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Identification and Characterization of a Novel Allele of *Escherichia coli dnaB* Helicase That Compromises the Stability of Plasmid P1

Roderick A. Slavcev and Barbara E. Funnell*

Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario, Canada

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Bacteriophage P1 lysogenizes *Escherichia coli* cells as a plasmid with approximately the same copy number as the copy number of the host chromosome. Faithful inheritance of the plasmids relies upon proper DNA replication, as well as a partition system that actively segregates plasmids to new daughter cells. We genetically screened for *E. coli* chromosomal mutations that influenced P1 stability and identified a novel temperature-sensitive allele of the *dnaB* helicase gene (*dnaB277*) that replaces serine 277 with a leucine residue (DnaB S277L). This allele conferred a severe temperature-sensitive phenotype to the host; *dnaB277* cells were not viable at temperatures above 34°C. Shifting *dnaB277* cells to 42°C resulted in an immediate reduction in the rate of DNA synthesis and extensive cell filamentation. The *dnaB277* allele destabilized P1 plasmids but had no significant influence on the stability of the F low-copy-number plasmid. This observation suggests that there is a specific requirement for DnaB in P1 plasmid maintenance in addition to the general requirement for DnaB as the replicative helicase during elongation.

The stability of the low-copy-number P1 plasmid depends on its replication system to ensure proper plasmid copy number during the bacterial cell cycle and on its partition system to properly localize and distribute the plasmid copies within the cell. Each of these systems is directed by plasmid-encoded proteins, but in general plasmids use these components to direct bacterial machinery to their chromosomes. In this study we sought to increase our understanding of bacterial involvement in plasmid maintenance and designed a genetic screening procedure to isolate *Escherichia coli* host mutations that reduced P1 stability. One of these mutations is a novel temperature-sensitive allele of *dnaB* that affects both chromosomal and plasmid DNA replication.

The P1 plasmid replication system is governed by the RepA replication initiator, an inactive dimer that is converted into active monomers by host DnaJ and DnaK molecular chaperones (55, 58, 59, 61). In monomeric form, RepA interacts with five direct imperfect 19-bp iteron sequences (*ori*) and autorepresses the promoter for its own gene that lies within this array (1, 10, 11). Further regulation of copy number is achieved by nine additional iteron sequences outside *ori* called *incA* that are proposed to regulate replication via RepA-mediated handcuffing of *ori* (25, 35, 38, 41, 56). In addition to P1 RepA, *E. coli* DnaA and HU proteins bind to the P1 origin (43). These proteins cooperate to melt an AT-rich sequence adjacent to *ori* and presumably promote the loading of the DnaB replication helicase (43).

The 1,413-bp *E. coli dnaB* gene encodes a 470-amino-acid polypeptide that assembles into a hexamer, forming DnaB helicase, which is essential for bacterial DNA replication. Functional domains of DnaB have been characterized in vitro by proteolysis and have been characterized in vivo by deletion analysis (3, 4, 39) (Fig. 1). Phage P1 encodes its own helicase (*ban*) that can functionally substitute for DnaB (12, 29). However, wild-type P1 prophages are repressed for *ban* expression and must rely on an alternate helicase for plasmid replication (12, 46). While it is evident that DnaB is required for P1 plasmid replication in vitro (60), there is little direct genetic evidence to confirm that it is required in vivo. Scott and Vapnek (48) reported a decrease in P1 replication in *dnaB* mutants, although no data or experimental procedures were provided. More compelling but indirect evidence came from Park et al. (43), who were able to accumulate P1 replication intermediates blocked at the initiation step by raising *dnaC*(Ts) mutants to a nonpermissive temperature, which presumably prevented loading of DnaB.

P1 partition requires two plasmid-encoded proteins, ParA and ParB, that act on the centromere-like site, *parS* (reviewed in reference 18). ParB binds to *parS* with the help of the *E. coli* IHF protein, forming a specific partition complex at *parS*. ParA is an ATPase that interacts with this complex to properly localize plasmids inside cells. IHF, which acts as an accessory factor to improve ParB affinity for *parS*, is the only host protein identified so far in the P1 partition reaction (17).

The stability requirements of the unit-copy F (sex factor) plasmid in *E. coli* closely resemble those of P1. The F plasmid-encoded RepE protein parallels P1 RepA in its autoregulation and role in replication initiation, binding to iterons in both the *ori* and *incC* region and subjecting the plasmid to the same handcuffing regulation that suppresses additional rounds of replication initiation in P1 plasmids (25, 35, 38, 41, 56). RepE has recently been shown to exhibit structural homology but not sequence homology with other P1-class replication initiator proteins, including P1, R6K, pSC101, pCU1, pPS10, pFA3, pGSH500, Rts1, and others, based on fold recognition program predictions (49). The genetic arrangement and function of the *sopA-sopB-sopC* partition locus is very similar to the genetic arrangement and function of the P1 *par* locus, and the

* Corresponding author. Mailing address: Department of Medical Genetics and Microbiology, University of Toronto, 1 Kings College Circle, Toronto, Ontario, Canada M5S 1A8. Phone: (416) 978-1665. Fax: (416) 978-6885. E-mail: b.funnell@utoronto.ca.

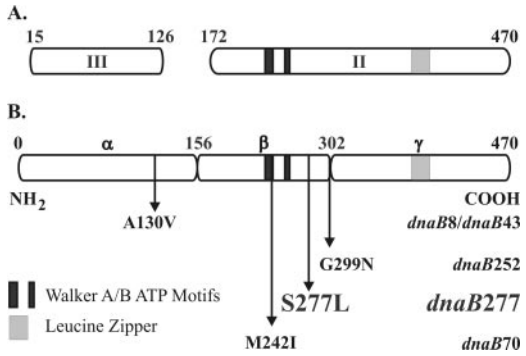


FIG. 1. Domains of DnaB designated by trypsin proteolysis (A) and deletion mapping (B). In vitro, limited tryptic digestion produces two stable protein fragments, a 12-kDa amino-terminal fragment (fragment III; amino acids 15 to ca. 126) that is required for interaction with DnaG and DnaC, as well as for helicase activity, and a C-terminal 33-kDa fragment (fragment II; amino acids 172 to 470) that retains ATPase, hexamerization, and single-stranded DNA binding activities (4, 39). Deletion analyses in vivo have also dissected the DnaB polypeptide into three regions: the α (amino acids 1 to 156), β (amino acids 157 to 302), and γ (amino acids 303 to 471) domains (3). The Walker ATP binding site is located in the β domain. A leucine zipper DNA binding motif is located in the γ domain. The positions of previously sequenced *dnaB*(Ts) mutations (45) are shown relative to the position of the *dnaB277* mutation, which was identified in this study. Sequences of the *dnaB6*, *dnaB22*, and *dnaB107* alleles have not been reported yet to our knowledge.

proteins (but not the *par* sites) exhibit limited sequence homology (18, 36).

Here, we describe a synthetic lethal approach to isolate *E. coli* mutations that compromise P1 maintenance. We found that two of the mutations isolated were identified as alleles of *ihfA* and *dnaB*. We further characterized a *dnaB* mutation, *dnaB277*, which is temperature sensitive for growth. This allele of *dnaB* reduced P1 stability but not F stability and thus appears to play a specific role in P1 plasmid maintenance.

MATERIALS AND METHODS

Reagents, media, and buffers. The suppliers for reagents were as follows: restriction enzymes and enzymes for DNA manipulations, New England Biolabs and Stratagene; ethane methylsulfonate (EMS) and antibiotics, Sigma; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), Roche; and radioisotopes, Amersham Biosciences. Bacterial cells were grown in Luria-Bertani (LB) medium or M9 medium (33). Antibiotics, when present, were used at the following concentrations: ampicillin, 100 μg/ml; chloramphenicol, 25 μg/ml; kanamycin, 25 μg/ml; and rifampin, 100 μg/ml. M9Xgal plates were M9 agar plates supplemented with glucose (0.2%), Casamino Acids (0.2%), tryptophan (50 μg/ml), adenine (70 μg/ml), thymine (40 μg/ml) and X-Gal (40 μg/ml). MinA buffer is 0.09 M potassium phosphate buffer (pH 7) with 1 g of $(NH_4)_2SO_4$ per liter and 0.5 g of sodium citrate per liter (33).

Bacterial strains, bacteriophages, and plasmids. *E. coli* K-12 strains and genotypes are listed in Table 1. *E. coli* N99 was used as the background strain for all genetic studies, and DH5 was used for plasmid construction. P1*rev6* and P1*c17* were the transducing phages used for strain construction (52). λNK1316 and λNK1323 encoded miniTn*10kan* and miniTn*10tet*, respectively (26). λ*lacI* was constructed by cloning the *lacI*^{q} gene from pMC9 (8) (the EcoRI fragment with HindIII synthetic linkers) into the HindIII site of the i^{21} phage λD69 (34).

To construct *E. coli* N99 *lacI*::*tet*, a Gal$^+$ derivative of N99 was first randomly mutagenized by miniTn*10tet* insertion by using λNK1323 infection as described previously (26). *lacI* mutants were isolated based on their ability to grow on phenyl-β-D-galactoside as the sole carbon source. Disruption of the *lacI* gene by miniTn*10* was confirmed by Southern hybridization. The *lacI*::*tet* allele was introduced into N99 by transduction with P1*rev6* to create strain BF226. The *lacI*::*cam* allele was constructed by linear transformation (62) by using a fragment of pMC9 in which the *lacI* coding sequence was interrupted at its MluI site by a chloramphenicol resistance cassette. The *lacI*::*cam* allele was then also transduced into N99, creating strain BF826.

TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
DH5	Δ(*argF-lac*)*169 glnV44 rfbD1 gyrA96 recA1 endA1 spoT1 thi-1 hsdR17*	
N99	*galK*	NIH[a]
BF226	*galK lacI*::*tet*	This study
N99 I1-18	*galK lacI*::*tet I1-18*	This study
N99 I1-20	*galK lacI*::*tet I1-20*	This study
BF826	*galK lacI*::*cam*	This study
BF833	*galK lacI*::*tet dnaB277* miniTn*10kan*	This study
BF834	*galK lacI*::*tet dnaB6* miniTn*10kan*	This study
BF835	*galK lacI*::*cam dnaB252 zjb504*::Tn*10*	This study
BF843	*galK lacI*::*tet dnaB8* miniTn*10kan*	This study
BF844	*galK lacI*::*tet dnaB*$^+$ miniTn*10kan*	This study
BF853	*galK lacI*::*tet dnaB22* miniTn*10kan*	This study
BF855	*galK lacI*::*tet dnaB70* miniTn*10kan*	This study
BF856	*galK lacI*::*tet dnaB107* miniTn*10kan*	This study
BF865	*galK dnaB252 zjb504*::Tn*10*	This study
BF890	*galK dnaB*$^+$ miniTn*10kan*	This study
BF891	*galK dnaB8* miniTn*10kan*	This study
BF892	*galK dnaB277* miniTn*10kan*	This study
BF915	*galK recA56 dnaB277* miniTn*10kan*	This study
E107[b]	*thr-1 leuB6 fhuA21 lacY1 glnV44 rfbD1 thyA6 rpsL67 thi-1 dnaB107 deoC1*	57
FA22[b]	*dnaB22 deoA21*	57
HfrH 165/70[b]	Hfr, *relA1 thyA11 spoT1 thi-1 dnaB70*	6
PC1[b]	*leuB6 thyA47 rpsL153 dnaC1 deoC3*	57
PC6[b]	*leuB6 thyA47 rpsL153 dnaB6 deoC3*	9
PC8[b]	*leuB6 thyA47 rpsL153 dnaB8 deoC3*	9
RS162[b]	*thr-1 leuB6 fhuA21 lacY1 glnV44 rfbD1 thyA6 rpsL67 thi-1 zbj-504*::Tn*10 dnaB252 deoC1*	47

[a] NIH, National Institutes of Health.
[b] Obtained from the *E. coli* Genetic Stock Center.

Alleles of *dnaB* were linked to miniTn*10kan* as follows. A library of miniTn*10kan* insertions in the *E. coli* chromosome was created by infecting λNK1316 (26) into N99 and pooling all Kan^r progeny. A P1*rev6* lysate was prepared with the library and used to transduce N99 I1-18 cells, selecting for Kan^r progeny that grew at 42°C (*dnaB*$^+$ miniTn*10kan*). This miniTn*10* insertion, which was over 90% linked to the *dnaB* gene, was then linked to all *dnaB* alleles used in this study (except *dnaB252* [Table 1]) and used to move them into N99 and/or N99 *lacI*::*tet*. The *dnaB252* mutation was already linked to Tn*10*(*tet*) in strain RS162 and was moved into N99 and N99 *lacI*::*cam*.

N99 *recA56 dnaB277* miniTn*10kan* was constructed by first transducing an *srl*::Tn*10* marker from an *srl*::Tn*10* derivative of MC4100 (50) into N99 *dnaB277* miniTn*10kan* and selecting for Tet^r colonies. Next, the *recA56* allele was transduced from MC4100 *recA56 srl*$^+$ into N99 *dnaB277* miniTn*10kan* with P1*c17*, selecting for Srl$^+$ transductants. One-half of the Srl$^+$ transductants (*srl* is 50% linked to *recA*) were confirmed to be *recA* by UV sensitivity screening.

Individual plasmids used in this study are listed in Table 2. pBEF224 was constructed by replacing the P1 KpnI fragment in pLG49 (which contains *rep* and *par* [20]) with the fragment from λ-mini-P1*par*S_{md2} (19). pBEF231 was constructed by replacing the Cam^r cassette of pBEF224 with a 1.2-kb Kan^r cassette from pUC4-K. pAJM4 was constructed by replacing the *kan* gene in pALA318*kan* with *lacI*^{q} from pMC9 (as BamHI fragments) and then replacing the *cam* region (a PstI fragment) with the 1.2-kb *kan* cassette from pUC4K. pDnaB2 was constructed as follows. A 1,676-bp fragment that included *dnaB* (with its promoter) was amplified from N99 cells by using primers 5′-GCGCG ACGTCCCACCGTGCTTGTGAAATTC-3′ and 5′-CGCGCCCGAGACCAG TTCACGAAGACGTTG-3′ and was inserted into pBR322 at its EcoRV site to produce pDnaB. The former primer contained an AatII restriction site; cleavage of pDnaB with AatII and subsequent religation deleted the pBR322 *tet* promoter and created pDnaB2. pDnaB277 was made in the same way as pDnaB2, except that the amplified DNA product from N99 I1-18 was used. To construct

TABLE 2. Plasmids

Plasmid	Description	Reference or source
pBR322	Vector, Amp^r	5
pST52	Vector compatible with pBR322, Cam^r	51
pUC4-K	Source of Kan^r cassette	Amersham Biosciences
pMC9	Source of *lacI*^{q} gene, Amp^r	8
p3d	*E. coli* library insert in pST52	This study
p4c	*E. coli* library insert in pST52	This study
pDnaB2	*dnaB*$^+$ in pBR322	This study
pDnaB277	*dnaB277* in pBR322	This study
pBEF260	pDnaB2 with in-frame deletion (bp 1 to 437) of *dnaB*	This study
pBEF251	P1 *parB* gene in pBR322	16
pLG44	Low-copy-number miniP1, Par$^+$ Cam^r	20
pLG49	pLG44 carrying *lacI*^{q}, Cam^r	20
pBEF224	pLG49 $parS_{md2}$, Cam^r	This study
pBEF231	pLG49 $parS_{md2}$, Kan^r	This study
pALA318Kan	miniP1 with elevated copy number but no partition system, Cam^r Kan^r	17
pAJM4	miniP1 with elevated copy number but no partition system, *lacI*^{q} Kan^r	This study
pBEF246	pLG44 Δ*parA-parB* (HindIII-XcmI deletion of P1 *par*), *parS*$^+$ Cam^r	This study
pMF3	miniF, Amp^r	31
pBEF215	pMF3 carrying *lacI*^{q}	This study

pBEF261, we used PCR primers 5′-CGCGAGATCTGCCATAGTGAATGGA GTTAC-3′ and 5′-CGCGAGATCTGGCTGAATCCCGCGTCTTTAAAAT T-3′ to amplify the entire pDnaB2 plasmid except the region in *dnaB* between the 1st and 158th codons. Each oligonucleotide also contained a BglII site, and both oligonucleotides were used to prime DNA synthesis with *Pfu* Turbo DNA polymerase. The PCR products were digested with BglII and religated to form pBEF261. Finally, a plasmid library of *E. coli* genes was constructed by insertion of a partial Sau3AI digest of *E. coli* DH5Δ*lac* DNA into the BglII site of pST52 (D. Xu and B. Funnell, unpublished results).

***E. coli* mutagenesis and isolation of mutations that compromise P1 stability.** *E. coli* N99 *lacI*::*tet*(pBEF224) cells were mutagenized with EMS essentially as described by Miller (33). Briefly, cells were grown until the A_{600} was approximately 0.6 in LB medium with chloramphenicol, collected by centrifugation, washed twice in MinA buffer, and resuspended in 0.75 volume of MinA buffer. The mixture was then treated with 7.5 μl of EMS per 0.5 ml of culture for 45 min at 37°C. Cells were again collected by centrifugation, washed twice in MinA buffer, and resuspended in an equal volume of MinA buffer. The culture was titrated to measure viability and diluted 20-fold into LB medium for overnight growth at 30°C. Cells were then plated on M9Xgal plates to screen for plasmid stability mutants and on plates containing LB medium with rifampin to select for rifampin resistance. This treatment with EMS resulted in approximately 80% viable cells and increased the frequency of Rif^r mutations about 1,000-fold (from approximately 10^{-7} to 10^{-4}) (data not shown).

All screening steps were performed at 30°C, and wild-type controls were included for comparison at each step. First, blue colonies on M9Xgal plates were transferred with toothpicks to plates containing LB medium with chloramphenicol so that only cells with miniP1 plasmids were sampled. The resulting colonies were divided into pools (usually 200 colonies per pool), and the pools were transformed by pBEF231 to Kan^r. This step effectively replaced pBEF224 with pBEF231 (due to incompatibility) so that mutations in the miniP1 plasmid pBEF224 were eliminated. The cultures (with pBEF231) were again plated on M9Xgal plates and screened for blue colonies. This time, individual blue colonies were picked and tested several times on M9Xgal plates following plasmid enrichment on plates containing LB medium with kanamycin. Colonies that were reproducibly blue were retained as described above. At least 1,000 colonies were screened for each pool, but mutants isolated from the same pool were considered to be siblings unless later phenotypes (extent of plasmid stability or growth phenotypes, for example) distinguished them. Plasmid-free segregants were isolated and lysogenized by λ*lacI*. Lysogens that were blue on M9Xgal plates (i.e., the blue color was not due to miniP1 plasmid stability) were discarded. Finally, cells were again transformed with pBEF231 to confirm that the phenotype was due to a host mutation.

Plasmid stability tests. A culture of *E. coli* cells containing either P1 or F plasmid was grown overnight in LB medium at 30°C from a single colony under selective conditions. This culture was diluted 10^4-fold into 5 ml of fresh LB medium without antibiotics, grown overnight at 30°C (about 15 generations), and then diluted and plated onto LB medium plates. The resulting colonies were transferred with toothpicks onto plates containing LB medium and LB medium with antibiotics to determine plasmid retention. A minimum of 50 colonies were tested for each strain, and each assay was repeated at least twice.

Measurement of DNA and protein synthesis. The rate of DNA synthesis was measured as described by Khidhir et al. (22). Culture aliquots (500 μl) were taken from log-phase cultures in M9-glucose medium and pulse-labeled for 2 min with [^{3}H]thymidine (6 μCi/ml; specific activity, ~70 Ci/mmol) at various times before and after culture cells were shifted from 30 to 42°C. For protein synthesis, cells were similarly pulse-labeled with [^{3}H]leucine (6 μCi/ml) for 2 min. Ice-cold 15% trichloroacetic acid was added to stop synthesis and precipitate macromolecules. The samples were filtered through glass fiber filters, and the amounts of incorporated ^{3}H-labeled thymidine or leucine were measured by scintillation counting.

RESULTS

Isolation of *E. coli* mutations that compromise P1 plasmid stability. We sought to identify new *E. coli* mutations that compromised P1 stability, and we wanted to be able to differentiate between mutations with effects on replication and mutations with effects on partition. Due to observations that components of the replication machinery are specifically localized in bacterial cells (28), we also considered the possibility that certain mutations might affect both processes. We designed a screening procedure for bacterial mutations that destabilize miniP1 plasmids with weakened Par systems. The rationale was that we would be more likely to uncover mutations that reduced plasmid stability (a synthetic lethal approach). The Par system was weakened due to the presence of a specific *parS* mutation, $parS_{md2}$ (19), which reduced ParB affinity for the site so that it was no longer sufficient for partition in the absence of IHF. The mutation had only a minor effect on plasmid stability in wild-type *E. coli*, but it drastically reduced partition in cells lacking IHF (19). Thus, another benefit of the approach is that we expected to find IHF mutants in this screening analysis as a positive control. In this analysis, the miniP1 plasmid pBEF224 contained the *E. coli lacI* repressor gene in addition to the $parS_{md2}$ mutation, and plasmids were propagated in *E. coli* that was *lacZ*$^+$ but *lacI*. On agar plates containing X-Gal (but with no selection for miniP1), cells in which P1 was stable produced white or very light blue colonies, while cells in which P1 was

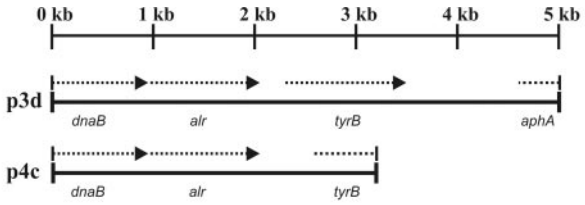
unstable produced darker blue colonies as the plasmid was lost during the growth of the colony. The intensity of the blue color approximately correlated with the number of cells in the colony containing miniP1 (colonies with no miniP1 were intensely blue, colonies with some miniP1 were less blue, etc.), so that we could qualitatively estimate the retention of miniP1 plasmids in each colony.

N99 *lacI*::*tet* cells containing pBEF224 were mutagenized with EMS and then screened for mutations that reproducibly produced blue colonies as described above (see Materials and Methods). The screening procedure and all initial tests were performed at 30°C so that we could recover conditional (temperature-sensitive) mutations in essential genes. Cells that produced blue colonies were retransformed by pBEF231, a Kan^r version of pBEF224, to eliminate plasmid mutations. Candidate mutants were then lysogenized by a λ phage carrying *lacI* to eliminate mutations that produced a blue phenotype independently of plasmid stability (blue λ*lacI* lysogens were eliminated). Using this approach, we screened approximately 65,000 mutagenized cells and identified nine mutants that reduced the stability of miniP1.

One mutant, designated N99 I1-18, was extremely temperature sensitive for growth (see below), while the other eight mutants were not. In this study, we focused on the I1-18 mutation, as it represented a change in a gene that is essential for *E. coli*. However, we also noted that one of the other mutations, designated I1-20, was most likely in *ihfA*, based on two major criteria. First, it could not be lysogenized by the λ*lacI* phage (IHF is required for λ integration [23]). Second, the plasmid stability defect was complemented by introduction (by mating) of F′506 or F′500, whose chromosomal inserts overlap for approximately five min of the *E. coli* map, including the region encoding the *ihfA* gene (37, 40) (data not shown).

We next characterized the stability of a higher-copy-number derivative of miniP1 in the color assay. This plasmid, pAJM4 (Table 2), which contained the *E. coli lacI* gene but not P1 *par*, was reasonably stable because its copy number was about 4 (derived from pALA318 [42]). N99 *lacI*::*tet*(pAJM4) cells were white on M9Xgal plates. N99 I1-20(pAJM4) cells were also white, which was consistent with the properties of an IHF mutant (IHF is involved in partition but not replication [17]). However, N99 I1-18(pAJM4) cells were blue, indicating that the I1-18 mutation did not depend on the P1 partition system for its plasmid stability phenotype and implying that I1-18 influenced the replication of miniP1.

Identification of *dnaB277*. We used an *E. coli* K-12 plasmid library to identify candidate genes altered by the I1-18 conditional mutation. Following transformation of N99 I1-18 by this library, we recovered two different transformants that allowed N99 I1-18 to grow at 42°C. These isolates (with pBEF231) also formed white colonies on X-Gal plates, indicating that miniP1 stability was restored in the cells (Fig. 2). The library plasmids from each isolate, designated p3d and p4c, were characterized by restriction mapping, and the ends of the chromosomal inserts were sequenced. Although the lengths of chromosomal DNA in p3d and p4c differed, the DNA sequences and restriction maps indicated that the two plasmids possessed an overlapping and contiguous region of *E. coli* DNA between 91.8 and 91.9 min of the chromosome (Fig. 2). One end of each of the inserts in p3d and p4c was identical and was located within



Strain	Cell Viability		Colony Color with pBEF231
	30°C	42°C	
N99 *lacI::tet*	+	+	(w)hite
N99-I1-18	+	-	blue
N99-I1-18 [3d]	+	+	w
N99-I1-18 [4c]	+	+	w

FIG. 2. Library plasmids that suppress the I1-18 mutation. As shown at the top, the inserts in plasmids p3d and p4c were identified by sequencing 500 to 700 bp at each end from the vector sequence, followed by BLAST, and were confirmed by restriction mapping. Note that the *dnaB* gene is incomplete and lacks the first 472 bp of coding sequence. The properties of the N99 I1-18(p3d) and N99 I1-18(p4c) isolates are indicated at the bottom. Single colonies of cells were streaked onto preheated LB medium plates at either 30 or 42°C and scored for viability after about 16 h. Single colonies of cells transformed by the pBEF231 miniP1 *lacI*^{q} plasmid (Table 2) were scored for blue color (loss of miniP1) after 8 h of growth on M9Xgal plates at 30°C.

the *dnaB* replication helicase gene (at position 473 of the 1,413-bp *dnaB* gene). Both plasmids also carried *alr*, whose product (alanine racemase) converts L-alanine to D-alanine for subsequent catabolism (30), and all (p3d) or a fraction (p4c) of *tyrB*, whose product (tyrosine aminotransferase) is involved in the biosynthesis of tyrosine and phenylalanine (15, 27). The p3d insert was about 1 kb longer than that of p4c and included the 5′ end of *aphA*, which encodes diadenosine tetraphosphatase, a class B acid phosphatase (54). Although the coding sequence of *dnaB* was not complete, *dnaB* was the only essential gene represented in the chromosomal inserts, and so we considered it to be the most likely gene mutated in N99 I1-18. To test this hypothesis, we cloned the *dnaB* gene with its natural promoter from wild-type strain N99 into the pBR322 vector (see Materials and Methods). The resulting plasmid, pDnaB2, was able to restore viability to N99 I1-18 at 42°C, indicating that *dnaB*$^+$ was sufficient to suppress the temperature-sensitive-lethal defect. We similarly cloned and sequenced the *dnaB* gene from N99 I1-18. Sequencing revealed a G-to-A transition at bp 830 in the coding strand of *dnaB*, corresponding to a change from serine to leucine at amino acid position 277 (Fig. 1). The allele was designated *dnaB277* and encoded DnaB S277L. Finally, pDnaB277 expressing the *dnaB277* allele could not restore viability to N99 I1-18 at 42°C, further supporting the hypothesis that the temperature-sensitive-lethal mutation in N99 I1-18 was in *dnaB*.

To confirm these results and to characterize *dnaB277* further, we moved the I1-18 mutation into a clean, un-

mutagenized N99 background by linking the Ts allele to miniTn*10kan* (see Materials and Methods), and we repeated viability and plasmid stability tests. We also compared it to a variety of other *dnaB*(Ts) alleles that we obtained from the *E. coli* Genetic Stock Center (Table 1). Most alleles were linked to the same miniTn*10kan* used for I1-18 and were transferred into N99 *lacI*::*tet* (Table 1). One allele, *dnaB252*, was already linked to Tn*10*(*tet*), which was used to transfer the allele into N99 and N99 *lacI*::*cam*.

The temperature sensitivities conferred by the various *dnaB* alleles varied slightly. The *dnaB252* and *dnaB277* alleles were the most severe alleles and inhibited colony formation at temperatures above 34°C, but all the *dnaB* mutants were incapable of growth at temperatures above 36°C (data not shown). N99 *lacI*::*tet dnaB277* was transformed by p4c and pST52 (its vector) at 30°C, but only p4c transformants grew when they were streaked out at 42°C, confirming the ability of p4c to suppress the temperature-sensitive-lethal defect in N99 I1-18. The suppression was not complete, however; when measured by plating, the ratio of colonies that grew at 42°C to colonies that grew at 30°C was 0.06. This ratio for pST52 transformants was $<10^{-6}$. In contrast, suppression by pDnaB2 was complete (the ratio was 1.0 [see below]). We constructed a *recA*56 derivative of N99 *dnaB277* (Table 1), tested the viability of p4c transformants at 42°C, and found that the viability dropped to $<10^{-5}$ in the absence of RecA function. We concluded that the p4c plasmid suppressed the *dnaB277* allele by marker rescue.

Interestingly, we observed that although the viability of wild-type cells was not significantly affected by p4c transformation (the 42°C/30°C viability ratio was 0.9), the plasmid did have a low cell growth rate so that N99(p4c) colonies were smaller than N99(pST52) colonies. This observation implied that p4c produced a *dnaB* product that was slightly deleterious to growth in the presence of wild-type DnaB. We created a similarly truncated *dnaB* gene in the pDnaB2 context that was deleted in frame between the *dnaB* start codon and bp 473 within *dnaB* but was expressed from the *dnaB* promoter signals, producing pBEF261 (see Materials and Methods). We found that this plasmid could transform wild-type *E. coli* at 37°C but not at 30°C (reduced by at least 1,000-fold). The 37°C transformants were cold sensitive and unable to form colonies at 30°C. We could not recover pBEF261 transformants in any of the *dnaB* mutant strain set at either temperature. These observations indicate that this fragment of DnaB is deleterious to cell growth in a wild-type background.

We measured the ability of the pDnaB2 plasmids to suppress *dnaB277* in the clean genetic background and confirmed that pDnaB2, but not pDnaB277, could completely suppress the temperature-sensitive viability defects (Table 3). We also tested the suppression of the other *dnaB* alleles by pDnaB2. This plasmid was able to efficiently suppress some alleles (*dnaB6*, *dnaB8*) but not all alleles (Table 3). We observed an incomplete ability of wild-type $dnaB^+$ in pDnaB2 to suppress the viability defects of the *dnaB22*, *dnaB70*, *dnaB107*, and *dnaB252* alleles. These observations suggest that in some contexts the mutant allele interferes with wild-type DnaB function, at least when it is expressed from a multicopy plasmid.

***dnaB277* is a fast-stop replication allele.** Conditional replication mutations are often divided into fast-stop mutations, which immediately inhibit the propagation of the replication fork, and slow-stop alleles, which prevent replication initiation. Since helicase function is required for replication elongation, most *dnaB* alleles, such as *dnaB8*, are fast-stop alleles. However, one allele originally misclassified as a *dnaA* allele is the exception (63). The *dnaB252* mutation is a slow-stop mutation; it does not influence helicase activity (45), and DNA propagation can be suppressed by overexpression of the DnaC replication initiation protein, suggesting that the *dnaB252* mutation may potentially interfere with initiation protein interactions (47). We measured the rates of DNA synthesis before and after a shift from a permissive temperature (30°C) to a nonpermissive temperature (42°C) (Fig. 3A). We compared *dnaB277* to both *dnaB252* (slow stop) and *dnaB8* (fast stop). First, we observed that the overall rate of DNA synthesis by the *dnaB277* mutant at 30°C was less than one-half that by the wild type or by mutants with other *dnaB* alleles for cultures at similar absorbance values (Fig. 3). However, the doubling time of *dnaB277* cells (162 min) was much longer than the doubling times of *dnaB8* cells (85 min) and *dnaB252* cells (106 min), implying that the *dnaB277* mutation was slowing the replication forks. Next, for each mutant we compared the rates of DNA synthesis before and after a shift to 42°C. Following the temperature shift, the *dnaB8* mutant immediately ceased DNA synthesis, as expected, while the *dnaB252* derivative continued DNA synthesis for 30 min following the shift to 42°C before a decline in replication began. The *dnaB277* mutant exhibited a replication fast-stop profile, although the cells did not cease replication quite as rapidly as the *dnaB8* mutant cells and exhibited >80% thymidine incorporation after 5 min at 42°C, compared to <3% for *dnaB8*.

Next, we asked whether the function of heat-labile DnaB S277L could be restored if a culture was shifted back to a permissive temperature. We monitored replication and protein synthesis in N99 *dnaB277* cells that were returned to 30°C following 30 min of incubation at 42°C (Fig. 3B). Prior to the shift back to 30°C, the 42°C cultures were divided, and one of the cultures was treated with chloramphenicol to prevent de novo protein synthesis. We monitored [^{3}H]thymidine incorporation in parallel with [^{3}H]leucine incorporation as measures of DNA and protein synthesis, respectively. Chloramphenicol reduced protein synthesis to less than 3% of the synthesis in untreated cells within 5 min. Following the shift back to the permissive temperature, the cells treated with chloramphenicol

TABLE 3. Suppression of *dnaB* alleles by plasmids carrying *dnaB*

Strain (*dnaB* allele)[a]	Cell viability (42°C/30°C)[b]		
	pBR322	pDnaB2	pDnaB277
BF844 ($dnaB^+$)	0.9	1.0	0.8
BF833 (*dnaB277*)	$<10^{-6}$	1.0	$<10^{-6}$
BF834 (*dnaB6*)	$<10^{-5}$	1.0	NT[c]
BF843 (*dnaB8*)	$<10^{-5}$	1.0	NT
BF853 (*dnaB22*)	3×10^{-5}	0.01	NT
BF855 (*dnaB70*)	2×10^{-5}	0.01	NT
BF856 (*dnaB107*)	6×10^{-5}	0.01	NT
BF865 (*dnaB252*)	4×10^{-5}	0.005	NT

[a] See Table 1. The sequence changes in *dnaB* mutations, if published, are shown in Fig. 1.
[b] Viable cell titer at 42°C divided by viable cell titer at 30°C.
[c] NT, not tested.

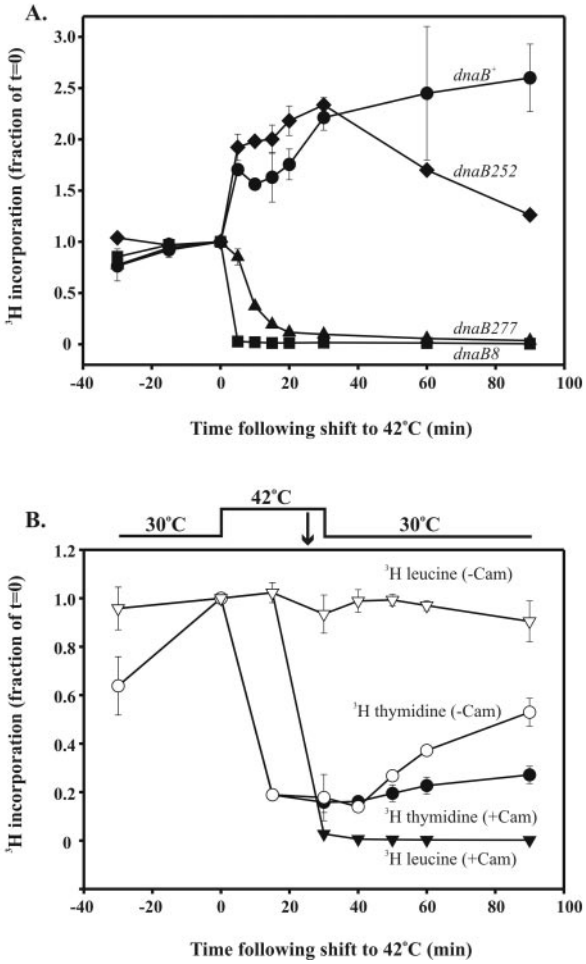


FIG. 3. DNA synthesis profile of *dnaB*(Ts) fast-stop, slow-stop, and *dnaB277* alleles. (A) DNA synthesis measured by [^{3}H]thymidine incorporation into N99 *dnaB*$^+$ (●), *dnaB8* (■), *dnaB252* (♦), and *dnaB277* (▲) before and after a shift from 30 to 42°C in rich media. Time zero was the time of temperature shift from 30 to 42°C. At −30 min, the average counts were as follows: for *dnaB*$^+$ (A_{600}, ~0.21), 43,492 cpm; for *dnaB8* (A_{600}, ~0.2), 45,239 cpm; for *dnaB252* (A_{600}, ~0.25), 59,459 cpm; and for *dnaB277* (A_{600}, ~0.29), 23,060 cpm. The plotted values are averages based on duplicate samples. (B) DNA synthesis and protein synthesis were measured in tandem by measuring incorporation of [^{3}H]thymidine and [^{3}H]leucine into untreated N99 *dnaB277* cells or chloramphenicol (Cam)-treated (125 μg/ml) cells shifted from 30 to 42°C and held at this temperature for 30 min before they were shifted back to 30°C. [^{3}H]leucine incorporation into untreated (▽) and chloramphenicol-treated (▼) cells and [^{3}H]thymidine incorporation into untreated (○) and chloramphenicol-treated (●) cells were determined. Time zero was the point at which cells were shifted from 30 to 42°C. The time of chloramphenicol addition is indicated by the arrow.

exhibited a small but reproducible increase in DNA synthesis. Therefore, the activity of temperature-labile DnaB S277L can be partially restored by shifting the protein back to a permissive temperature, although the activity resumes slowly and recovery is incomplete.

Influence of *dnaB* alleles on P1 and F plasmid stability. We assayed the stability of unit-copy miniP1 (pBEF224) and miniF (pMF3) plasmids in the *dnaB* mutant set by measuring plasmid retention after growth for approximately 15 generations in the absence of selection (Table 4). The *dnaB277* allele compromised P1 stability but not F stability at 30°C, while none of the other *dnaB* alleles assayed reduced the stability of either plasmid. The effect of the *dnaB277* allele was even more dramatic at 34°C (8% P1 retention, compared to >85% P1 retention for all other *dnaB* derivatives [data not shown]). These results were a quantitative measure of the instability of miniP1 that we observed in the initial genetic screening with N99 I1-18. We also measured the stability of the higher-copy-number but Δ*par* miniP1 derivative pALA318Kan (which had the same origin and copy number as pAJM4 [Table 2]). This miniP1 derivative was also destabilized by *dnaB277* but not by the other *dnaB*(Ts) alleles.

TABLE 4. Influence of *dnaB* on P1 and F plasmid stability

Strain (*dnaB* allele)	Plasmid retention (30°C, ~15 generations) (%)		
	pBEF224[a]	pALA318Kan	pMF3
BF844 (*dnaB*$^+$)	91 ± 6	98 ± 2	>98 ± 2
BF833 (*dnaB277*)	44 ± 4	47 ± 5	>98 ± 2
BF834 (*dnaB6*)	93 ± 4	>98 ± 2	>98 ± 2
BF843 (*dnaB8*)	96 ± 2	95 ± 2	>98 ± 2
BF853 (*dnaB22*)	94 ± 2	>98 ± 2	>98 ± 2
BF855 (*dnaB70*)	96 ± 2	>98 ± 2	>98 ± 2
BF856 (*dnaB107*)	93 ± 4	>98 ± 2	>98 ± 2
BF835 (*dnaB252*)	99 ± 2	98 ± 2	>98 ± 2

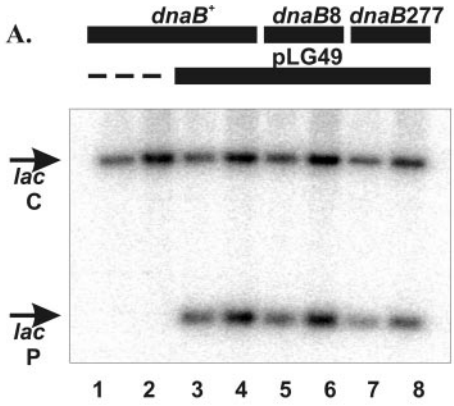
[a] miniP1 *parS*$_{md2}$ pBEF224 (Cam^r) was used for stability assays for all strains except N99 *lacI*::*cam dnaB252*, which is Cam^r. For this strain miniP1 *parS*$_{md2}$ stability was tested with pBEF231 (Kan^r).

Since a plasmid expressing *dnaB*$^+$ (pDnaB2) could fully complement the *dnaB277* allele and restore viability at 42°C, we asked whether it could restore P1 stability in the cells in the type of plasmid retention assay described above (Table 5). We observed that pDnaB2 could restore P1 stability to nearly wild-type levels in N99 *dnaB277*. This finding confirmed that the *dnaB277* allele is responsible for both the TS-lethal and P1 instability phenotypes seen in this strain. Interestingly, pDnaB2 and (to a lesser extent) pDnaB277 destabilized miniP1 in wild-type cells at 30°C. These results support a role for DnaB in P1 plasmid stability and further suggest that overexpression of *dnaB*$^+$ somehow compromises plasmid maintenance.

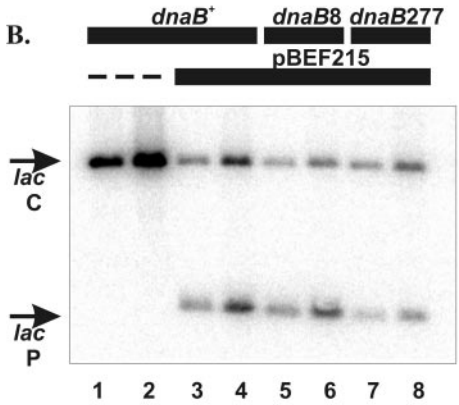
Finally, because the miniP1 plasmid pBEF224 contains a weakened *parS* site (*parS*$_{md2}$), we assayed the effect of *dnaB277* on the *parS*$^+$ miniP1 plasmid pLG49 (which had the same replicon and copy number as pBEF224 [Table 2]). We

TABLE 5. miniP1 (pBEF224) stability in the presence of plasmids carrying *dnaB*

Strain (*dnaB* allele)	Plasmid carrying *dnaB*	miniP1 pBEF224 retention (30°C, ~15 generations) (%)
BF844 (*dnaB*$^+$)	None	91 ± 6
	pBR322 (vector)	99 ± 1
	pDnaB2	24 ± 2
	pDnaB277	48 ± 5
BF833 (*dnaB277*)	None	44 ± 4
	pBR322 (vector)	49 ± 4
	pDnaB2	95 ± 4
	pDnaB277	53 ± 4



Strain	Plasmid	Plasmid copy number
dnaB$^+$	---	---
dnaB$^+$	pLG49	0.9 ± 0.1
dnaB8	pLG49	0.9 ± 0.2
dnaB277	pLG49	0.7 ± 0.2



Strain	Plasmid	Plasmid copy number
dnaB$^+$	---	---
dnaB$^+$	pBEF215	1.0 ± 0.5
dnaB8	pBEF215	1.3 ± 0.6
dnaB277	pBEF215	0.5 ± 0.1

FIG. 4. Determination of plasmid (miniP1) copy number in N99 *dnaB*(Ts) mutants. DNA was isolated, cut with different restriction enzymes, and analyzed by Southern hybridization as described by Funnell and Gagnier (20). (A) Lanes 1 and 2, N99 *dnaB*$^+$ (BF890); lanes 3 and 4, *dnaB*$^+$(pLG49); lanes 5 and 6, *dnaB8* (pLG49) (BF891); lanes 7 and 8, *dnaB277* (pLG49) (BF892). (B) Lanes 1 and 2, N99 *dnaB*$^+$; lanes 3 and 4, *dnaB*$^+$ (pBEF215); lanes 5 and 6, *dnaB8* (pBEF215); lanes 7 and 8, *dnaB277* (pBEF215). Total genomic DNA was digested with EcoRI and EcoRV. The amount of DNA in lanes 2, 4, 6, and 8 was twice the amount in lanes 1, 3, 5, and 7. The *lacI* probe was a 942-bp EcoRI-HincII fragment from pMC9 (8). The arrows indicate the positions of the chromosomal 1.5-kb (*lac* C) and plasmid 0.85-kb (*lac* P) bands hybridizing to the *lacI* probe. The plasmid-to-chromosome (P/C) ratio was calculated for each lane by dividing the intensity of the *lac* P band by the intensity of the *lac* C band. The copy number results for pLG49 were averaged and corrected for the percentage of cells that lost pLG49 (*dnaB*$^+$, <1%; *dnaB8*, <1%; *dnaB277*, 12%).

found that 37% ± 7% of N99 *dnaB277* cells retained pLG49 after 15 generations of growth in nonselective rich medium at 30°C, compared to 98% ± 1% for wild-type N99 cells. These results indicate that destabilization of miniP1 by the *dnaB277* mutation is similar for pLG49 and pBEF224 and thus is not dependent on the specific *parS* site present in the plasmid. In addition, the difference between P1 stability and F stability is not due to the weakened *par* system of pBEF224 and pBEF231.

Next, we measured and compared the copy numbers of a miniP1 plasmid (pLG49) and a miniF plasmid (pBEF215) in *dnaB277* mutants and wild-type cells by Southern hybridization (Fig. 4). These versions of miniP1 and miniF plasmids each contain the *E. coli lacI* gene (Table 2), and *lacI* sequences were used as a probe to measure the ratio of plasmid DNA to chromosomal DNA in wild-type and *dnaB277* cells. Within the error of the experiment, the copy numbers of miniP1 and miniF relative to chromosomal DNA were slightly reduced by the *dnaB277* mutation. This result indicates that this *dnaB* allele influenced replication of both P1 and F plasmids. In addition, overall copy number differences do not simply explain the differential effects of *dnaB277* on miniP1 and miniF (see Discussion).

Plasmid localization. Since components of the replication machinery are known to be specifically localized in bacterial cells, we considered the possibility that *dnaB277* could affect P1 plasmid localization. In addition, genetic evidence has suggested a role for DnaB in the partition of plasmid pSC101 (32). We examined P1 positioning by immunofluorescence of ParB, which forms large foci that colocalize with *parS* and thus with P1 (14). In wild-type cells these foci colocalize with bacterial nucleoids (14) (Fig. 5 and Table 6). In contrast, in the absence of a complete partition system (no ParA), the ParB foci are usually located between and outside the space occupied by the nucleoids. We reasoned that if DnaB were influencing partition as well as replication, ParB foci may also be mislocalized. We observed a 10- to 15-fold-greater number of filamentous N99 *dnaB277* cells than of wild-type cells at 30°C, although the majority of mutant cells exhibited a healthy morphology at this temperature (Table 6). However, shifting *dnaB277* cells to 42°C resulted in extensive filamentation by 60 min (data not shown). We noted that a greater number of *dnaB277* cells had lost the P1 plasmid at 30°C and that the number of foci per plasmid-containing cell was lower than the number seen in wild-type cells (74% of the wild-type value). However, we observed no irregular positioning of foci in the N99 *dnaB277* strain at 30°C (Fig. 5D and E), indicating that P1 localization, as measured by its ability to colocalize with the bacterial nucleoid, is not significantly affected by the *dnaB277* allele.

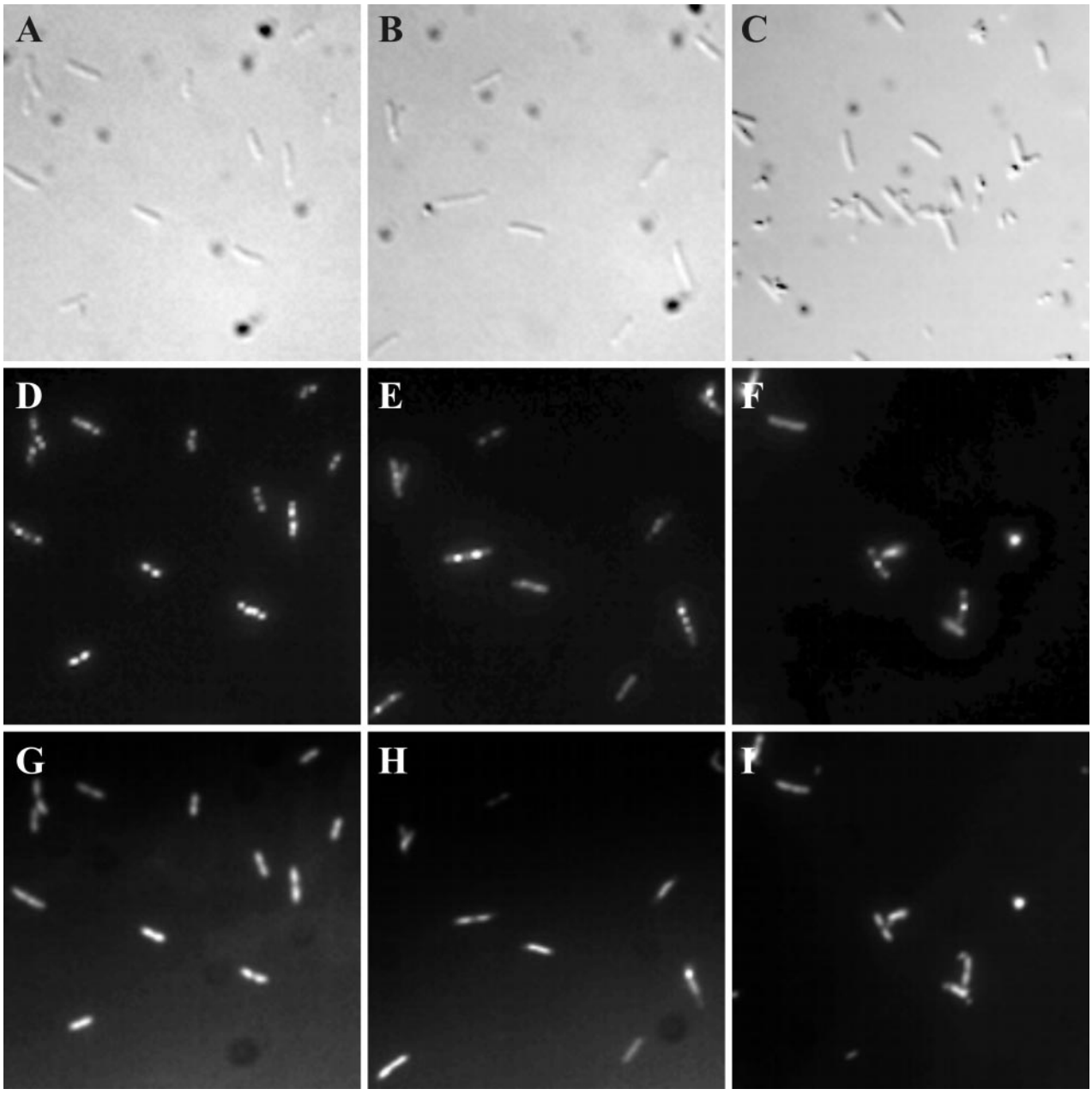


FIG. 5. Visualization of P1 ParB by immunofluorescence. *E. coli* cells were collected and fixed by treatment with paraformaldehyde plus glutaraldehyde (7, 14). The fixed cells were stained with affinity-purified rabbit anti-ParB and then with Cy3-conjugated goat anti-rabbit immunoglobulin G and 0.01% 4′,6-diamidino-2-phenylindole (DAPI) prior to mounting. *E. coli* N99 *dnaB277* (strain BEF892) (Table 1) cells containing the Par^+ pLG44 plasmid ($ParA^+$ $ParB^+$ $parS^+$) were examined and compared to N99 wild-type cells containing pLG44 or Par^- pBEF246 ($parS^+$) in the presence of only ParB (pBEF251). (A, D, and G) Log-phase N99(pLG44) cells. (B, E, and H) Log-phase N99 *dnaB277*(pLG44) cells. (C, F, and I) Log-phase N99(pBEF246/pBEF251) cells. Panels A to C are Nomarski images of cells. Panels D to F show ParB visualized by Cy3-labeled secondary antibodies. Panels G to I show DAPI staining of cells to visualize nucleoids. In the absence of ParA, the pBEF246 plasmid was unstable and resulted in a large number of cells that had lost the plasmid (I).

DISCUSSION

In this paper we describe a genetic screening procedure in which a synthetic lethal approach was used to identify mutations that compromise plasmid maintenance functions. We used a weakened partition system as the background for potential mutations. The rationale was that a strong partition system could compensate for mutations that eliminated accessory factors or that reduced but did not eliminate the functions of essential factors. The screening analysis yielded nine mutants, two of which we identified, *ihfA* and *dnaB*. We point out that *ihfA* would not have been picked up by more traditional screening procedures as IHF is an accessory factor for the P1 partition system (17). The *dnaB277* mutation is a new allele of *dnaB* that exhibits several interesting

TABLE 6. Cell morphology and P1 positioning

Strain (allele of *dnaB*) (plasmid[s])	% of filamentous cells	% of cells with foci	Avg no. of foci/cell[a]	% of foci not overlapping nucleoid	No. of cells
N99 (*dnaB*$^+$) (pLG44)	0.7	98.5	3.1 (3.0)	0.1	457
BF892 (*dnaB277*) (pLG44)	12.1	79.3	2.3 (1.8)	0.6	290
N99 (*dnaB*$^+$) (pBEF246/pBEF251)	1.2	12.2	2.0 (0.2)	95	82

[a] Total number of foci divided by the total number of cells that possess at least one focus. The value in parentheses is the total number of foci divided by the total number of cells.

properties with respect to plasmid stability, so we have begun to characterize it further.

Effects of *dnaB277* on *E. coli*. The *dnaB277* mutation reduces the rate of total DNA replication and exhibits a fast-stop DNA synthesis phenotype (Fig. 3), which indicates a defect during elongation of DNA replication. The DnaB S277L mutation identified in this study is 15 residues C terminal to the conserved aspartate of the Walker B ATP binding motif (Fig. 1). The mutation is within fragment II (defined by partial proteolysis) and the β domain (defined by deletion analysis) of the DnaB polypeptide. The proximity of the ATP binding site and the observation that *dnaB277* is a fast-stop replication mutation (Fig. 3A) may suggest that the change affects helicase activity, perhaps by affecting ATP binding or hydrolysis. Alternatively, other interactions at the replication fork may be compromised so that the fork disassembles at nonpermissive temperatures. DnaB interacts with a variety of chromosomal replication proteins (for example, DnaA and DnaC) during initiation of replication and the τ subunit of DNA polymerase III holoenzyme to coordinate DNA replication on the leading and lagging strands (24, 53).

Effects of *dnaB277* on plasmid maintenance. Based on our observations that the *dnaB277* allele reduced the plasmid copy number (Fig. 4) and destabilized P1 plasmids that do not depend on *par* for their stability (pALA318kan [Table 4]), we inferred that the destabilization of P1 is primarily due to a reduction in plasmid replication. The phenotypes provide direct genetic evidence that DnaB is required for plasmid replication, which is consistent with its requirement for replication in vitro (60). However, although the *dnaB277* allele confers similar reductions in P1 and F copy numbers, it is interesting that F remains relatively stable in the cells and P1 does not. One possibility is that the *sop* system of F may be more effective than P1 *par* in plasmid distribution and thus can suppress the slight reduction in plasmid copy number. Alternatively, the *dnaB277* allele may differentially influence the copy number distribution of F and P1 in the population, without affecting the average copy number. A wider distribution of P1 could result in a greater number of cells that lose the plasmid. Finally, we cannot formally rule out the possibility that *dnaB277* also interferes with P1 partition either directly or indirectly (for example, by delaying the separation of plasmids due to delayed replication).

Hyperrecombination mutants with plasmid stability defects have also been identified (2), since an increase in the number of plasmid dimers and multimers reduces the number of partitionable units. Recently, DnaB has also been shown to drive branch migration at Holliday junctions and thus may be involved in the resolution of recombination intermediates (21). However, we think that it is unlikely that *dnaB277* exerts its effect via recombination for two reasons. We saw no difference in the pattern of plasmid monomers versus multimers when plasmids were isolated from *dnaB*$^+$ and *dnaB277* cells, and miniP1 plasmids were unstable in the Rec$^-$ *dnaB277* derivative (unpublished results). In addition, it is difficult to explain why miniP1 would be more affected than miniF in this regard.

The β domain also contains the regions that interact with pSC101 RepA (amino acids 208 to 261) (13) and R6K π (amino acids 151 to 189) (44). In light of these observations, it is possible that the specific defect in P1 plasmid maintenance involves a weakened interaction between DnaB S277L and the P1 replication initiator, RepA. Interestingly, we also observed that high-copy expression of *dnaB*$^+$ from pDnaB2 in a *dnaB*$^+$ host destabilized miniP1 plasmids (Table 5). We speculated that this destabilization could result from an excess of DnaB titrating RepA away from other initiation factors.

Interactions of mutant and wild-type *dnaB* alleles. We were able to suppress the *dnaB277* allele with a multicopy *dnaB*$^+$ plasmid that restored viability and P1 stability to wild-type levels. These results imply that the *dnaB277* allele is recessive, although we have not confirmed this using single-copy *dnaB*$^+$ in a complementation test. Alleles *dnaB252*, *dnaB22*, *dnaB70*, and *dnaB107* were only partially complemented by the *dnaB*$^+$ plasmid at 42°C. These alleles may compete for protein interactions, with wild-type DnaB diluting out available interactions, or may have a negative dominant influence, generating heteromultimers with wild-type DnaB at their nonpermissive temperatures that reduce DnaB function. Alternatively, the promoter present on the pDnaB2 plasmid may influence *dnaB* gene regulation by diluting out transcriptional stimulators or repressors.

We also found that wild-type cells carrying multicopy plasmids that encoded only the βγ domains of DnaB (p4c and pBEF261) interfered with cell growth. pBEF261 conferred a cold-sensitive phenotype to cells. We concluded that the βγ fragment interferes with wild-type DnaB function, possibly by competing with DnaB for a protein interaction. This effect may be particularly detrimental at lower temperatures, at which the rate of replication is lower.

We designed a genetic screening procedure to isolate host mutations that compromise the stability of plasmid P1 with the goal of improving our understanding of the mechanisms of P1 stability. We used a destabilized miniP1 in order to design a synthetic lethal screening procedure, with which we could isolate mutations that might not have been identified by using a wild-type P1 system. We think that the isolation of an *ihfA* mutation validates this approach, and we suggest that the method could be extended to identify other factors that play

accessory roles in plasmid maintenance in this and other plasmid systems. It should of course yield mutations in genes that are essential, such as the identification here of the *dnaB277* allele. We continue to investigate the involvement of DnaB in P1 plasmid stability, and we are extending this screening analysis to search for other factors that play roles in plasmid maintenance.

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REFERENCES

1. **Abeles, A. L., K. M. Snyder, and D. K. Chattoraj.** 1984. P1 plasmid replication: replicon structure. J. Mol. Biol. **173:**307–324.
2. **Biek, D. P., and S. N. Cohen.** 1986. Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:**594–603.
3. **Biswas, E. E., and S. B. Biswas.** 1999. Mechanism of DnaB helicase of *Escherichia coli*: structural domains involved in ATP hydrolysis, DNA binding, and oligomerization. Biochemistry **38:**10919–10928.
4. **Biswas, S. B., P. H. Chen, and E. E. Biswas.** 1994. Structure and function of *Escherichia coli* DnaB protein: role of the N-terminal domain in helicase activity. Biochemistry **33:**11307–11314.
5. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–113.
6. **Bonhoeffer, F.** 1966. DNA transfer and DNA synthesis during bacterial conjugation. Z. Vererbungsl. **98:**141–149.
7. **Buddelmeijer, N., M. E. G. Aarsman, A. H. J. Kolk, M. Vicente, and N. Nanninga.** 1998. Localization of cell division protein FtsQ by immunofluorescence microscopy in dividing and nondividing cells of *Escherichia coli*. J. Bacteriol. **180:**6107–6116.
8. **Calos, M. P., J. S. Lebkowski, and M. R. Botchan.** 1983. High mutation frequency in DNA transfected into mammalian cells. Proc. Natl. Acad. Sci. USA **80:**3015–3019.
9. **Carl, P. L.** 1970. *Escherichia coli* mutants with temperature-sensitive synthesis of DNA. Mol. Gen. Genet. **109:**107–122.
10. **Chattoraj, D. K., R. J. Mason, and S. H. Wickner.** 1988. Mini-P1 plasmid replication: the autoregulation-sequestration paradox. Cell **52:**551–557.
11. **Chattoraj, D. K., and T. D. Schneider.** 1997. Replication control of plasmid P1 and its host chromosome: the common ground. Prog. Nucleic Acid Res. Mol. Biol. **57:**145–186.
12. **D'Ari, R., A. Jaffe-Brachet, D. Touati-Schwartz, and M. B. Yarmolinsky.** 1975. A *dnaB* analogue specified by bacteriophage P1. J. Mol. Biol. **94:**341–366.
13. **Datta, H. J., G. S. Khatri, and D. Bastia.** 1999. Mechanism of recruitment of DnaB helicase to the replication origin of the plasmid pSC101. Proc. Natl. Acad. Sci. USA **96:**73–78.
14. **Erdmann, N., T. Petroff, and B. E. Funnell.** 1999. Intracellular localization of P1 ParB protein depends on ParA and *parS*. Proc. Natl. Acad. Sci. USA **96:**14905–14910.
15. **Fotheringham, I. G., S. A. Dacey, P. P. Taylor, T. J. Smith, M. G. Hunter, M. E. Finlay, S. B. Primrose, D. M. Parker, and R. M. Edwards.** 1986. The cloning and sequence analysis of the *aspC* and *tyrB* genes from *Escherichia coli* K12. Comparison of the primary structure of the aspartate aminotransferase and aromatic aminotransferase of *E. coli* with those of the pig aspartate aminotransferase isozymes. J. Biochem. **234:**593–604.
16. **Fung, E., J.-Y. Bouet, and B. E. Funnell.** 2001. Probing the ATP-binding site of P1 ParA: partition and repression have different requirements for ATP binding and hydrolysis. EMBO J. **20:**4901–4911.
17. **Funnell, B. E.** 1988. Participation of *Escherichia coli* integration host factor in the P1 plasmid partition system. Proc. Natl. Acad. Sci. USA **85:**6657–6661.
18. **Funnell, B. E., and R. A. Slavcev.** 2004. Partition systems of bacterial plasmids, p. 81–103. *In* B. E. Funnell and G. J. Phillips (ed.), Plasmid biology. ASM Press, Washington, D.C.
19. **Funnell, B. E., and L. Gagnier.** 1993. The P1 plasmid partition complex at *pars*. II. Analysis of ParB protein binding activity and specificity. J. Biol. Chem. **268:**3616–3624.
20. **Funnell, B. E., and L. Gagnier.** 1995. Partition of P1 plasmids in *Escherichia coli mukB* chromosomal partition mutants. J. Bacteriol. **177:**2381–2386.
21. **Kaplan, D. L., and M. O'Donnell.** 2002. DnaB drives DNA branch migration and dislodges proteins while encircling two DNA strands. Mol. Cell **10:**647–657.
22. **Khidhir, M. A., S. Casaregola, and I. B. Holland.** 1985. Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated *E. coli*: inhibition is independent of *recA* whilst recovery requires RecA protein itself and an additional, inducible SOS function. Mol. Gen. Genet. **199:**133–140.
23. **Kikuchi, Y., and H. A. Nash.** 1978. The bacteriophage λ*int* gene product. J. Biol. Chem. **253:**7149–7157.
24. **Kim, S., H. G. Dallman, C. S. McHenry, and K. J. Marians.** 1996. Coupling of a replicative polymerase and helicase: a tau-DnaB interaction mediates rapid replication fork movement. Cell **84:**643–650.
25. **Kittell, B. L., and D. R. Helinski.** 1991. Iteron inhibition of plasmid RK2 replication in vitro: evidence for intermolecular coupling of replication origins as a mechanism for RK2 replication control. Proc. Natl. Acad. Sci. USA **88:**1389–1393.
26. **Kleckner, N., J. Bender, and S. Gottesman.** 1991. Uses of transposons with emphasis on Tn*10*. Methods Enzymol. **204:**139–180.
27. **Kuramitsu, S., K. Inoue, H. Ogawa, and H. Kagamiyama.** 1985. Aromatic amino acid aminotransferase of *Escherichia coli*: nucleotide sequence of the *tyrB* gene. Biochem. Biophys. Res. Commun. **133:**134–139.
28. **Lemon, K. P., and A. D. Grossman.** 1998. Localization of bacterial DNA polymerase: evidence for a factory model of replication. Science **282:**1516–1519.
29. **Lemonnier, M., G. Ziegelin, T. Reick, A. M. Gomez, R. Diaz-Orejas, and E. Lanka.** 2003. Bacteriophage P1 Ban protein is a hexameric DNA helicase that interacts with and substitutes for *Escherichia coli* DnaB. Nucleic Acids Res. **31:**3918–3929.
30. **Lilley, P. E., N. P. Stamford, S. G. Vasudevan, and N. E. Dixon.** 1993. The 92-min region of the *Escherichia coli* chromosome: location and cloning of the *ubiA* and *alr* genes. Gene **129:**9–16.
31. **Manis, J. J., and B. C. Kline.** 1977. Restriction endonuclease mapping and mutagenesis of the F sex factor replication region. Mol. Gen. Genet. **152:**175–182.
32. **Miller, C., and S. N. Cohen.** 1999. Separate roles of *Escherichia coli* replication proteins in synthesis and partitioning of pSC101 plasmid DNA. J. Bacteriol. **181:**7552–7557.
33. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
34. **Mizusawa, S., and D. F. Ward.** 1982. A bacteriophage lambda vector for cloning with BamHI and Sau3A. Gene **20:**317–322.
35. **Morrison, P. F., and D. K. Chattoraj.** 2004. Replication of a unit-copy plasmid F in the bacterial cell cycle: a replication rate function analysis. Plasmid **52:**13–30.
36. **Motallebi-Veshareh, M., D. A. Rouch, and C. M. Thomas.** 1990. A family of ATPases involved in active partitioning of diverse bacterial plasmids. Mol. Microbiol. **4:**1455–1463.
37. **Movva, N. R., E. Katz, P. L. Asdourian, Y. Hirota, and M. Inouye.** 1977. Gene dosage effects of the structural gene for a lipoprotein of the *Escherichia coli* outer membrane. J. Bacteriol. **133:**81–84.
38. **Mukherjee, S., I. Patel, and D. Bastia.** 1985. Conformational changes in a replication origin induced by an initiator protein. Cell **43:**189–197.
39. **Nakayama, N., N. Arai, M. W. Bond, Y. Kaziro, and K. Arai.** 1984. Nucleotide sequence of *dnaB* and the primary structure of the protein from *Escherichia coli*. J. Biol. Chem. **259:**97–101.
40. **Novel, M., and G. Novel.** 1976. Regulation of β-glucuronidase synthesis in *Escherichia coli* K-12: constitutive mutants specifically derepressed for *uidA* expression. J. Bacteriol. **127:**406–417.
41. **Pal, S. K., and D. K. Chattoraj.** 1988. P1 plasmid replication: initiator sequestration is inadequate to explain control by initiator-binding sites. J. Bacteriol. **170:**3554–3560.
42. **Pal, S. K., R. J. Mason, and D. K. Chattoraj.** 1986. P1 plasmid replication. Role of initiator titration in copy number control. J. Mol. Biol. **192:**275–285.
43. **Park, K., S. Mukhopadhyay, and D. K. Chattoraj.** 1998. Requirements for and regulation of origin opening of plasmid P1. J. Biol. Chem. **273:**24906–24911.
44. **Ratnakar, P. V. A. L., B. K. Mohanty, M. Lobert, and D. Bastia.** 1996. The replication initiator protein π of the plasmid R6K specifically interacts with the host-encoded helicase DnaB. Proc. Natl. Acad. Sci. USA **93:**5522–5526.
45. **Saluja, D., and G. N. Godson.** 1995. Biochemical characterization of *Escherichia coli* temperature-sensitive *dnaB* mutants *dnaB8*, dnaB252, *dnaB70*, *dnaB43*, and *dnaB454*. J. Bacteriol. **177:**1104–1111.
46. **Schaefer, T. S., and J. B. Hays.** 1991. Bacteriophage-P1 Bof protein is an indirect positive effector of transcription of the phage bac-1 *ban* gene in some circumstances and a direct negative effector in other circumstances. J. Bacteriol. **173:**6469–6474.
47. **Sclafani, R. A., and J. A. Wechsler.** 1981. Deoxyribonucleic acid initiation mutation *dnaB252* is suppressed by elevated *dnaC*$^+$ gene dosage. J. Bacteriol. **146:**418–421.
48. **Scott, J. R., and D. Vapnek.** 1980. Regulation of replication of the P1 plasmid prophage, p. 335–345. *In* B. Alberts (ed.), Mechanistic studies of DNA replication and genetic recombination. Academic Press, Toronto, Canada.
49. **Sharma, S., B. K. Sathyanarayana, J. G. Bird, J. R. Hoskins, B. Lee, and S. Wickner.** 2004. Plasmid P1 RepA is homologous to the F plasmid RepE class of initiators. J. Biol. Chem. **279:**6027–6034.

50. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Som, T., and J. Tomizawa.** 1982. Origin of replication of *Escherichia coli* plasmid RSF1030. Mol. Gen. Genet. **187:**375–383.
52. **Sternberg, N. L., and R. Maurer.** 1991. Bacteriophage-mediated transduction. Methods Enzymol. **204:**19–43.
53. **Sutton, M. D., K. M. Carr, N. Vicente, and J. M. Kaguni.** 1998. *Escherichia coli* DnaA protein—the N-terminal domain and loading of DnaB helicase at the *E. coli* chromosomal origin. J. Biol. Chem. **273:**34255–34262.
54. **Thaller, M. C., Schippa, S., Bonci, A., Cresti, S., and G. M. Rossolini.** 1997. Identification of the gene (*aphA*) encoding the class B acid phosphatase/phosphotransferase of *Escherichia coli* MG1655 and characterization of its product. FEMS Microbiol. Lett. **146:**191–198.
55. **Tilly, K., and M. Yarmolinsky.** 1989. Participation of *Escherichia coli* heat shock proteins DnaJ, DnaK, and GrpE in P1 plasmid replication. J. Bacteriol. **171:**6025–6029.
56. **Uga, H., F. Matsunaga, and C. Wada.** 1999. Regulation of DNA replication by iterons: an interaction between the *ori2* and *incC* regions mediated by RepE-bound iterons inhibits DNA replication of mini-F plasmid in *Escherichia coli*. EMBO J. **18:**3856–3867.
57. **Wechsler, J. A., and J. D. Gross.** 1971. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. Mol. Gen. Genet. **113:**273–284.
58. **Wickner, S., J. Hoskins, and K. McKenney.** 1991. Monomerization of RepA dimers by heat shock proteins activates binding to DNA replication origin. Proc. Natl. Acad. Sci. USA **88:**7903–7907.
59. **Wickner, S. H.** 1990. Three *Escherichia coli* heat shock proteins are required for P1 plasmid DNA replication: formation of an active complex between *E. coli* DnaJ protein and the P1 initiator protein. Proc. Natl. Acad. Sci. USA **87:**2690–2694.
60. **Wickner, S. H., and D. K. Chattoraj.** 1987. Replication of mini-P1 plasmid DNA *in vitro* requires two initiation proteins, encoded by the *repA* gene of phage P1 and the *dnaA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84:**3668–3772.
61. **Wickner, S. H., J. Hoskins, and K. McKenney.** 1991. Function of DnaJ and DnaK as chaperones in origin-specific DNA binding by RepA. Nature **350:**165–167.
62. **Winans, S. C., S. J. Elledge, J. H. Krueger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with cloned fragments in *Escherichia coli*. J. Bacteriol. **161:**1219–1221.
63. **Zyskind, J. W., and D. Smith.** 1977. Novel *Escherichia coli dnaB* mutant: direct involvement of *dnaB252* gene product in the synthesis of an origin-ribonucleic acid species during initiation of a round of deoxyribonucleic acid replication. J. Bacteriol. **129:**1476–1486.