transduced to TS$^+$ whether or not they were λ$^+$ lysogens. Therefore, data obtained with λ*cI857tna* phages suggest, but do not prove, that the SUZ, CS mutations are in *dnaA*.

A second approach was the use of a transducing phage that carries a *dnaA*(TS) mutation. Schaus *et al.* (10) have isolated a λ*tna*$^+$ *dnaA*$^+$ *dnaN*$^+$ phage and a missense mutant λ*tna*$^+$ *dnaA*46(TS) *dnaN*$^+$. Transduction by these phages depended on recombination, as shown by the fact that neither transduced *tna*, *dnaA*, or *dnaN* strains, which also carry a *recA* mutation. λ*tna*$^+$ *dnaA*46(TS)*dnaN*$^+$ phage did not transduce *dnaA*177(TS), *dnaA*508(TS), or SUZ, CS mutants in spot tests, although the λ*tna*$^+$ *dnaA*$^+$ *dnaN*$^+$ phage did. The λ*tna*$^+$ *dnaA*46(TS)*dnaN*$^+$ phage transduced *tna*$^+$ and *dnaN*$^+$ in similar tests. These data also suggest that the SUZ, CS mutations are in *dnaA*.

A third mapping approach was the use of deletion mutants of λ*imm*^{21} *dnaA*$^+$ *dnaN*$^+$ (11, 16). A phage known to carry intact *dnaA* and *N* (λ*imm*^{21} △22) transduced both recipients with frequencies of 2–4 × 10^{-4} per phage (Table 2). A deletion (△33) that removed part of *dnaA*$^+$ but none of *dnaN*$^+$ reduced the frequency of transduction of SUZ, CS$^+$ by a factor of 1/100 without altering the *dnaN*$^+$ transduction frequency. If the SUZ, CS mutations were in *dnaN*, phage △33 should transduce SUZ, CS with the same frequency as it transduces *dnaN*.

Complementation of *dnaN* and SUZ, CS by these phages also was tested. They carry the wild-type immunity region of phage 21 and therefore readily form lysogens that are stable even at elevated temperature. Strains that were *dnaN*(TS) and SUZ, CS were infected with λ*imm*^{21} △22 and λ*imm*^{21} △33; lysogens were identified on the basis of phage immunity at the permissive temperature (30 or 34°C). These lysogens were tested for complementation of *dnaN* and SUZ, CS using, as an assay, efficiency of plating at nonpermissive temperatures (Table 3). The *dnaN*$^+$ *A*$^+$ phage complemented *dnaN*(TS) and two SUZ, CS strains, indicating that *N*$^+$ and CS$^+$ are dominant over the mutant alleles. The *dnaN*$^+$ △A phage complemented a *dnaN*(TS), but not SUZ, CS strains. These data are consistent with the interpretation that the SUZ, CS mutations are located in *dnaA*. All three independent SUZ, CS alleles behaved similarly in transduction tests and the two strains tested by complementation also responded similarly. These three alleles have been designated *dnaA*71(SUZ2016, CS), *dnaA*72(SUZ2016, CS), and *dnaA*73 (SUZ2016, CS).

Table 2. Transduction by λ*imm*^{21} *dnaA dnaN* and its deletion mutants

Phage	Transduction test			
	dnaN(TS)	*dnaA*508(TS)	SUZ, CS	*tna*
λ*imm*^{21} *tna*$^+$	−	−	−	+
λ*imm*^{21} *tna*$^+$ *dnaA*$^+$ #2	+	+	+	+
λ*imm*^{21} △22 (*dnaN*$^+$ *dnaA*$^+$)	+ (3 × 10^{-4})	+ (4 × 10^{-4})	+ (2.3 × 10^{-4})	−
λ*imm*^{21} △33 (*dnaN*$^+$ *dnaA*$^-$)	+ (1.6 × 10^{-4})	− (<1 × 10^{-7})	− (1.1 × 10^{-6})	−

+, Positive spot test; −, negative spot test. Results in parentheses are transductants per phage in quantitative tests.

Table 3. Complementation by λ*imm*^{21} *dnaA dnaN* and its deletion mutants

Lysogen	Complementation (efficiency of plating)			
	20°C	30°C	34°C	42°C
dnaN(TS)	NT	1.0	NT	3 × 10^{-7}
dnaN(TS) (λ*imm*^{21} △22)	NT	1.0	NT	0.99
dnaN(TS) (λ*imm*^{21} △33)	NT	1.0	NT	1.02
dnaA(TS)	NT	1.0	NT	2 × 10^{-5}
dnaA(TS) (λ*imm*^{21} △22)	NT	1.0	NT	0.30
dnaA(TS) (λ*imm*^{21} △33)	NT	1.0	NT	6 × 10^{-5}
*dnaA*71(SUZ, CS)	1.2 × 10^{-7}	NT	1.0	NT
*dnaA*71(SUZ, CS) (λ*imm*^{21} △22)	0.77	NT	1.0	NT
*dnaA*71(SUZ, CS) (λ*imm*^{21} △33)	5 × 10^{-4}	NT	1.0	NT
*dnaA*73(SUZ, CS)	3 × 10^{-5}	NT	1.0	NT
*dnaA*73(SUZ, CS) (λ*imm*^{21} △22)	1.2	NT	1.0	NT
*dnaA*73(SUZ, CS) (λ*imm*^{21} △33)	1.1 × 10^{-5}	NT	1.0	NT

Efficiency of plating at 30°C or 34°C is defined as 1.0. NT, not tested.

Residual Increase in DNA in an SUZ, CS Mutant at 19°C. The mapping and complementation data suggest that SUZ, CS strains are defective in *dnaA* function at low temperature. This was verified by analysis of residual DNA synthesis after shifting to 19°C. The residual increase in DNA in a *dnaZ*$^+$ *dnaA*(SUZ, CS) strain at 19°C was compared with the amount synthesized when initiation was inhibited at 34°C by rifampicin, a known inhibitor of initiation (7–9). On shifting to 19°C, the rate of DNA synthesis decreased immediately, but synthesis continued and the DNA amount increased 50–60% over a 7-hr period (Fig. 3*B*). The immediate decrease in rate of DNA synthesis (Fig. 3*B*)

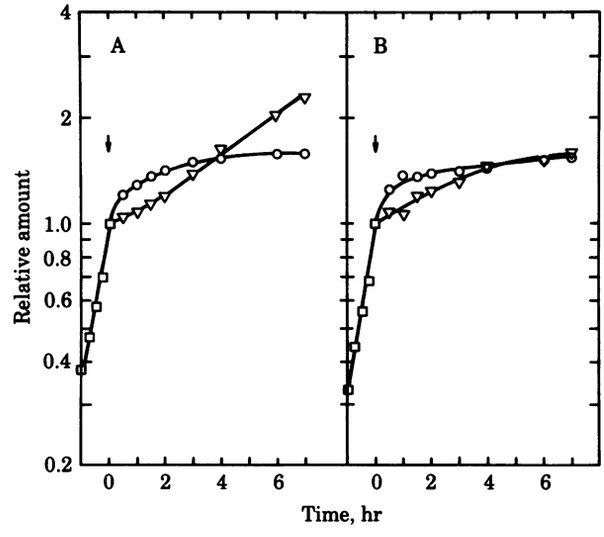

FIG. 3. Effect of incubation at 19°C or addition of rifampicin at 34°C on mass increase (*A*) and DNA synthesis (*B*) of *dnaZ*$^+$ *dnaA*(SUZ, CS) strain DK4. A culture was grown at 34°C in yeast extract/tryptone medium containing [^{3}H]thymine (□) and divided at 0 time (↓) into two parts. One was shifted to 19°C (▽) and the other was maintained at 34°C and treated with rifampicin (○). Relative amount 1.0 represents 3,000 cpm of trichloroacetic acid-insoluble material per 0.5 ml and OD = 0.2. Strain DK4 was prepared by P1 transduction of *ilv*$^+$ SUZ, CS from strain S1 into strain KL198 (*ilv*, *dnaA*$^+$, *dnaZ*$^+$).

probably was not an effect of the SUZ, CS mutation on polymerization. It probably resulted from an effect of the shift from 34°C to 19°C on chemical reactions in general for the following reasons. (*i*) The rate of growth as measured by optical density also decreased immediately after the shift (Fig. 3*A*). Growth, however, continued for at least 7 hr while DNA synthesis stopped after ≈4 hr. (*ii*) In a wild-type strain similarly shifted from 34°C to 19°C, the rates of both growth and DNA synthesis also decreased immediately (data not shown); however, both continued indefinitely at the lower rates.

The residual increase of 50–60% in DNA after the SUZ, CS strain was shifted to 19°C was compared with that when rifampicin was added to inhibit initiation at the permissive temperature. After rifampicin addition, the DNA synthesis rate decreased gradually and the final increment was 50–60% (Fig. 3*B*), as expected if no new rounds of replication were begun. There was no immediate decrease in rate of DNA synthesis or growth because the culture temperature remained at 34°C (Fig. 3). Two other SUZ, CS mutants similarly synthesized a 50–60% increase in DNA at 19°C.

The SUZ, CS mutants are probably defective in initiation of replication at 19°C because of the gradual stopping of DNA synthesis, the 50–60% increment, and the fact that the increment was similar to that observed when rifampicin was added at the permissive temperature.

DISCUSSION

We have studied an *in vivo* property of the *dnaA* product. Three independently isolated extragenic suppressors of a *dnaZ*(TS) mutation were localized in the *dnaA* region by a set of λ*tna* transducing phages (9) and mapped close to or within *dnaA* by deletion phages (11, 16). The mapping data were corroborated by complementation tests. The conclusion that the SUZ, CS mutations are within *dnaA* is also supported by physiological studies. Both the double mutant *dnaA* (SUZ, CS) *dnaZ*(TS) and the *dnaA*(SUZ, CS) *dnaZ*$^+$ strain were cold sensitive and responded to incubation at 19°C as initiation-defective strains. DNA synthesis stopped slowly at 19°C; the final increment of DNA was 50–60%, which was the same as observed after addition of rifampicin was added to the culture growing at the permissive temperature. Thus, if the SUZ, CS mutation is not in *dnaA*, one would have to propose that it lies in a previously undiscovered DNA replication gene that is closely linked to *dnaA* and has properties similar to those of *dnaA*.

The polymerization gene *dnaN* is adjacent to *dnaA* and Sako and Sakakibara (20) have proposed that *dnaA* and *N* constitute an operon in which *dnaA* is proximal to the operator. Maximum *dnaN* expression requires an intact *dnaA* gene, but *dnaN* has its own weak promoter. It is possible to argue, therefore, that the suppression provided by the SUZ, CS mutation results from an alteration of *dnaN* expression. This seems very unlikely because the SUZ, CS mutations probably are missense.

The finding that mutations in the *dnaA* gene suppress the *dnaZ*(TS) defect suggests that an alteration in the dnaA protein stabilizes the dnaZ(TS) protein at 39°C to 40°C. Presumably the mutant dnaA protein, altered by the SUZ, CS mutation, accomplishes this by physical association with the dnaZ(TS) protein. A potential association between dnaA and Z is a novel finding; the *dnaA* product is required for initiation and is thought to act once per initiation (before the *dnaC* product functions) (21, 22), while the *dnaZ* product is required continuously for chromosome polymerization (8). If the SUZ, CS mutations suppress the *dnaZ*(TS) defect directly, the mutant dnaA initiation protein must interact with the polymerization mutant dnaZ protein continuously during polymerization. This would suggest interaction between wild-type dnaA and Z proteins continuously during polymerization. Alternatively, the *dnaA*(SUZ, CS) product could stabilize the mutant dnaZ(TS) protein indirectly through an interaction with other replication factors. A model that reconciles a continuous interaction (direct or indirect) between a protein required for initiation and a second that functions in polymerization is the possibility that both proteins are components of a multienzyme replication complex. We propose, therefore, that *dnaA* has at least two functions. First, there is the active initiation function. Second, the dnaA protein might form part of the replication complex, a structure in which initiation and polymerization can occur. The active initiation function is thought to involve RNA synthesis because an extragenic suppressor of a *dnaA*(TS) mutation maps in *rpoB* (23), the structural gene for RNA polymerase β subunit (24, 25).

This proposal for dnaA function does not explain why the usual *dnaA* mutants have initiation, rather than polymerization, defects. Perhaps the usual TS mutations in *dnaA* affect the initiation function without influencing the replication complex. Another explanation is that *dnaA* participates in the assembly of the replication complex only once per round of replication, i.e., at initiation. Functional complexes assembled with or by dnaA(TS) protein would continue to function in polymerization even at high temperature. Also, it should be pointed out that the *dnaA*(SUZ, CS) strains might be expected to be a special class of mutants because they account for <1% of the TS$^+$ revertants.

There are several indications from other systems that replication *in vivo* occurs in a complex, that precursor biosynthesis occurs in a complex, and even that precursor biosynthesis and replication can be coupled. In T4-infected *E. coli* cells and in two different animal cells, DNA precursors are synthesized in a complex of protein; these precursors are then channeled directly to the replication points (26–29). Replication of T4 DNA in a protein complex has been proposed (26) and interaction of T4 replication proteins is required for initiation of DNA synthesis (30). At least one of the T4 DNA precursor biosynthetic enzymes is also required for DNA synthesis, which suggests (26) an interaction between the precursor biosynthetic complex and a replication complex. DNA polymerase holoenzyme proteins of *E. coli* can be extracted in a complex (31) (although there is no evidence that dnaA protein participates *in vitro* in a replication complex). A DNA polymerase and DNA precursor biosynthetic enzymes can be prepared as an aggregate from animal cells (29, 32) and Reddy and Pardee (29) have proposed the term "replitase" to refer to a complex that synthesizes DNA precursors, channels them to replication points, and replicates DNA.

Recent attempts to transfer the *dnaA*(SUZ, CS) mutation into other *dna*(TS) strains have detected another property of the suppressor. Phage P1 was used to transduce the SUZ, CS mutation along with a tightly linked (80–98% cotransducible) Tn*10* into wild-type or *dnaZ*(TS) strains. However, it has not been possible to isolate *dnaA*(SUZ, CS) transductants of *dnaC*(TS) or *G*(TS) mutants even at an intermediate permissive temperature. *dnaA*(SUZ, CS) transductants of *dnaB*(TS) and *E*(TS) strains were formed at the permissive temperature but with very low frequency. These findings are interpreted to result from lethality (or a high probability of lethality) of combinations of *dnaA*(SUZ, CS) with some other *dna* alleles. Possibly this lethality results from protein–protein interactions.

We thank Richard Meyer for providing strain 1307 before publication of its properties, Andrew Wright for providing the transducing phages in advance of publication of their properties, Kasper von Meyenburg for λ*cI857tna* phages, Y. Sakakibara for λ*imm*^{21} *dnaA* phages, and Barbara Bachmann for strains. This work was supported by American Can-

cer Society Grant NP169 and in part by National Science Foundation Grant PCM 78-07808 and National Institutes of Health Grant AI08286.

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