Isolation and Characterization of Suppressors of Two *Escherichia coli* *dnaG* Mutations, *dnaG2903* and *parB*

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Manuscript received September 11, 1996
Accepted for publication December 20, 1996

ABSTRACT

The *dnaG* gene of *Escherichia coli* encodes the primase protein, which synthesizes a short pRNA that is essential for the initiation of both leading and lagging strand DNA synthesis. Two temperature-sensitive mutations in the 3′ end of the *dnaG* gene, *dnaG2903* and *parB,* cause a defect in chromosome partitioning at the nonpermissive temperature 42°. We have characterized 24 cold-sensitive suppressor mutations of these two *dnaG* alleles. By genetic mapping and complementation, five different classes of suppressors have been assigned: *sdgC, sdgD, sdgE, sdgG* and *sdgH.* The genes responsible for suppression in four of the five classes have been determined. Four of the *sdgC* suppressor alleles are complemented by the *dnaE* gene, which encodes the enzymatic subunit of DNA polymerase III. The *sdgE* class are mutations in *era,* an essential GTPase of unknown function. The *sdgG* suppressor is likely a mutation in one of three genes: *ubiC, ubiA* or *yjbI.* The *sdgH* class affects *rpsF,* which encodes the ribosomal protein S6. Possible mechanisms of suppression by these different classes are discussed.

THE *dnaG* gene of *Escherichia coli* encodes the protein primase, which synthesizes a short primer RNA that is essential for the initiation of both leading- and lagging-strand DNA synthesis (LARK 1972; VAN DER ENDE *et al.* 1985). The enzymatic function of primase has been well established, yet the molecular interactions within the replisome that determine when and where primase synthesizes a primer RNA for the initiation of an Okazaki fragment remain largely unknown. Biochemical studies have implicated functional interactions between primase and both DnaB and DNA polymerase III holoenzyme (KORNBERG and BAKER 1992; MARIANS 1992; ZECHNER *et al.* 1992; TOUGU *et al.* 1994), although no direct physical interactions have been demonstrated between these proteins. A model to describe the interactions between primase, helicase, and *Pol*III has been proposed (MARIANS 1992; ZECHNER *et al.* 1992).

Five conditional-lethal temperature-sensitive alleles of *dnaG* have been isolated. Three of these mutants, *dnaG3, dnaG308* and *dnaG399,* were identified in screens for temperature-sensitive mutations affecting DNA replication. All three mutations, found in the middle of the *dnaG* gene, cause substitutions near the region of the primase that is highly conserved in different bacterial species (GROMPE *et al.* 1991; VERSALOVIC and LUPSKI 1993; SUN *et al.* 1994; MUSTAEV and GODSON 1995). This region also has homology with RNA polymerases and is where the synthesis of the primer RNA is believed to occur (VERSALOVIC and LUPSKI 1993). The other two alleles, *dnaG2903* and *parB,* were isolated in two different screens. The *dnaG2903* mutation was found in a screen for phenethyl alcohol-resistant mutants while the *parB* mutation was isolated in a screen for chromosome partitioning defective mutations (HIROTA *et al.* 1968; WADA and YURA 1974). These *par* mutants are unable to segregate their nucleoids but do not convey a defect in DNA synthesis at the nonpermissive temperature (VERSALOVIC and LUPSKI 1997).

The mutations in *dnaG2903* and *parB* are found in the 3′ end of the gene, 9 bp apart, each causing a different Glu-to-Lys substitution, which represents a significant change in charge (GROMPE *et al.* 1991). The SOS response is induced by both mutations, but filamentation and the defect in chromosome partitioning is only partially caused by the induction of SOS (VERSALOVIC and LUPSKI 1997). Thus it is unclear whether or not the partitioning defective phenotype of *dnaG2903* and *parB* reflects an active role of primase in chromosome partitioning or if this phenotype is caused by perturbed DNA replication. The latter possibility seems more likely. MARIANS and coworkers have proposed that the COOH terminus of primase plays a regulatory role for the priming activity of primase (TOUGU *et al.* 1994). They further suggest that a functional interaction between the carboxy-terminus of primase and DnaB is important for priming activity. The *dnaG2903* allele may affect this regulatory region of primase and the functional interaction with DnaB (TOUGU *et al.* 1994). However, mutations affecting the genes encoding primase in *Saccharomyces cerevisiae* result in chromosome segrega-

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tion defects (LONGHESE *et al.* 1993). It is therefore possible that primase has an undetermined role in chromosome partitioning.

Suppressors of the *dnaG2903* and *parB* mutations occur at a high rate (KATAYAMA *et al.* 1989; BRITTON and LUPSKI 1995). KATAYAMA and coworkers characterized 100 suppressors of *dnaG2903* in an attempt to identify proteins that directly interact with primase (KATAYAMA *et al.* 1989). They found two classes, *sdgA* and *sdgB* (KATAYAMA *et al.* 1989). No other classes were found. Suppressors in the *sdgA* class were point mutations in the transcription terminator T_1, which precedes the *dnaG* gene. The *sdgB* class were mapped to the *rpoB* gene, which encodes the enzymatic subunit of RNA polymerase. Both classes result in the overexpression of the mutant primase, causing suppression of the temperature-sensitive phenotype (KATAYAMA *et al.* 1989; BRITTON and LUPSKI 1995). We have previously shown that *parB* can also be suppressed by mutations in T_1, and both *dnaG2903* and *parB* can be suppressed by a transcription termination defective *rpoB* allele (BRITTON and LUPSKI 1995). Thus *parB* and *dnaG2903* likely have a similar effect on primase function.

This paper describes an approach to identify new classes of suppressors of *dnaG2903* and *parB* in an attempt to find proteins that directly interact with primase during DNA replication, govern primase function indirectly, or are involved in the regulation of *dnaG* expression. To find novel suppressors it was important to devise a screen that would eliminate the two previously described classes of suppressors, *sdgA* and *sdgB*. Because all of the *sdgA* and *sdgB* suppressors grow well at 25°, suppressors of *dnaG2903* and *parB* that cured the heat-sensitive defect but caused a cold-sensitive phenotype were isolated. The characterization of these cold-sensitive suppressors and the possible mechanisms of suppression are discussed.

MATERIALS AND METHODS

Strains: Strains are listed in Table 1. RAB2903 and RABPARB were constructed by making a P1 lysate of N407 (kindly provided by NAOMI FRANKLIN) and transducing KY1378 (KATAYAMA *et al.* 1989) and JV53 (VERSALOVIC and LUPSKI 1997) to tetracycline resistance, respectively. A collection of Hfr strains and strains containing defined markers created for genetic mapping in *E. coli* were used in this study (Table 2) (SINGER *et al.* 1989). In addition, marker *hfq2*::Ωkan was used for mapping the *sdgH* class of suppressors (kindly provided by Dr. MALCOLM WINKLER). Strains containing *dnaE* mutations that are antimutators were kindly provided by Dr. ROEL SCHAAPER (FIJALKOWSKA and SCHAAPER 1993).

Genetic techniques and reagents: Map locations were determined based on EcoMap7 (BERLYN *et al.* 1996). Hfr conjugations and P1 transductions using P1*vir* were performed as described (MILLER 1972). Electroporations were done using the Gene Pulser II apparatus (Bio-Rad) as per manufacturer specifications. Cells were grown in Luria-Bertani (LB) broth (10 g tryptone, 5 g yeast extract, 10 g NaCl). Bactoagar (15 g) was added to LB broth to make plates. All media reagents were purchased from Difco. Antibiotics Timentin (Tm) (50 μg/ml), kanamycin (Km) (30 μg/ml), chloramphenicol (Cm) (30 mg/ml) and tetracycline (Tc) (15 μg/ml) were added when necessary. Restriction enzymes were purchased from New England Biolabs and Boehringer Mannheim. *Taq* polymerase was purchased from Cetus.

TABLE 1

Bacterial strains

Strain	Genotype
KY1378	W3110 *dnaG2903*
JV53	MG1655 *parB*
N407	Tn*10* Tc^r *proC*$^-$ *lacZ*$^-$
RAB2903	W3110 *dnaG2903* Tn*10* Tc^r *proC*$^-$ *lacZ*$^-$
RABPARB	MG1655 *parB* Tn*10* Tc^r *proC*$^-$ *lacZ*$^-$
SDG1	RAB2903 *sdgE1*
SDG3	RAB2903 *sdgC3*
SDG6	RABPARB *sdgD6*
SDG7	RAB2903 *sdgC7*
SDG8	RABPARB *sdg8*
SDG9	RAB2903 *sdg9*
SDG10	RAB2903 *sdgG10*
SDG11	RAB2903 *sdgC11*
SDG12	RAB2903 *sdg12*
SDG13	RAB2903 *sdg13*
SDG14	RAB2903 *sdg14*
SDG15	RAB2903 *sdgE15*
SDG16	RAB2903 *sdgD16*
SDG17	RABPARB *sdgC17*
SDG18	RABPARB *sdgC18*
SDG19	RABPARB *sdgC19*
SDG20	RABPARB *sdgC20*
SDG21	RABPARB *sdg21*
SDG22	RABPARB *sdg22*
SDG23	RABPARB *sdgH23*
SDG24	RABPARB *sdgH24*
SDG27	RAB2903 *sdgC27*
SDG28	RAB2903 *sdgD28*
SDG32	RABPARB *sdgH32*
SDG33	RABPARB *sdgC33*
SDG34	RABPARB *sdgD34*

Isolation of cold-sensitive suppressors: Strains containing the *dnaG2903* or *parB* mutations were grown in 5 ml of LB broth at 30° until an OD_{600} of 0.6–0.8. Fifty and 500 μl of each culture were then plated on prewarmed LB plates at 42°. Plates were checked 1, 2 and 3 days after incubation at 42° for suppressors. Individual colonies were patch plated at 42° and 25° to confirm the suppression and to identify suppressors that were cold-sensitive. Once a cold-sensitive suppressor was identified, any other suppressors isolated from the same culture were discarded to ensure that siblings were not chosen.

Plasmids: A shotgun library of the entire *E. coli* chromosome was obtained from Drs. GENSHI ZHAO and MALCOLM WINKLER. Plasmid pDS4-26, which contains a wild-type copy of the *dnaE* gene, was provided by Dr. ROBB MOSES (SHEPARD *et al.* 1984). Plasmids pBS*priB*op and pBSΔ*priB* were obtained from Dr. KEN MARIANS (ZAVITZ *et al.* 1991). The genes carried on these latter two plasmids are *rpsF-priB-rpsR-rplI* and *rpsF-rpsR-rplI*, respectively. In pBSΔ*priB*, the deletion removes the entire *priB* gene and therefore should have little or no polar effect on *rpsR* and *rplI*. The plasmids containing amber nonsense suppressors, pDS1, pGFIB-tRNA^{cys}, and pGFIB-tRNA^{phe} were provided by Dr. GEORGE WEINSTOCK.

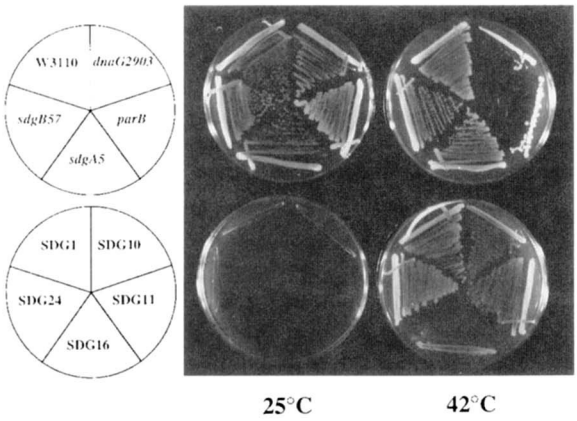

FIGURE 1.—Growth of cold-sensitive suppressors of *dnaG2903* and *parB*. W3110 is a wild-type *E. coli* strain. The *dnaG2903* and *parB* alleles are temperature-sensitive mutations in the *dnaG* gene. The *sdgA5* and *sdgB57* alleles are mutations in the *sdgA* and *sdgB* classes of suppressors, respectively. The SDG strains are cold-sensitive suppressors of *dnaG2903* or *parB*.

Sequencing: The sequencing of the *rpsF* and *priB* genes was performed by the direct sequencing of PCR products amplified from the respective genes. Primers used to amplify and sequence the *priB* gene were priB1 (GTGAGCGTCGCGATGATTTCGC) and priB2 (ACGGCAGAACTTGCGACGACGG). Primers used to amplify and sequence the *rpsF* gene were rpsF1 (GCTGACTTTGTGTGCTCTCCTTCC), rpsF2 (AGTGTAGCGCTCGATCATGCCC), rpsF3 (CGGCTGACCCAGACAGGAGGCG) and rpsF4 (CGATGCTCAAGCACGAACTGGC). PCR products were amplified from *E. coli* genomic DNA using either biotinylated rpsF1, rpsF4, priB1, or biontinylated priB2. Single-strand DNA preparation and sequencing was performed essentially as described (ROA *et al.* 1993).

Kohara hybridization: Hybridizations of probes to the Kohara filter were performed as per manufacturer specifications (Takara Biochemical Inc.)

RESULTS

Isolation of cold-sensitive suppressors of *dnaG2903* and parB: Two mutations affecting *dnaG, dnaG2903* and *parB,* convey chromosome partitioning defects and yield spontaneous suppressors at a high rate (10^{-5}–10^{-6}) (KATAYAMA *et al.* 1989; BRITTON and LUPSKI 1995; VERSALOVIC and LUPSKI 1997). To identify novel suppressors of *dnaG2903* and *parB,* it was necessary to eliminate the two previously described classes of suppressors, *sdgA* and *sdgB.* Because the *sdgA* and *sdgB* class of suppressors were the only two suppressor classes found previously and grow well at 25°, cold-sensitive suppressors of *dnaG2903* and *parB* were isolated. A total of 6100 suppressors were screened (2550 of *dnaG2903* and 3550 of *parB*) and 35 (0.5%) were found to be cold-sensitive (22 were suppressors of *dnaG2903* and 13 were suppressors of *parB*).

Figure 1 demonstrates typical results for the cold-sensitive suppressor isolation screen. The reference wild-type strain W3110 grows well at both 25° and 42°, while *dnaG2903* and *parB* are unable to form colonies at 42°. The alleles *sdgA5* and *sdgB57* are representative mutations of the two previously described classes of suppressors (KATAYAMA *et al.* 1989). Both restore the ability of *dnaG2903* to grow at 42° and can form colonies at 25° after 2 days. The SDG (suppressor of *dnaG*) strains are suppressors of either *dnaG2903* or *parB* that restore colony formation at 42° but are unable to form colonies at 25° after 2 days. It is noted that although the suppressor strains do not form colonies after 2 days at 25°, many of them are not cold-sensitive lethal mutations and do form colonies after several days.

Genetic mapping of sdg cold-sensitive suppressors: The suppressor mutations were mapped within 15-min segments of the *E. coli* chromosome by Hfr conjugation (SINGER *et al.* 1989). Suppressor strains were mated with the seven different donor strains for 35 min and then matings were disrupted by diluting the culture 10-fold and vigorous vortexing for 2–3 min. Exconjugants were plated at 25° for 2 days on LB media containing Km to select for strains receiving the Km^r marker and Tc to select against the donor strains. Exconjugants that formed colonies at 25° after 48 hr were screened for temperature-sensitivity (original *dnaG* phenotype) to insure replacement of the suppressor locus. Of the 35 suppressors isolated, 23 were localized to four different regions of the chromosome (Table 2).

Markers (Km^r) from these different regions of the genome were then transduced by P1 generalized transduction into the suppressor strains. Transductants that received a Km^r marker and were able to form large colonies at 25° after 2 days were tested for temperature sensitivity (the original $dnaG^{ts}$ phenotype). Linked markers that transduced the cells to cold resistance and temperature sensitivity are listed in Table 2. As a control for the P1 mapping, a second unlinked marker was used to ensure that the recovery of the original $dnaG^{ts}$ phenotype was due to replacement of the suppressor allele near the Km^r marker and not reversion of the suppressor mutation. Based on these data, five novel classes of suppressors have been identified and mapped. They are as follows: *sdgC* (4.75′), *sdgD* (20.0′), *sdgE* (58.2′), *sdgG* (91.5′), and *sdgH* (94.8′) (Table 2).

***sdgC* class of suppressors:** The *sdgC* class of suppressors mapped near marker *zae-3095*::Tn*10*kan, which is located at the 4.75′ region of the *E. coli* chromosome. The *dnaE* gene, which encodes the enzymatic subunit of DNA polymerase III, is located at 4.6′ on the *E. coli* chromosome. To test if *dnaE* is the gene responsible for suppression in the *sdgC* class of suppressors, the plasmid pDS4-26, which contains a wild-type copy of the *dnaE* gene, was electroporated into six of the *sdgC* suppressors. Four of these suppressors, *sdgC11, sdgC17, sdgC20,* and *sdgC27,* became temperature-sensitive when the *dnaE* gene was provided *in trans* demonstrating that *dnaE* could reverse the suppression (Figure 2). In addition, the cold sensitivity of the strains was

TABLE 2

Summary of map positions of the suppressor mutations in the SDG strains

Strain	Map location based on Hfr mapping	Linked marker determined by P1 transduction	Map location of marker	Class
SDG1	45-60′	*nadB3140*::Tn*10*kan	58.2′	*sdgE*
SDG3	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG6	10-28′	*zbj-3111*::Tn*10*kan	20.0′	*sdgD*
SDG7	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG8	97-8′	*cycA3185*::Tn*10*kan	95.75′	NA
SDG9	10-28′	ND	—	—
SDG10	82-97′	*malF3180*::Tn*10*kan	91.5′	*sdgG*
SDG11	95-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG14	97-8′	*cycA3185*::Tn*10*kan	95.75′	NA
SDG15	45-60′	*nadB3140*::Tn*10*kan	58.2′	*sdgE*
SDG16	10-28′	*zbj-3111*::Tn*10*kan	20.0′	*sdgD*
SDG17	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG18	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG19	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG20	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG21	45-60′	*zfc-3071*::Tn*10*kan	52.75′	NA
SDG22	ND	*zgh-3159*::Tn*10*kan	67.0′	NA
SDG23	82-97′	*hfq2*::Ωkan	94.8′	*sdgH*
SDG24	82-97′	*hfq2*::Ωkan	94.8′	*sdgH*
SDG27	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG28	10-28′	*zbj-3111*::Tn*10*kan	20.0′	*sdgD*
SDG32	82-97′	*hfq2*::Ωkan	94.9′	*sdgH*
SDG33	97-8′	*zae-3095*::Tn*10*kan	4.75′	*sdgC*
SDG34	10-28′	*zbj-3111*::Tn*10*kan	20.0′	*sdgD*

ND, No interpretable data; NA, Not assigned to a class.

lost when the *dnaE* gene was present. Table 3 shows a summary of the complementation tests for the *sdgC* class of suppressors. While four suppressors were reversed by the *dnaE* gene on a plasmid, two others were not. The other two suppressors are either dominant or potentially do not reside within the *dnaE* gene.

To test whether known *dnaE* mutations isolated as suppressors of *dnaG2903* and *parB* are similar to previously identified *dnaE* alleles, several *dnaE* antimutator alleles (FIJALKOWSKA and SCHAAPER 1993) were tested for suppression of *dnaG2903* and *parB*. These *dnaE* alleles are flanked by Cm^r and Tc^r markers (FIJALKOWSKA and SCHAAPER 1993) and were transduced by P1 into *dnaG2903* and *parB*. Transductants of *dnaG2903* and *parB* that were Cm^r and Tc^r were then tested for growth at 42°. Because the Cm^r and Tc^r markers flank the *dnaE* gene, it is likely that such transductants carry the *dnaE* mutation. None of the *dnaE* antimutator alleles tested were able to suppress *dnaG2903* or *parB*, suggesting that the *dnaE* mutations isolated as suppressors of *dnaG2903* and *parB* may be different from the previously characterized *dnaE* antimutator alleles (data not shown).

***sdgG* suppressor:** One suppressor mapped near the marker *malF3180*::Tn*10*kan, that is located at the 91.5-minute region of the chromosome and was designated

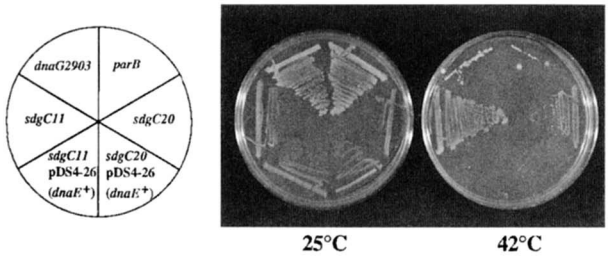

FIGURE 2.—The *dnaE* gene provided *in trans* can reverse suppression in the *sdgC* class of suppressors. The *sdgC11* and *sdgC20* alleles are cold-sensitive suppressors of *dnaG2903* and *parB*, respectively. Plasmid pDS4-26, which contains the *dnaE* gene, is able to reverse the suppression in both *sdgC11* and *sdgC20*.

TABLE 3

Effect of plasmid pDS4-26 (*dnaE*$^+$) on the *sdgC* class of suppressors

Strain	Phenotype[a]	Phenotype with pDS4-26 (*dnaE*$^+$)
SDG7	Tr, Cs	Tr, Cs
SDG11	Tr, Cs	Ts, Cr
SDG17	Tr, Cs	Ts, Cr
SDG20	Tr, Cs	Ts, Cr
SDG27	Tr, Cs	Ts, Cr
SDG33	Tr, Cs	Tr, Cs

[a] Tr, temperature-resistant (grows at 42°); Ts, temperature-sensitive (no growth at 42°); Cr, cold-resistant (grows at 25° after 2 days); Cs, cold-sensitive (no growth at 25° after 2 days).

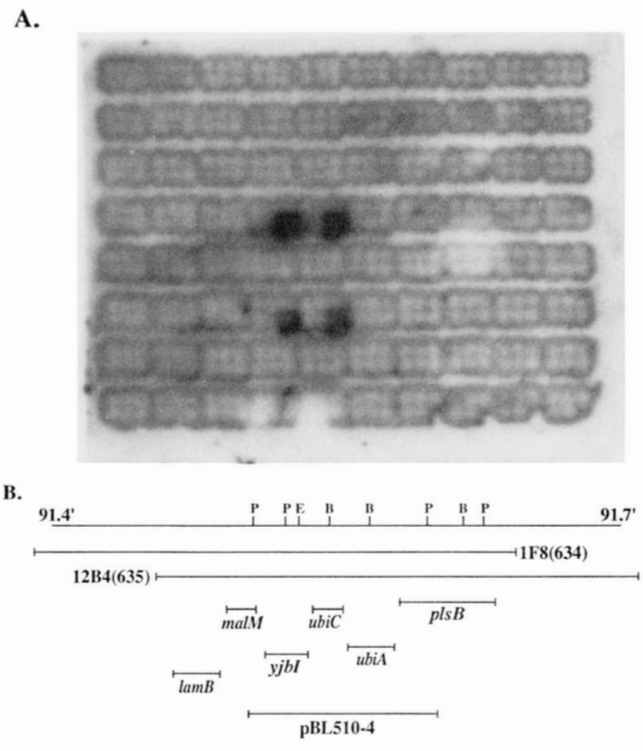

FIGURE 3.—(A) Hybridization of pBL510-4 to the Kohara filter. Two contiguous λ clones, 634 and 635, hybridized to the *E. coli* genomic DNA in pBL510-4. (The signals corresponding to 634 and 635 are located in the middle of the filter. The two signals at the bottom are from a previous hybridization. This is because we do not strip the filters so that the DNA will last longer.) (B) Location of pBL510-4 with respect to the 91.4′–91.7′ region of the *E. coli* genome. pBL510-4, as determined by restriction mapping, likely contains the *ubiC*, *ubiA*, and *o442*(*yjbI*) genes. P, *Pvu*II; E, *Eco*RI; B, *Bam*HI.

sdgG10. Because the *dnaB* gene is located at 91.9′ on the *E. coli* chromosome and a functional interaction between the DnaB protein (helicase) and primase has been demonstrated (TOUGU *et al.* 1994), this suppressor was characterized further. However, the tests shown below demonstrate that *sdgG10* is not an allele of the *dnaB* gene.

Isolation of plasmids that complement *sdgG10*: A shotgun library of the entire *E. coli* chromosome was used to isolate plasmids that reversed the suppression of *sdgG10*. The plasmid library was electroporated into *sdgG10* and transformants were plated at 25°. Colonies that were visible after 2 days were tested for temperature sensitivity at 42°. Four plasmids were isolated from transformants that were temperature-sensitive. Their DNA were hybridized to Kohara filters, containing 476 ordered and overlapping λ clones that contain 99% of the *E. coli* genome (KOHARA *et al.* 1987). Figure 3A shows the hybridization of plasmid pBL510-4 to the Kohara gene mapping filter. Two contiguous λ clones containing DNA from the 91.4′–91.6′ region of the chromosome, 634 (1F8) and 635 (12B4), hybridized to pBL510-4. The other plasmids tested also hybridized to

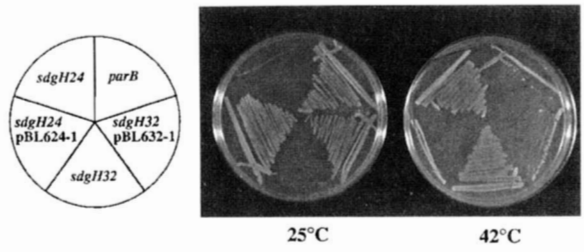

FIGURE 4.—Plasmids that reverse suppression of *sdgH24* and *sdgH32*. The *sdgH24* and *sdgH32* alleles are suppressors of *parB*. The plasmids pBL624-1 and pBL632-1 were isolated from *sdgH24* and *sdgH32*, respectively.

λ clones 634 and 635. The plasmid pBL510-4 had the smallest insert and its DNA map is shown (Figure 3B) in relation to genes identified in this region of the chromosome. Restriction mapping of the plasmids demonstrated that each contained three genes, *ubiA*, *ubiC*, and *yjbI* (*o442*) (Figure 3B).

***sdgH* class of suppressors:** A third class of suppressors was designated *sdgH* and were mapped to the 82′–97′ region of the chromosome by Hfr conjugation. The three alleles in this class, *sdgH23*, *sdgH24* and *sdgH32*, were unable to be further localized on the *E. coli* chromosome using Tn*10*kan markers. To identify the gene affected in this class of suppressors, the shotgun library of the *E. coli* chromosome was electroporated into two of these strains in an attempt to isolate plasmids that reverse the suppression.

Several plasmids that reverse the suppression of *sdgH24* and *sdgH32* were isolated. pBL624-1, which reversed the suppression of *sdgH24*, and pBL632-1, which reversed the suppression in *sdgH32*, are shown in Figure 4. Strains containing *sdgH24* and *sdgH32* are able to form colonies at 42° but are unable to form colonies after 2 days at 25°. The plasmids pBL624-1 and pBL632-1 restore the original *parB* temperature-sensitive phenotype in *sdgH24* and *sdgH32*, respectively. A control plasmid, pBR322, did not have any effect on either strain (data not shown). Since the plasmid pBL624-1 was also able to reverse the suppression caused by *sdgH32*, the *sdgH24* and *sdgH32* mutations likely affect the same gene.

Plasmids that reversed the suppression in both strains were then hybridized to the Kohara filter to determine their location on the *E. coli* physical and genetic map. Figure 5 shows the hybridization of plasmid pBL624-1 to the Kohara filter. For pBL624-1 and for the other plasmids tested, overlapping clones 654 (1G10) and 655 (7E9) hybridized. These λ clones contain DNA from the 95.2′–95.4′ region of the chromosome, which is consistent with the genetic mapping data. The only identified genes in this region constitute the *rpsF* operon: *rpsF-priB-rpsR-rplI*. The *rpsF*, *rpsR* and *rplI* genes encode the ribosomal proteins S6, S18, and L9, respectively. The *priB* gene encodes the PriB protein, which is essential for the replication of ΦX174 phage and

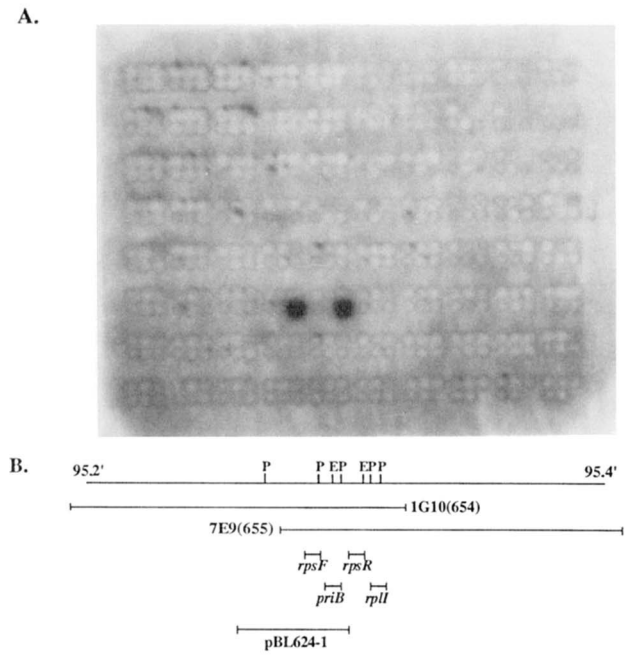

FIGURE 5.—(A) Hybridization of pBL624-1 to the Kohara filter. Two contiguous λ clones, 654 and 655, hybridized to the *E. coli* genomic DNA in pBL510-4. (B) Location of pBL624-1 with respect to the 95.2′–95.4′ region of the *E. coli* genome. pBL624-1, as determined by restriction mapping, contains the *rpsF* and *priB* genes. P, *Pvu*II; E, *Eco*RI.

likely participates in DNA replication in *E. coli* (KORNBERG and BAKER 1992; MARIANS 1992). Presently it is unknown if any of these genes are essential for *E. coli* viability.

All plasmids tested contained genes of the *rpsF* operon, suggesting that one of these genes was responsible for suppression. One plasmid, pBL624-1, contained only the *rpsF* and *priB* genes, thereby refining the candidate gene for this class to one of these two genes (Figure 5). To confirm that the mutations in *sdgH23, sdgH24* and *sdgH32* are located at the 95.3′ region of the chromosome, marker *hfq*Ω2::kan (located at 94.8′ on the chromosome) was transduced into SDG23, SDG24, and SDG32. In all three cases, the strains that became Km^r were also frequently temperature-sensitive (original $dnaG^{ts}$ phenotype). Other unlinked markers tested did not produce the same effect, confirming that the suppressor mutations in SDG23, SDG24, and SDG32 are at the 95.3′ region of the chromosome.

Complementation of *sdgH24* and *sdgH32* by *rpsF* and *priB*: To determine if either *rpsF* or *priB* was responsible for suppression, complementation tests were performed to examine which gene could reverse suppression. The pBS*priB*op plasmid contains all four genes of the operon, while pBSΔ*priB* is the same plasmid with only the *priB* gene deleted. Electroporation of these plasmids into strains SDG24 (*sdgH24*) and SDG32 (*sdgH32*) yielded conflicting results. Suppression was reversed in *sdgH24* only with pBS*priB*op, while in *sdgH32* suppression was reversed by both plasmids (Figure 6), therefore the gene responsible for suppression could be either *rpsF* or *priB*. However, the plasmid pBSΔ*priB* yielded 100- to 1000-fold less transformants in *sdgH24* than did pBS*priB*op, suggesting that this plasmid is lethal when placed into this strain and that either a rearrangement in the plasmid or a suppressor mutation on the chromosome is necessary for pBSΔ*priB* and *sdgH24* to coexist. Restriction digestion analysis of the pBSΔ*priB* plasmid isolated from *sdgH24* transformants showed substantial rearrangements in the plasmid (data not shown).

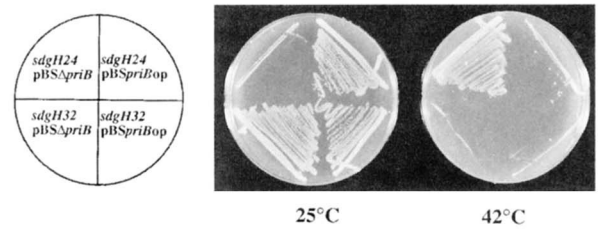

FIGURE 6.—Complementation of suppression with plasmids containing or lacking the *priB* gene. The *sdgH24* and *sdgH32* alleles are suppressors of *parB*. The plasmid pBS*priB*op contains the entire *rpsF* operon. The plasmid pBSΔ*priB* contains the *rpsF* operon with the *priB* gene deleted. See text for details.

DNA sequence analysis of *rpsF* and *priB* in *sdgH24* and *sdgH32*: To determine which gene is involved in the suppression of the *parB* phenotype, the *rpsF* and *priB* gene in each suppressor strain was sequenced. No mutations were detected in the *priB* gene. Analysis of the *rpsF* gene revealed a single point mutation in each strain. The location of these mutations are shown in Figure 7. The *sdgH24* allele contains a G-T mutation at position 49 of the *rpsF* gene, creating a TAG nonsense codon at position 17 of the protein. This mutation results in the truncation of the S6 protein from 130 amino acids to 16 amino acids and is likely a null mutation. The *sdgH32* allele has a deletion of one of two tandem G residues found at nucleotides 141 and 142, resulting

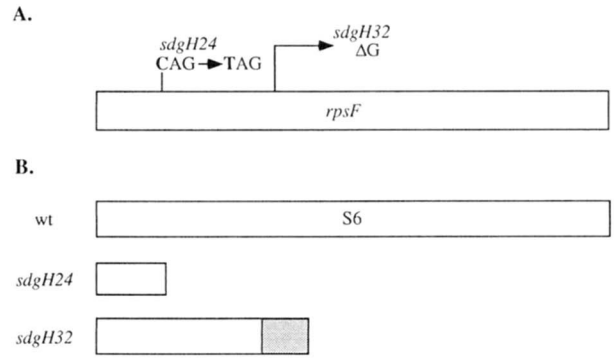

FIGURE 7.—Summary of mutations within *rpsF* that suppress *parB*. (A) Locations of the *sdgH24* and *sdgH32* mutations in the *rpsF* gene. See text for details. (B) Truncated S6 proteins that are likely produced due to the mutations in *sdgH24* and *sdgH32*. □ indicates the 13 new amino acids produced due to the frameshift in *sdgH32*.

TABLE 4

Ability of tRNA nonsense suppressors to reverse suppression in *sdgH24*

Allele	Ability of plasmid to reverse suppression			
	pDS1 ($tRNA^{ser}_{CUA}$)	pGFIB-$tRNA^{phe}_{CUA}$	pGFIB-$tRNA^{cys}_{CUA}$	pBR322
sdgH24	Yes	Yes	Yes	No
sdgH32	No	No	No	No

in a frameshift at this position. This mutation creates a new *Bbv*I restriction site and the presence of this mutation in *sdgH32* was confirmed by restriction analysis with *Bbv*I (data not shown). Tandem in-frame stop codons were identified at codons 61 and 62 of the *rpsF* gene, which by conceptual translation result in a mutant S6 protein in which the first 47 amino acids are wild type and the remaining 13 are frameshifted. In both cases the *sdgH* mutation is likely to have a polar effect on the downstream genes of the operon in addition to having an absolute defect in S6.

Reversion of suppression in *sdgH24* by tRNA nonsense suppressors: To demonstrate that the nonsense mutation in *sdgH24* was responsible for the suppression of *parB,* plasmids carrying nonsense suppressors were electroporated into *sdgH24.* Three different plasmids, pDS1, pGFIB-$tRNA^{phe}$, and pGFIB-$tRNA^{cys}$, which contain the amber nonsense suppressors $tRNA^{ser}$, $tRNA^{phe}$, and $tRNA^{cys}$, respectively, were tested. In all three cases, SDG24 (*sdgH24*) became temperature-sensitive (Table 4). The plasmids did not have a similar effect on strains harboring the *sdgH32* allele. These data confirm the sequencing results and demonstrate that the mutation creating the nonsense codon at codon 17 of *rpsF* is responsible for the suppression of *parB.*

***sdgD* and *sdgE* class of suppressors:** Four SDG suppressors were mapped at 20.0′ by the marker *zbj3111*::Tn*10*kan and define the *sdgD* class of suppressors. A shotgun library of the *E. coli* chromosome was transformed into two of the strains, SDG6 and SDG16, in an effort to identify plasmids that could reverse the suppression in this class. Repeated attempts to isolate such plasmids were unsuccessful. Further characterization is needed to identify the gene responsible for suppression in this class.

The *sdgE* mutations mapped near marker *nadB3140*::Tn*10*kan, which is located at the 58.2 minute region of the *E. coli* chromosome. Further characterization of these suppressors identified the *era* gene, which encodes an essential GTPase, as being responsible for suppression. Characterization of these two *sdgE* suppressors will be presented elsewhere.

DISCUSSION

Cold-sensitive suppressors of *dnaG2903* and *parB* were isolated in an attempt to identify novel suppressors of these alleles. Thirty-five cold-sensitive suppressors were isolated and of these none were of the previously described *sdgA* and *sdgB* classes, demonstrating that this new screen was effective. It is noted that in this type of screen any genes that could suppress *dnaG2903* or *parB* but cannot yield a cold-sensitive phenotype would not be found.

Of the 35 suppressors isolated, 24 have been mapped to the *E. coli* chromosome. The other suppressors were unable to be localized to a specific region of the chromosome. Of the 24 suppressors mapped, 19 have been assigned to five new classes. The putative genes responsible for the suppression in each class are shown in Table 5.

The *sdgC* class of suppressors may reside within *dnaE,* which is the enzymatic subunit of DNA polymerase III. Suppressor strains containing the *sdgC11, sdgC17, sdgC20* and *sdgC27* alleles all become temperature-sensitive when *dnaE* is provided *in trans* on a plasmid, demonstrating that these four alleles are recessive when wild-type *dnaE* is present in multi-copy. Marker rescue experiments and/or direct determination of mutations within the *dnaE* gene in the suppressor strains are necessary to prove that *dnaE* is responsible for suppression. Two other suppressors are not reversed by the *dnaE* gene, indicating that either these mutations are dominant or are not *dnaE* mutations and may be in another gene linked to *dnaE.* Additional mapping experiments should be able to discern between these two possibilities.

FIJALKOWSKA and SCHAAPER have characterized several *dnaE* mutations that have antimutator properties (FIJALKOWSKA and SCHAAPER 1993). These mutants are unable to suppress *dnaG2903* and *parB,* suggesting that the *dnaE* mutations isolated in this study may affect different properties of the DnaE protein. DNA polymerase III has been proposed to directly interact with pri-

TABLE 5

Genes identified in the suppression of *dnaG2903* and *parB*

Class	Gene responsible for suppression
sdgC	*dnaE*
sdgD	?
sdgE	*era*
sdgG	*ubiA, ubiC,* or *yjbI*
sdgH	*rpsF*

mase during lagging strand DNA replication. When primase is used in a general priming reaction, it will synthesize RNA molecules ranging in size from eight to 70 nucleotides. When DNA polymerase III is added to this reaction, RNA molecules ranging from eight to 14 nucleotides are produced, which is the size of primer RNA molecules found *in vivo* (ZECHNER *et al.* 1992). This finding has led to the formation of a model suggesting that *Pol*III regulates primer size *in vivo* via a direct interaction with primase (MARIANS 1992; ZECHNER *et al.* 1992). Perhaps the *dnaE* mutations isolated in this study affect this proposed interaction with primase. Alternatively, the *dnaE* mutations may slow down the replisome allowing more time for the defective primase to attach to the replisome and synthesize a primer RNA. A third possibility is that the suppressor mutation lies in another gene located in the 4–6′ region of the chromosome and that overexpressing *dnaE* from a multicopy plasmid bypasses the suppressor effect.

The *sdgG* class, which is defined by a single suppressor, is either located in the *ubiA, ubiC,* or *yjbI* gene. The ORF *yjbI* is a potential coding region that shows no significant homology to any known protein and has not been proven to be a functional gene. The *ubiA* and *ubiC* genes encode the proteins 4-hydroxybenzoate polyprenyltransferase and chorismate lyase, respectively. Both proteins are involved in the first two steps of the biosynthesis of ubiquinone-8, which is a component of the electron transport chain and the major ubiquinone expressed in *E. coli* during aerobic growth (MEGANATHAN 1996). Strains that are defective in ubiquinone-8 biosynthesis have 30% growth yield when compared to wild type (MEGANATHAN 1996). Because of the role of ubiquinone-8 in the electron transport chain, the *sdgG10* allele may be defective in generating energy potential for the cell. It is possible that these mutations could suppress *dnaG2903* by slowing down cell growth due to decreased energy in the cell, thereby allowing the mutant primase extra time to complete its function. Alternatively, overexpression of one or more of these three genes may bypass the effect of the real suppressor mutation in this strain.

The *sdgH* class of suppressors are two mutations that affect the ribosomal protein S6. Both mutations result in two different truncated proteins. In fact, the *sdgH24* mutation in predicted to produce only a 16-amino acid peptide of S6 and likely represents a null mutation of *rpsF*. This demonstrates that *rpsF* is dispensable for viability in *E. coli*.

The function of S6 in *E. coli* is still largely unknown. The protein is present in one copy per ribosome and interacts with the 16S rRNA subunit of the 30S ribosome (BARTSCH *et al.* 1982; GREGORY *et al.* 1984). Reconstitution studies demonstrated that S6 is not required for normal ribosome functions *in vitro* (NOMURA and HELD 1974), perhaps suggesting S6 plays a regulatory rather than structural role in the ribosome.

The S6 protein is regulated at the posttranslational level by the *rimK* gene product, which adds glutamic acid residues to the carboxy terminus of the protein (NOMURA and HELD 1974). The reason for this posttranslational modification is unclear but such regulation suggests that S6 is important for ribosome function *in vivo*. S6 is also phosphorylated by the phage T7 kinase (gp0.7 PK) during T7 infection, again suggesting a regulatory role for S6 (ROBERTSON *et al.* 1994). T7 phosphorylates several *E. coli* proteins during infection, presumably to regulate their functions (ROBERTSON *et al.* 1994). It is still unclear whether or not S6 is phosphorylated under normal conditions in *E. coli*.

How do the *rpsF* mutations suppress *dnaG2903* mutations? One possible mechanism is that the S6 mutations slow down total cellular growth by slowing down translation. This seems unlikely since other ribosomal mutations were not found in this screen. S6 may play a regulatory role in the translating ribosome that may explain why other ribosomal mutations were not found. An alternative possibility is that S6 may have another function independent of its role in ribosome activity. Ribosomal protein S10 (NusE) has been shown to function in the antitermination of transcription terminators in both λ and *E. coli* (NODWELL and GREENBLATT 1993). The strains carrying the S6 mutations are an excellent resource for determining S6 function *in vivo* in *E. coli*.

In summary, the isolation of cold-sensitive suppressors of *dnaG2903* and *parB* has identified five novel classes of suppressors. These classes may affect genes involved in diverse processes such as DNA replication, translation, and possibly the electron transport chain. Further characterization is needed to determine exactly how each class is involved in suppressing the *dnaG2903* and *parB* mutations.

We appreciate the critical review of this manuscript by Dr. DONALD L. COURT.

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Communicating editor: R. MAURER