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Multicopy Suppressors of the Cold-Sensitive Phenotype of the *pcsA68* (*dinD68*) Mutation in *Escherichia coli*

TAKESHI YASUDA, TOSHIO NAGATA, AND HARUO OHMORI*

Institute for Virus Research, Kyoto University, Kyoto, Kyoto 606-01, Japan

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The *Escherichia coli* strain cs2-68 is a cold-sensitive (cs) mutant that forms a long filamentous cell at 20°C with a large nucleoid mass in its central region. We have recently shown that the *pcsA68* mutation causing the cs phenotype is a single-base substitution within the *dinD* gene, a DNA damage-inducible gene which maps at 82 min. Since null mutants of the *pcsA* (*dinD*) gene are viable, with no discernible defect in cell growth, the cs phenotype is attributed to a toxic effect by the mutant protein. In an attempt to identify a target(s) for the toxic *pcsA68* mutant protein, we screened for chromosomal fragments on multicopy plasmids that could suppress the cs phenotype. Three different *Bam*HI fragments were found to suppress cold sensitivity, and the *lexA*, *dinG*, and *dinI* genes were identified to be responsible for the suppression in each fragment. DinG shares multiple motifs with many DNA helicases. The complete sequence of *dinI* revealed that DinI is a small protein of 81 amino acids. It is similar in size and sequence to ImpC of the *Salmonella typhimurium* plasmid TP110 and to a protein (ORFfs) of the retronphage φR67, both of which are also under the control of LexA.

Most organisms have a genetic system(s) to ensure a tight coordination between chromosomal replication and cell division even if normal processes in the cell cycle are accidentally disturbed (10, 33, 46, 48). In *Escherichia coli* cells, a central role in this coordination is performed by RecA, a multifunctional protein that is also essential in genetic recombination (6). If replication is interrupted by DNA damage, RecA is activated by binding to the single-stranded DNA region resulting from the damage (43). Activated RecA promotes self-cleavage of LexA (28), which is a general repressor for more than 20 SOS genes dispersed on the chromosome and plasmids (6, 23, 46). Some induced proteins directly participate in repairing DNA damage (6), but other products contribute to the repair only indirectly, as exemplified by the *sulA* gene product (10). SulA prevents cell division by binding to FtsZ protein (4), a key element in septation, thus providing more time for completion of DNA repair, and its degradation by Lon protease leads to resumption of the bacterial cell cycle (32).

We have recently shown (37) that the *pcsA68* mutant, which is a cold-sensitive (cs) mutant forming a long filament with a large nucleoid mass at the midcell (20), has a single-base substitution within the *dinD* gene (13), an SOS gene located at 82 min (23, 30). When the *pcsA68* mutant cells were exposed to 20°C, the SOS response was induced in the cells without any treatment that damages DNA (37), similarly to conditional mutants defective in DNA replication that are placed under nonpermissive conditions (43). However, the *pcsA* null mutants were fully viable with no discernible defect, indicating that the *pcsA* gene product played no essential role in replication or partition of replicated daughter chromosomes under normal growth conditions. These results implied that under the nonpermissive temperatures the *pcsA68* mutant protein interfered with the normal replication process, probably at some late stage, and consequently induced the SOS response. Once induced in the *pcsA68* mutants at 20°C, the SOS response should result in more production of the toxic *pcsA68* mutant protein, thus amplifying its adverse effect to eventual formation of long filaments. In agreement with this idea, the cs phenotype of the *pcsA68* mutation was suppressed by *recA* or *lexA* (Ind^-) mutations that impair induction of the SOS response (37).

The *pcsA68* mutant protein could interfere with normal chromosomal replication directly by binding to DNA or indirectly through interaction with another protein(s) which binds to DNA. If the latter were the case, we would predict that the cs phenotype should be suppressed when the target protein(s) was produced at higher levels by increasing the gene dosage. To test this prediction, we screened for multicopy plasmids carrying a chromosomal fragment that could suppress the cs phenotype of the *pcsA68* mutant. We describe the isolation and characterization of the three genes which act as *pcsA68* suppressors when present in multicopy.

MATERIALS AND METHODS

Bacteria and plasmids. The bacterial strains used are all derivatives of *E. coli* K-12 and are listed in Table 1. Gene disruption was carried out by the positive and negative selection method that we developed (37). In short, an *E. coli* fragment with a Kan-disrupted gene(s) was placed on pKH5002 (Amp^r) carrying the *E. coli* $rpsL^+$ gene (the target of streptomycin), the vector plasmid which replicates in RNase H-deficient (*rnhA*) cells but not in wild-type cells. Transformation of $rnhA^+$ *rpsL* (Str^r) cells by such a plasmid generates Str^s Amp^r Kan^r cointegrates, which upon selection by streptomycin yield the revertants Str^r Amp^s Kan^s and disruptants Str^r Amp^s Kan^r. Bacteriophage P1*vir* was used for generalized transduction. The plasmids used are listed in Table 2. pTZ19ULC is a low-copy-number derivative of pTZ19U, which was constructed by sequence-directed mutagenesis (35) to revert the high-copy-number mutation present in pTZ19U and other pUC-like plasmids (27). A pUC9 derivative carrying the *impC* gene from plasmid R64, which contains a sequence identical to that in the *impC* gene of TP110, was provided by Teruya Komano at Tokyo Metropolitan University. Another pUC9 derivative containing an 11-kb *Eco*RI fragment of retrophage φR67 (9) was provided by Sumiko Inouye at the Robert Wood Johnson Medical School and was used for further subcloning.

Media. LB (1% Bacto Tryptone, 0.5% yeast extract, 0.5% NaCl [pH 7.4]) was used for cultivation of bacteria. L agar contained 1.5% agar. If necessary, ampicillin (50 μg/ml), kanamycin (20 μg/ml), tetracycline (20 μg/ml), or streptomycin (100 μg/ml) was added. $CaCl_2$ at a final concentration of 2.5 mM was supplemented to LB and L agar for P1 transduction.

DNA manipulation and sequencing. Most procedures for DNA manipulation and bacterial transformation with plasmid DNA followed the standard protocols (41). For nucleotide sequence analysis by the dideoxy chain terminator method (42), DNA fragments were cloned in $pSKII^+$ (obtained from Stratagene, La Jolla, Calif.) or pTZ19U (obtained from Bio-Rad, Richmond, Calif.) phagemid

* Corresponding author. Mailing address: Institute for Virus Research, Kyoto University, 53, Kawahara-Machi, Shogoin, Sakyo-ku, Kyoto, Kyoto 606-01, Japan. Phone: 81-75-751-4033. Fax: 81-75-751-3989. Electronic-mail address: hohmori@virus.kyoto-u.ac.jp

TABLE 1. Bacterial strains used in this study

Strain	Genotype	Source, construction, or reference
cs2-68	F$^-$ *pcsA68 thr leu trp his thy thi ara lac gal xyl mtl strA*	20
JH137	HfrC *phoA*(Am) (λ$^-$) *dinD1*::Mud1 (Ap *lac*)	7
MC1061	F$^-$ Δ*lacX74 strA araD139* Δ(*ara*, *leu*)*7697 galU galK hsr hsm*$^+$	Laboratory stock
KN2007	MC1061 *pcsA68 zic-11*::Tn*10*	37
SY50	MC1061 Δ*dinG1*::*kan*	This work[a]
SY51	MC1061 *ybiB1*::*kan*	This work[b]
SY52	MC1061 Δ(*dinG-ybiB*)*101*::*kan*	This work[c]
SY117	MC1061 *dinI1*::*kan*	This work[d]
SY118	MC1061 *pcsA68 zic-11*::Tn*10* Δ*dinG1*::*kan*	KN2007 × P1(SY50)
SY121	MC1061 *pcsA68 zic-11*::Tn*10* *dinI1*::*kan*	KN2007 × P1(SY117)

[a] The 1.8-kb region flanked by the *Aat*II and *Bcl*I sites was replaced by *kan* (see line 6 in Fig. 2).
[b] The *kan* marker was inserted at the *Nde*I site in the *ybiB* gene (see line 5 in Fig. 2).
[c] The 2.3-kb region flanked by the *Eco*RV and *Eco*RV sites was replaced by *kan* (see line 7 in Fig. 2).
[d] The *kan* marker was inserted at the *Pvu*II site in the *dinI* gene (see Fig. 4).

vector. Single-stranded DNAs were prepared by infection of the helper phage M13KO7 (45) and were used as templates for reactions with the Sequenase kit from U.S. Biochemicals (Cleveland, Ohio).

In vitro protein synthesis and analysis of the products. Plasmid-encoded proteins were synthesized and labeled with [^{35}S]methionine by using the coupled transcription-translation system described by Zubay (49) with approximately 1 μg of plasmid DNA, as described elsewhere (1). The cell extracts were kindly supplied to us by Y. Akiyama at this institute. Reactions (15-μl volume) were run at 37°C for 40 min and were stopped by adding 15 μl of ice-cold 10% trichloroacetic acid solution. The precipitates were collected by centrifugation, washed with 500 μl of cold acetone, and resuspended in the sample buffer (21) for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The 19.6% acrylamide gel system described by Ito et al. (12) was used to separate small proteins. Products were visualized by Fujix bioimaging analyzer BAS2000 (Fuji Film, Tokyo, Japan).

Computer analysis of DNA or protein sequence. The presence of coding frames in DNA sequences was examined with the GENEMARK program (5). Homology search analysis of DNAs or proteins encoded in DNA sequences was done by using the BLAST (2), FASTA (38), and MPsrch (44) programs.

Nucleotide sequence accession number. The DNA sequences determined in the present study have been deposited in DNA Data Bank of Japan under accession number D31709.

RESULTS

Isolation of multicopy suppressors. We previously noticed that the cs phenotype of the *dinD68* (for uniformity, *dinD* is used instead of *pcsA* hereafter) mutation was suppressed by high-copy-number plasmids containing a segment as short as 111 bp near the 5′ end of the *dinD* coding region, as well as by high- or low-copy-number plasmids carrying the intact *dinD* gene (37). For isolation of multicopy suppressors, we chose JH137 (7), a *dinD1*::Mud1(Ap *lac*) strain, as the source of chromosomal DNA. Since the Mu phage insertion occurred approximately 500 bp downstream of the segment described above (30, 37), we anticipated that the *Bam*HI fragment from this strain that contained the short segment and the *dinD-lacZ* fusion would function as a multicopy suppressor and that the plasmids carrying this fragment should be easily distinguished from the rest for its Lac$^+$ phenotype.

The chromosomal DNA of JH137 was digested by *Bam*HI and ligated with *Bam*HI-cleaved DNA of pTZ19ULC, a low-copy-number derivative of pTZ19U. KN2007 (*dinD68* Δ*lacX74*)

TABLE 2. Plasmids used in this study

Plasmid	Vector or precursor	Relevant feature or construction	Derivation, source, or reference
pUC4K	pUC4	*amp*$^+$ *kan*$^+$	Pharmacia
pSKII$^+$	pUC	*amp*$^+$	Stratagene
pTZ19U	pUC19	*amp*$^+$	Bio-Rad
pTZ19ULC	pTZ19U	Sequence-directed mutagenesis	This work
pMspA	pTZ19ULC	φ(*dinD1-lacZ*)(Hyb)	This work
pMspB	pTZ19ULC	*dinI*$^+$	This work
pMspC	pTZ19ULC	*lexA*$^+$ *dnaB*$^+$	This work
pMspD	pTZ19ULC	*dinG*$^+$ *ybiB*$^+$	This work
pKH5002	pHSG664	*rpsL*$^+$	37
pYP16a	pTZ19ULC	*dinG*$^+$ (the fragment in line 1 in Fig. 2)	This work
pYP16b	pTZ19ULC	*ybiD*$^+$ (the fragment in line 4 in Fig. 2)	This work
pYP17a	pTZ19ULC	*dinG*$^+$ *ybiB*$^+$ (the fragment in line 2 in Fig. 2)	This work
pYP17b	pTZ19ULC	*ybiC*$^+$ (the fragment in line 3 in Fig. 2)	This work
pYP21	pYP17a	Δ(*dinG-ybiB*)::*kan* (the fragment in line 7 in Fig. 2)	This work
pYP22a	pYP17a	*dinG*$^+$ *ybiB*::*kan* (the fragment in line 5 in Fig. 2)	This work
pYP23	pKH5002	Δ(*dinG-ybiB*)::*kan* (the fragment in line 7 in Fig. 2)	This work
pYP24	pKH5002	*dinG*$^+$ *ybiB*::*kan* (the fragment in line 5 in Fig. 2)	This work
pYP27a	pYP17a	Δ*dinG*::*kan ybiB*$^+$ (the fragment in line 6 in Fig. 2)	This work
pYP32a	pKH5002	Δ*dinG*::*kan ybiB*$^+$ (the fragment in line 6 in Fig. 2)	This work
pYP26	pTZ19ULC	*ORFf ORFg* of φR67 (1.2-kb *Hpa*I$_{6098}$-*Hpa*I$_{7258}$ fragment)[a]	This work
pYP28	pTZ19ULC	*ORFf* of φR67 (0.8-kb *Hpa*I$_{6098}$-*Bcl*I$_{6874}$ fragment)[a]	This work
pKH5601a	pSKII$^+$	Fragment in line 1 in Fig. 3	This work
pKH5601b	pSKII$^+$	*dinI*$^+$ (the fragment in line 2 in Fig. 3)	This work
pKH5601c	pSKII$^+$	*dinI*$^+$ (the fragment in line 3 in Fig. 3)	This work
pKH5601d	pSKII$^+$	Fragment in line 4 in Fig. 3	This work
pYP47	pKH5002	*dinI*::*kan* (the *kan* marker was inserted at the *Pvu*II site in the 2.2-kb *Bam*HI fragment)	This work
pYP78	pTZ19ULC	*impC*$^+$ of R64 (0.8-kb *Eco*RI$_1$-*Cla*I$_{824}$ fragment)[b]	This work

[a] The base position refers to that in reference 9.
[b] The base position refers to that in reference 29.

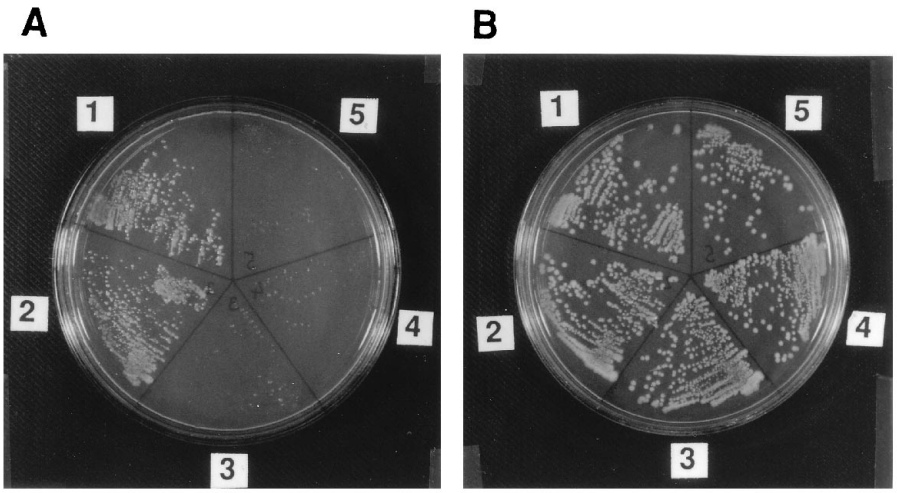

FIG. 1. Multicopy suppressors of the *dinD68* mutation. cs2-68 cells harboring pYP16a (*dinG*$^+$ [1]), pMspB (*dinI*$^+$ [2]), pYP78 (*impC*$^+$ [3]), pYP28 (*ORFf*$^+$ of φR67 [4]), and pTZ19ULC (vector [5]) were streaked on L agar plates containing ampicillin, and the plates were incubated at 20°C for 4 days (A) or at 37°C for 1 day (B).

was used as the host for transformation with the reaction product. Plasmid DNAs were isolated from the transformants that appeared on plates containing ampicillin after incubation at 20°C for several days. The plasmids were reexamined for their ability to suppress the cs phenotype of KN2007 and also of the original *dinD68* strain cs2-68 that showed a more strict cs phenotype than KN2007. In the first round of screening, four different *Bam*HI fragments were found to exhibit suppressor activity (Fig. 1). The plasmids carrying each of the four fragments were designated pMspA to pMspD, respectively, among which pMspA exhibited the Lac$^+$ phenotype and served as a standard for measuring suppressor activity. None of them suppressed the cs phenotype completely, and the ratios of the numbers of colonies formed at 20°C to those formed at 37°C were approximately 0.2 to 0.5. The same procedures were repeated, but no fragments other than the four described above were found among the candidates in the second screening, so that we characterized the three plasmids (pMspB, pMspC, and pMspD) in more detail.

Identification of *lexA* and *dinG* as the suppressor genes in plasmids pMspC and pMspD, respectively. We determined the DNA sequence of each insert in the above three plasmids from both directions. The results, through homology search analysis against the *E. coli* sequences in GenBank, allowed us to locate the fragments in the *E. coli* chromosome. pMspC was found to carry an approximately 10-kb *Bam*HI fragment derived from the 92-min region, which contained the *lexA* gene near one end and the *dnaB* gene near the other end (16, 40). Derivatives of pMspC lacking the portions near *dnaB* retained suppressor activity, but deletions entering into the *lexA* gene, for example a C-terminal deletion retaining the promoter and operator region and the N-terminal 122 amino acids, abolished this activity (not shown). Furthermore, we observed that a plasmid carrying only the *lexA* gene intact (pTH227 [8]) could suppress the cs phenotype of the *dinD68* mutation (not shown).

Similarly, pMspD was found to carry the 5.7-kb *Bam*HI fragment derived from the 18-min region, the whole sequence of which we determined previously (36). The fragment contains the *dinG* (*rarB*, in our former designation) gene, which is an SOS gene containing an atypical LexA binding sequence (24, 25), whose predicted product has multiple motifs commonly found among DNA helicases (17). The *Bam*HI fragment also contains two downstream open reading frames (ORFs) in the same direction (*ybiB* and *ybiC* [Fig. 2]). The *ybiB* gene is probably cotranscribed with *dinG*, because the distance between *dinG* and *ybiB* is only 27 base pairs, and a GC-rich palindromic sequence with 4 T residues, similarly to transcriptional terminators or attenuators, is found 10 bp after the *ybiB* termination codon. The predicted sequence for the *ybiB* gene product (YbiB [320 amino acids]) shows a weak similarity to anthranilate phosphoribosyltransferases from many bacterial species. Various derivative plasmids containing a subfragment of the 5.7-kb *Bam*HI fragment were constructed and examined for their ability to suppress the *dinD68* cs phenotype. The result, as summarized in Fig. 2, indicated that the intact *dinG* gene was necessary and sufficient for the activity.

Identification of *dinI* as the suppressor gene in pMspB. The DNA sequence at one end of the 2.2-kb insert in pMspB coincided with that of the *Bam*HI fragment containing the 3′ region of the *pyrC* gene at 24.2 min (16, 40). Of the *Bam*HI fragment, only about a 900-bp sequence had been determined previously (3, 47), which included the 120-bp sequence downstream of the *pyrC* coding region (Fig. 3). In this downstream

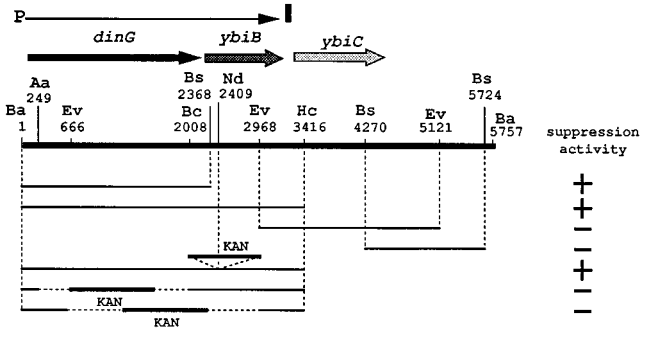

FIG. 2. Suppression by *dinG*. Fragments depicted by horizontal lines were cloned in pTZ19ULC and examined for suppression activity. In the fifth line, the kanamycin resistance (KAN) gene was inserted at the *Nde*I site. In the last two lines, the DNA segments depicted by broken lines were deleted and replaced by the kanamycin resistance gene. Base positions were numbered, starting with the left *Bam*HI site as 1. Ba, Aa, Ev, Bc, Bs, Nd, and Hc, *Bam*HI, *Aat*II, *Eco*RV, *Bcl*I, *Bss*HII, *Nde*I, and *Hin*cII sites, respectively. Thin horizontal line with P, transcription of *dinG* and *ybiB*, which probably stops at a putative terminater (denoted by a thick vertical bar); horizontal boxes, coding regions.

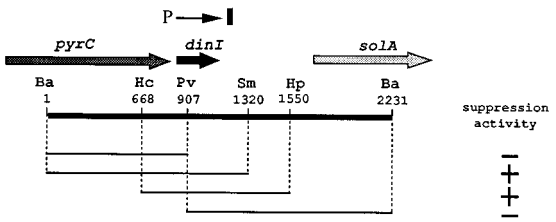

FIG. 3. Suppression by *dinI*. The ORF at the right was designated the *solA* (sarcosine oxidase-like) gene (18). Pv, Sm, and Hp, *Pvu*II, *Sma*I, and *Hpa*I sites, respectively. Other symbols are as described in the legend to Fig. 2.

sequence, Lewis et al. (23) found one of the high-affinity binding sites for LexA, which overlapped with a probable −10 signal sequence for transcription initiation. Furthermore, they showed that a single-base substitution at the conserved base within the LexA binding site abolished the binding activity in vitro and designated the putative gene under LexA regulation *dinI*. We determined the sequence of the remaining 1.3-kb region of the 2.2-kb *Bam*HI fragment. The result indicated that the *dinI* coding region consisted of 81 codons and it was followed by a GC-rich palindromic sequence with a downstream T stretch, reminiscent of ρ-independent termination signals (Fig. 4). After an approximately 600-bp noncoding region, a partial coding sequence was found (Fig. 3), the expected product of which showed a strong similarity with the N-terminal sequence of *Bacillus* sarcosine oxidase (18, 19).

The DNA segment capable of suppressing the *dinD68* cs phenotype was narrowed down to a small region of approximately 650 bp flanked by the *Hinc*II site at 668 and the *Sma*I site at 1320 (Fig. 3). Since deletion of either the N-terminal or the C-terminal portion of the *dinI* gene abolished suppressor activity, we conclude that the intact *dinI* gene is required for suppression. By in vitro protein synthesis coupled with transcription, we observed that pMspB yielded a product of approximately 9 kDa, the size expected for DinI from the DNA sequence (Fig. 5, lane 2).

Proteins homologous to DinI. Homology search analysis of DinI revealed that DinI has two homologs of similar size. One

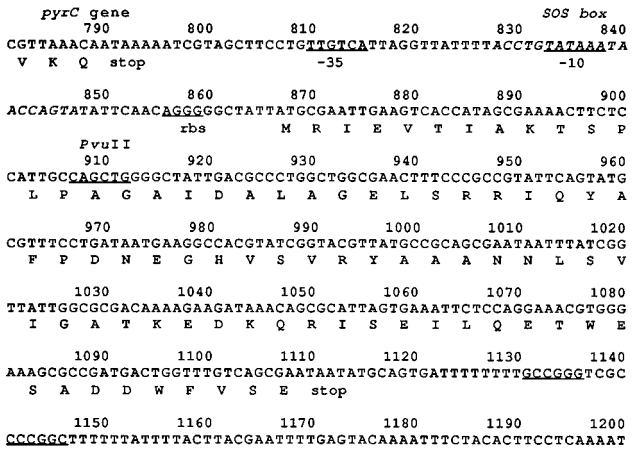

FIG. 4. Nucleotide sequence of the *dinI* gene. The probable −35 and −10 signals for transcription initiation, ribosome-binding site (rbs), *Pvu*II site used for insertion of the kanamycin resistance gene, and GC-rich palindromic sequence downstream of the coding region are underlined in this order. The SOS box sequence at positions 828 to 847 is depicted in italics. The amino acid residues deduced from the DNA sequence are indicated by the single-letter codes below the DNA sequence.

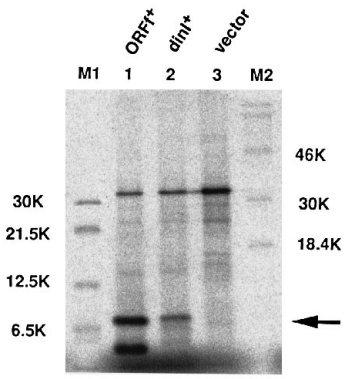

FIG. 5. In vitro translation analysis. Reactions with pYP28 (*ORFf* of ϕR67 [lane 1]), pMspB (*dinI* [lane 2]), or pTZ19ULC (vector [lane 3]) as template were carried out, and the reaction products were analyzed, as described in Materials and Methods. In lanes M1 and M2, different sets of molecular weight markers were loaded. The topmost bands in lanes 1, 2, and 3 are β-lactamase (32 kDa). The arrow indicates the positions of the bands corresponding to DinI and ORFfs (approximately 9 kDa). The lowest band in lane 1 is probably a degradation product of ORFfs, since it was not consistently observed.

is ImpC (82 amino acids), which is encoded by the promoter-proximal gene of the LexA-regulated *impCAB* operon in the *Salmonella typhimurium* plasmid TP110, which is involved in SOS-induced mutagenesis (29). The function of *impC* is not known, since its deletion did not affect mutagenesis (29).

Another protein homologous to DinI is that encoded by *ORFf* in the retronphage ϕR67, whose function is unknown (9, 11). Lewis et al. (23) found a sequence similar to the SOS box consensus sequence also upstream of this *ORFf* (5′-TACTGT AAATATAAACAGTG-3′ at positions 6182 to 6201 in Fig. 6). This sequence probably corresponds to the operator sequence for *ORFf* transcription, because a probable combination of the −35 and −10 signals overlaps with it (Fig. 6), as in many other SOS genes (6, 23). ORFf was originally expected to start with

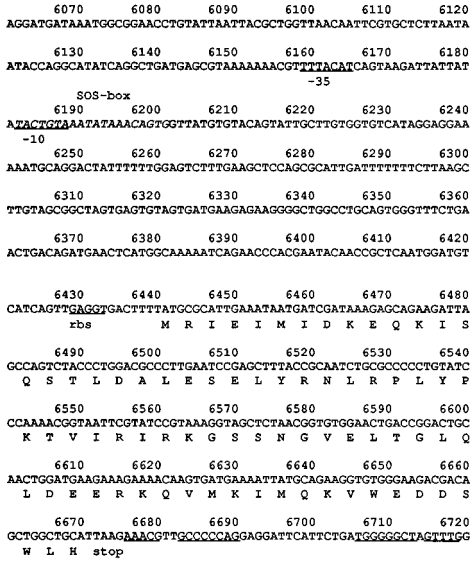

FIG. 6. Nucleotide sequence around *ORFf* of ϕR67. This sequence was taken from Hsu et al. (9). The probable −35 and −10 signals for transcription initiation, ribosome-binding site (rbs), and palindromic sequence downstream of the coding region are underlined in this order. The SOS box-like sequence at positions 6182 to 6201 is depicted in italics. ORFf was originally thought to start with an ATG at 6369 to 6371, but the downstream ATG at 6441 to 6443 is more likely to be the initiation codon (see text).

```
consensus    RIE               AL  E        P

 DinI    1   MRIEVTIAK--TSPLPAGAIDALAGELSRRIQYAFPDNEGHVSVRYAAANN
              ***.  ...  *. * .*..**..*...*..  .*  *  ...  ..* .
 ImpC    1   MIRIEILFDRQSTKNLKSGTLQALQNEIEQRLKPHYP--EIWLRIDQGSAPS
             .****..*.  .....  .**.**..*. ..*.* **  .. .** .**. .
 ORFfs   1   MRIEIMIDK--EQKISQSTLDALESELYRNLRPLYP--KTVIRIRKGSSNG
              ...* .**  .*** *  .** . ** . .. ..*   * . ..***
 Tum    25   VKVNITLDK--DQKIGQPVIDAFQCELTKRIQSVFP--STRVTVKKGS

consensus        G                W   DD W

 DinI    50  LSVIGAT-KEDKQRISEILQETWESADD-WFVSE
             .**.** .. **.** ..*.*.*.  ** *.
 ImpC    51  VSVTGARNDKDKERILSLLEEIWQ--DDSWLPAA
             *..** . *.............*.  *****
 ORFfs   48  VELTGLQLDEERKQVMKIMQKVWE--DDSWLHX
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FIG. 7. Similarities among DinI, ImpC, ORFfs, and Tum proteins. The FASTA program was used to analyze the similarity between each combination of two proteins. Identical and similar residues are indicated by asterisks and periods, respectively. The consensus residues among DinI, ImpC, and ORFfs are indicated at the tops of the sequences.

the ATG codon at positions 6369 to 6371 and code for a protein of 101 amino acids (9); however, our experiment with in vitro protein synthesis indicated that the gene product was slightly smaller than DinI, which has 81 amino acids (Fig. 5, lane 1). Therefore, it seems more likely that the ORFf starts with the ATG codon at 6441 to 6443, which is preceded by a good candidate for the ribosome-binding site (the 5′-GAGG-3′ sequence at 6429 to 6432), because the product is then expected to consist of 77 amino acids, in agreement with the above result by in vitro protein synthesis. An example of multiple alignment among DinI, ImpC, and ORFfs (the smaller product of ORFf) is shown in Fig. 7. The residues among the three proteins are only 29 to 36% identical, but the similar residues amount to 78 to 87%.

To examine whether the *impC* or *ORFf* gene was able to function as a multicopy suppressor for the *dinD68* mutation, we constructed derivatives of pTZ19ULC containing *impC* or *ORFf* with their cognate promoter and operator region. Neither of them appeared to suppress the cs phenotype (Fig. 1).

Phenotypes of null mutants of *dinG* and *dinI*. Prior to this study, we had isolated two mini-Tn*10* (Kan) insertion mutants of the *dinG* gene (*rarB11* and *rarB19* [36]) which showed temperature-sensitive (ts) cell growth under special conditions; that is, in *dnaA5*(Ts) *rnhA* genetic backgrounds and in rich media such as L broth. In the present study, we constructed some deletion and insertion mutants in the *dinG-ybiB* region (Fig. 2, lines 5 to 7) from MC1061 ($dnaA^+$ $rnhA^+$). All of the mutants isolated were fully viable, with no difference in UV sensitivity from that of the wild type (data not shown). We then transferred the Δ*dinG1*::*kan* mutation into KN2007 (*dinD68*) by P1 phage transduction to construct a *dinD68* Δ*dinG1* double mutant (SY118). If the *dinG* gene product were the target for the toxic *dinD68* gene product, such a double mutant would be expected to become cold resistant. However, the double mutant appeared to be more cold sensitive than the *dinD68* single mutant (not shown). Similarly, we isolated a null mutation in the *dinI* gene by inserting the Kan resistance gene at the *Pvu*II site near the 5′ end (Fig. 4). The *dinI1*::*kan* mutant showed UV sensitivity similar to that of its parental strain, and a *dinD68 dinI1*::*kan* double mutant (SY121) remained cold sensitive (not shown).

DISCUSSION

Although null mutations in the *dinD* gene do not affect bacterial cell growth, one particular mutation in it (*dinD68*) restricts growth at low temperatures, inducing the SOS response. Intriguingly, the cs phenotype was suppressed by a short segment within its coding region when the segment was placed on multicopy plasmids (37). One plausible explanation for this observation was that a sequence in the segment might serve as an entry site for DinD to bind DNA, and a multicopy plasmid containing the sequence might titrate out the toxic DinD68 proteins. No sequence similar to the segment described above was found among the *E. coli* sequences available from DNA databases. To know how many other segments on the *E. coli* genome could exhibit such an activity, we undertook isolation of multicopy suppressors for the cs phenotype, also hoping that a target of the *dinD68* toxic protein might be identified among such multicopy suppressors. However, none of the suppressors newly isolated was found to contain a sequence similar to that of the above segment within the *dinD* gene or a target for DinD68. Thus, we have no definitive answer yet to the questions of why the *dinD68* mutant is cs and why the short segment described above can function as a multicopy suppressor. Nevertheless, the present study has revealed that each of three additional genes, *lexA*, *dinG*, and *dinI* (all SOS genes), could function as a multicopy suppressor and that DinI shows similarity in size and sequence to two other LexA-regulated proteins. Repression of *dinD68* by overexpression of *lexA* could account for its roles. We now discuss how the other two multicopy suppressor genes *dinG* and *dinI* suppress the *dinD68* cs phenotype.

The *dinG* gene product shares multiple motifs with many DNA helicases, especially with yeast RAD3 and human XPD helicases (17), which play crucial roles in transcription-coupled DNA repair (6). We isolated various *dinG* mutants, which are neither UV sensitive nor defective for cell growth under normal conditions. The only phenotype that we have observed with *dinG* mutations is that they confer *dnaA5*(Ts) *rnhA* strain ts growth in rich media. Since such a phenotype was observed under conditions in which RNA transcription activities were highly enhanced and the RNAs hybridized with the template DNA were not degraded by RNase H, we have speculated that DinG might function to displace the RNA transcription complex or RNA hybridized to DNA, or a special form of them, which otherwise might inhibit rapid progression of replication forks (31). Our result showing that the *dinD68* Δ*dinG1* double mutant is more cold sensitive than the *dinD68* single mutant seems to exclude a possibility that DinD68 poisons DinG DNA helicase to form an inactive complex and impedes the movement of the replication fork and rather favors another possibility that DinG helicase serves as a sweeper to remove various obstacles, for example, a protein complex containing DinD68, from the path of the advancing replication fork.

The results described in the present study are not sufficient to explain how *dinI* functions as a multicopy suppressor of the *dinD68* mutation. However, we may infer a possible mechanism on the basis of the present finding that DinI has at least two homologs with similar sizes (ImpC from TP110 and ORFfs from ϕR67) which are also under control of LexA. The *impC* gene is followed by *impA* and *impB*, which are homologous in both structure and function to the *umuD* and *umuC* genes on the *E. coli* chromosome, respectively, which are involved in SOS-induced mutagenesis (15, 39). A gene such as *impC* is found neither in the *E. coli umuDC* operon nor in the homologous operons *mucAB* and *samAB* carried by the *S. typhimurium* plasmids pKM101 and pSLT, respectively (34, 39), implying that *impC* is not essential for SOS-induced mutagenesis. The pTZ19ULC derivatives carrying either *impC* or *ORFfs* with their cognate regulatory region were unable to suppress the *dinD68* cs phenotype (Fig. 1). However, since we have not examined how ImpC or ORFfs is expressed from these plasmids without any SOS-inducing signal, the results described above do not necessarily eliminate the possibility that ImpC and ORFfs have an activity similar to that of DinI when they are produced at amounts equivalent with DinI. It

was suggested that ImpC interferes with the SOS induction process when it is produced constitutively in large quantities, although no experimental data were presented (29). If DinI also had such an effect, it would explain why *dinI* is able to function as a multicopy suppressor for the *dinD68* mutation. We are currently pursuing this possibility.

Lastly, it may be noteworthy that the ORFfs protein of ϕR67 shows a similarity to the known N-terminal amino acid sequence of the phage 186 Tum protein (Fig. 7), which is also under control of LexA and functions as an antirepressor upon UV induction (22). It has been pointed out that the two ORFs in the retronphage ϕR67 (ORFi and ORFb) have significant similarities to the *c*I and *c*II (*cp76*) gene products, respectively, of the phage 186 (9). Another retronphage, ϕR86, which is very similar to ϕR67, was found to be inducible in *recA730* strains under some conditions, while it was not detectably inducible in *recA*$^+$ strains (14). Further experiments are necessary to test whether the ORFfs protein of ϕR67 has a function similar to that of the Tum protein (14, 26).

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