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Suppression of Thermosensitive Initiation of DNA Replication in a *dnaR* Mutant of *Escherichia coli* by a Rifampin Resistance Mutation in the *rpoB* Gene

YOSHIMASA SAKAKIBARA*

Department of Biochemistry and Cellular Biology, National Institute of Health, 1-23-1, Toyama, Shinjuku, Tokyo 162, Japan

Received 11 July 1994/Accepted 21 November 1994

The thermosensitivity of the *Escherichia coli dnaR130* mutant in initiation of DNA replication was suppressed by a spontaneous rifampin resistance mutation in *rpoB*, the gene for the β subunit of RNA polymerase. Among the *dnaR*-suppressing *rpoB* alleles obtained was *rpoB22*, which was able to suppress the thermosensitivity of the *dnaA46* or *dnaA167* mutant, but not that of the *dnaA5* mutant, in initiation of replication. Some *dnaA*-suppressing *rpoB* alleles obtained from rifampin-resistant derivatives of the *dnaA* mutants were able to suppress the *dnaR* defect. The *dnaR* mutant with the *rpoB22* allele was deprived of thermoresistance by the *dnaA5* mutation and of viability at low and high temperatures by the *dnaA46* but not the *dnaA167* mutation. The results show that the *rpoB*-mediated suppression of the *dnaA* or *dnaR* defect depends on the functions of both *dnaA* and *dnaR* products. I propose that the *dnaR* product has a key role in transcriptional activation of the replication origin for the *dnaA*-dependent initiation of DNA replication.

Replication of the *Escherichia coli* chromosome is initiated from a fixed site, *oriC*, at a specific stage of the cell cycle. The initiation of chromosome replication is sensitive to rifampin, an inhibitor of RNA synthesis, at a stage when the initiation is already insensitive to a protein synthesis inhibitor (9, 12). Thus, RNA synthesis, besides de novo protein synthesis, is required for the initiation of replication. It has been shown that replication of *oriC* plasmids is influenced by transcription toward or away from the *oriC* site (1, 3, 10, 11, 14, 22, 24). Transcription around *oriC* by RNA polymerase has been shown to assist DnaA protein to trigger the initiation of replication (7, 21). It was recently shown that the genes adjacent to *oriC*, *mioC* and *gidA*, are transcribed in a cell cycle-dependent manner (15, 25).

Transcription affecting replication of *oriC* plasmids has been shown to be modulated by the *dnaA* product, which binds to DnaA box sequences within or around *oriC* (1, 10, 11, 13, 20, 22). Involvement of the *dnaA* product in the transcription that is obligatory for the initiation of chromosome replication has been proposed on the basis of genetic evidence that the temperature-sensitive phenotype of some *dnaA* mutants is reversed by a rifampin resistance (Rif^r) mutation in the *rpoB* gene (4, 5).

Here I report that a spontaneous Rif^r mutation in the *rpoB* gene can suppress the *dnaR* mutation in the *prs* gene, which codes for phosphoribosylpyrophosphate (PRPP) synthetase. The *dnaR* mutant has been shown to be thermosensitive in initiation, but not propagation, of chromosome replication (16, 17). Suppression of the *dnaR* defect by an *rpoB* mutation depends on *dnaA* function, and the *rpoB*-mediated suppression of the *dnaA* defect depends on *dnaR* function. A functional interaction between the *dnaR* and *dnaA* products is suggested from suppression of the *dnaR* defect by certain *dnaA* mutations (18). On the basis of these results, I discuss the possibility that the transcriptional activation of *oriC* for the initiation of chromosome replication may require the *dnaR* product in cooperation with the *dnaA* product.

* Mailing address: Department of Biochemistry and Cellular Biology, National Institute of Health, 1-23-1, Toyama, Shinjuku, Tokyo 162, Japan. Phone: 3-5285-1111. Fax: 3-5285-1157.

MATERIALS AND METHODS

Bacterial strains and growth medium. The *E. coli* K-12 strains used are listed in Table 1. The strains were constructed by transduction with P1 bacteriophage. Some of them were previously described (16, 18). The growth medium was L broth, which contained 1.0% (wt/vol) tryptone (Difco Laboratories, Detroit, Mich.), 0.5% (wt/vol) yeast extract (Difco), and 0.1 M NaCl. Thymine was added at 4 μg/ml, except when cells were labeled with radioactive thymine. For L-broth plates, Bacto Agar (Difco) was added at 1.5% (wt/vol). Rif^r mutants were selected on plates supplemented with rifampin (Boehringer GmbH, Mannheim, Germany) at 80 μg/ml. The sources of other drugs used were described previously (18).

Measurement of DNA synthesis. Cells were labeled with [2-^{14}C]thymine (55 mCi/mmol; ICN Radiochemicals, Irvine, Calif.) as indicated in the legend to Fig. 2. The cells were precipitated with trichloroacetic acid at a final concentration of 10% (wt/vol), and the acid-insoluble material was collected to measure the radioactivity, as described previously (18).

Immunoblot analysis. Cells were grown to logarithmic phase as indicated in the legend to Fig. 4 and harvested from 1.0 ml of the culture by centrifugation at 10,000 × *g* for 5 min at 4°C. The cells were washed with 0.15 M NaCl and suspended in 50 μl of the lysis buffer described by Laemmli (8). After treatment in a boiling water bath for 5 min, the cell lysate was centrifuged at 15,000 × *g* for 10 min. A 2-μl portion of the supernatant was subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The acrylamide concentrations in the stacking and separation gels were 3.5 and 12% (wt/vol), respectively. Electrophoresis was done at 18 mA for 90 min in a slab gel (10 by 10 by 0.1 cm). One of the two gels was stained with a Coomassie brilliant blue R solution to see the total protein separated. Proteins in another gel were electrotransferred to a GV nitrocellulose membrane (Millipore Corp., Bedford, Mass.) and reacted with rabbit anti-PRPP synthetase antiserum. The cross-reacting proteins were detected by use of peroxidase-conjugated goat anti-rabbit immunoglobulin G antibody (Jackson Immunoresearch Laboratories, Inc., West Grove, Pa.). The PRPP synthetase used for preparation of the antiserum was purified according to the procedure of Switzer and Gibson (23). Molecular weight markers were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

RESULTS

Suppression of the *dnaR* mutation by a spontaneous Rif^r mutation. Rif^r derivatives of the *dnaR* mutant were spontaneously obtained at an efficiency of about 3×10^{-7} when examined at 30°C on a rifampin-containing L-broth plate. About one-third of them were able to grow at a high temperature

TABLE 1. Bacterial strains used

Strain	Relevant marker(s)	Source
HC5101	*thr leu thi thyA*	C600
HC5103	*dnaR130*	HC5101
HC5111	*ilv zid-167*::Tn*10*[a]	HC5101
HC5112	*dnaA167*	HC5111
HC5118	*dnaA46*	HC5111
HC5119	*dnaA5*	HC5111
HC5121	*dnaR130 ilv zid-167*::Tn*10*	HC5103
HC5133	*dnaR130 dnaA167*	HC5121
HC5134	*dnaR130 dnaA46*	HC5121
HC5146	*dnaR130 argE86*::Tn*10*[b] *metA*[c]	HC5103
HC5152	*rpoB11 argE86*::Tn*10 dnaR130*	HC5103
HC5153	*rpoB11 argE86*::Tn*10 dnaA167*	HC5112
HC5154	*rpoB11 argE86*::Tn*10 dnaA46*	HC5118
HC5162	*rpoB11 dnaR130*	HC5152
HC5167	*rpoB11 dnaR130* Δ*recA306*::Tn*10*[d]	HC5162
HC5172	*rpoB22 argE86*::Tn*10 dnaR130*	HC5103
HC5173	*rpoB22 argE86*::Tn*10 dnaA167*	HC5112
HC5174	*rpoB22 argE86*::Tn*10 dnaA46*	HC5118
HC5182	*rpoB22 dnaR130*	HC5172
HC5187	*rpoB22 dnaR130* Δ*recA306*::Tn*10*	HC5182
HC5192	*rpoB55 argE86*::Tn*10 dnaR130*	HC5103
HC5193	*rpoB55 argE86*::Tn*10 dnaA167*	HC5112
HC5194	*rpoB55 argE86*::Tn*10 dnaA46*	HC5118
HC5195	*rpoB55 argE86*::Tn*10 dnaA5*	HC5119

[a] Derived from strain HC5106 (16).
[b] Derived from strain ME8474, which was obtained from A. Nishimura (Genetic Stock Research Center, National Institute of Genetics, Mishima, Japan).
[c] Derived from strain JE6895, which was obtained from A. Nishimura.
[d] Derived from strain MV1190 (26).

(42°C) nonpermissive for the parental strain, similarly to Rif^r derivatives of some temperature-sensitive *dnaA* mutants (4, 6). Among spontaneous temperature-resistant revertants of the *dnaR* mutant, which appeared at an efficiency of about 10^{-5} at the high temperature on an L-broth plate, Rif^r strains were found at a level of a few percent. These results showed that a spontaneous Rif^r mutation conferred temperature resistance to the *dnaR* mutant.

To examine whether the Rif^r mutation took place in the *rpoB* gene, a Tn*10* element existing in the *argE* gene, which was closely linked to the *rpoB*$^+$ allele, was introduced into each of 16 isolated temperature-resistant Rif^r strains by P1-mediated transduction. About half of the tetracycline-resistant (Tet^r) transductants of each Rif^r strain had become sensitive to both rifampin and temperature, showing that the *dnaR*-suppressing Rif^r mutations resided in close proximity to *argE*.

For representative mutants Rif^r-11 and Rif^r-22, the mutations were precisely mapped by use of *argE* and *metA* markers, which were located on either side of *rpoB* (Fig. 1). Transduction of HC5146 (*dnaR130 argE*::Tn*10 metA*) cells with phage P1 grown on the Rif^r-22 mutant showed that the Rif^r mutation was cotransduced with *argE* and *metA* at efficiencies of 47 and 65%, respectively. All Rif^r strains among the Arg$^+$ or Met$^+$ transductants tested were temperature resistant, and none of the Rif^s strains was temperature resistant. All of the Arg$^+$ Met$^+$ transductants obtained were Rif^r and temperature resistant. A similar result was obtained for the Rif^r-11 mutation (data not shown). These results showed that the *dnaR* mutation was suppressed by a spontaneous Rif^r mutation in the *rpoB* gene. The mutant alleles assigned will be referred to as *rpoB11* and *rpoB22*.

The *dnaR* mutant with the *rpoB11* or *rpoB22* allele formed normal colonies at both high and low temperatures on L-broth plates (Table 2). The growth rates of these strains at the high

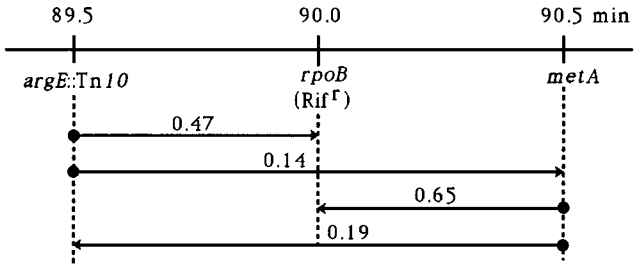

FIG. 1. Genetic linkage map of the Rif^r-22 mutation. HC5146 (*dnaR130 argE*::Tn*10 metA*) cells were infected with phage P1 grown on the Rif^r-22 mutant. Arg$^+$ and Met$^+$ transductants were independently selected at 30°C and examined for methionine or arginine requirement and sensitivity to rifampin and temperature. The numbers of Arg$^+$ and Met$^+$ transductants tested were 105 and 89, respectively. Transduction efficiencies presented are fractions of the numbers of transductants with unselected markers. The selected and unselected markers are indicated by closed circles and arrow heads, respectively. The scale at top shows minutes on the *E. coli* chromosome map.

temperature were comparable to the growth rate of isogenic wild-type cells. The colony-forming ability of the *rpoB dnaR* double mutants at the high temperature was not abolished by the Δ*recA306* mutation (Table 2), indicating that the suppression was not due to elicitation of the *recA*-dependent alternative pathway of chromosome replication, called stable DNA replication (for a review, see reference 2). The *dnaR*$^+$ cells with the *rpoB11* or *rpoB22* allele formed normal colonies at the low and high temperatures (data not shown).

Ability of *dnaR*-suppressing *rpoB* alleles to suppress *dnaA* mutations. The *dnaR*-suppressing *rpoB11* and *rpoB22* alleles were examined for their abilities to suppress the temperature sensitivity of the *dnaA5*, *dnaA46*, and *dnaA167* mutants. The ability of each *rpoB dnaA* double mutant to form colonies at 42°C is summarized in Table 3. The results showed that the *rpoB11* allele did not suppress any of the *dnaA* mutations and that the *rpoB22* allele suppressed the *dnaA46* and *dnaA167* mutations but not the *dnaA5* mutation. The *dnaA5* mutation was not suppressed by any *dnaR*-suppressing *rpoB* alleles isolated. The ability of the *rpoB22* allele to suppress the *dnaA46* or *dnaA167* mutation was low compared with the *dnaR*-suppressing ability, as seen from the growth rate and plating efficiency of the double mutant at the high temperature (Table 3).

DNA synthesis in the *dnaR* and *dnaA* suppressor mutants. When the initiation-defective *dnaR130* or *dnaA167* cells grown at 30°C were exposed to 42°C, DNA synthesis stopped with a net increase of about 1.5-fold (Fig. 2A and D). In the *dnaR* mutant with the *rpoB11* allele, DNA synthesis continued with a significant lag at a rate similar to that observed before the upshift (Fig. 2B). In contrast, the *dnaA167* mutant with the

TABLE 2. Suppression of the *dnaR* mutation by *rpoB* mutant alleles

Strain	Relevant marker(s)	Relative plating efficiency[a] (42°C/30°C)
HC5103	*dnaR130*	0.00001
HC5162	*dnaR130 rpoB11*	0.99
HC5182	*dnaR130 rpoB22*	1.00
HC5167	*dnaR130 rpoB11* Δ*recA306*	0.79
HC5187	*dnaR130 rpoB22* Δ*recA306*	0.88

[a] The numbers of colonies formed at 30 and 42°C on L-broth plates were scored after about 15 h of incubation of cells grown at 30°C in L broth to a cell density of about 3×10^8/ml.

TABLE 3. Abilities of *rpoB* mutant alleles to suppress *dnaR* or *dnaA* mutations

Strain	Relevant marker	Relative plating efficiency of *rpoB* mutant derivative[a]		
		rpoB11	*rpoB22*	*rpoB55*[b]
HC5103	*dnaR130*	0.99	1.00	0.99
HC5112	*dnaA167*	<0.0001	0.80	0.84
HC5118	*dnaA46*	<0.0001	0.16	0.60
HC5119	*dnaA5*	<0.0001	<0.0001	(0.85)[c]

[a] Each *rpoB* derivative was constructed by means of cotransduction of the Rif^r mutation with *argE86*::Tn*10*. The plating efficiencies at 30 and 42°C were measured as described in Table 2, footnote *a*, except that those of the *dnaA rpoB* double mutants at the high temperature were determined after about 40 h of incubation. The colony-forming abilities of all strains at the low temperature were normal.

[b] This allele was isolated from a Rif^r derivative of the *dnaA5* strain.

[c] Relative efficiency at 41°C. This double mutant did not grow normally at 42°C. The *dnaA5* strain with the *rpoB11* or *rpoB22* allele was unable to grow even at 41°C.

rpoB11 allele showed thermosensitive DNA synthesis after the shift, like the *dnaA* mutant with the *rpoB*$^+$ allele (Fig. 2E).

DNA synthesis in the *rpoB22 dnaR* double mutant exposed to the high temperature continued without a lag at a rate significantly faster than the preshift rate (Fig. 2C). Synthesis in the *rpoB22 dnaA167* double mutant was also thermoresistant, although to a much lesser extent than that in the *rpoB dnaR* double mutant (Fig. 2F). The *rpoB22 dnaA46* double mutant showed inefficient synthesis after the upshift, similarly to the *rpoB22 dnaA167* double mutant (data not shown).

Suppression of the *dnaR* mutation by *dnaA*-suppressing *rpoB* alleles. Mutant *rpoB* alleles obtained from temperature-resistant Rif^r derivatives of the *dnaA5*, *dnaA46*, or *dnaA167* strain were examined for the ability to suppress the *dnaR* mutation. All of the 53 independently isolated mutant alleles displayed the *dnaR*-suppressing ability to various degrees. The result for one of the alleles that completely suppressed the *dnaR* mutation, *rpoB55*, is shown in Table 3. This allele, derived from the *dnaA5* strain, was also able to suppress the *dnaA46* and *dnaA167* mutations. The *dnaR* mutant with the *rpoB55* allele grew well at the high temperature at a rate comparable to that of isogenic wild-type cells, while either of the *dnaA* mutants with the *rpoB55* allele grew at a lower rate at the high temperature. DNA synthesis in the *rpoB55 dnaR* double mutant, but not that in any of the *rpoB dnaA* double mutants, was efficient after temperature upshift, as was that in the *rpoB22 dnaR* double mutant (data not shown). All of the other *rpoB* mutant derivatives of the three *dnaA* mutants showed inefficient DNA synthesis upon exposure to 42°C.

Involvement of *dnaA* and *dnaR* functions in *rpoB*-mediated suppression. To examine whether the *rpoB*-mediated suppression of the *dnaR* defect was dependent on *dnaA* function, the *dnaA5* mutation was introduced into the suppressor mutant by means of cotransduction with the *zid-167*::Tn*10* element, which was closely linked to the *dnaA* mutation. The *dnaA5* derivatives of the *rpoB11 dnaR* and *rpoB22 dnaR* double mutants were temperature sensitive, in contrast to the *dnaA*$^+$ parental

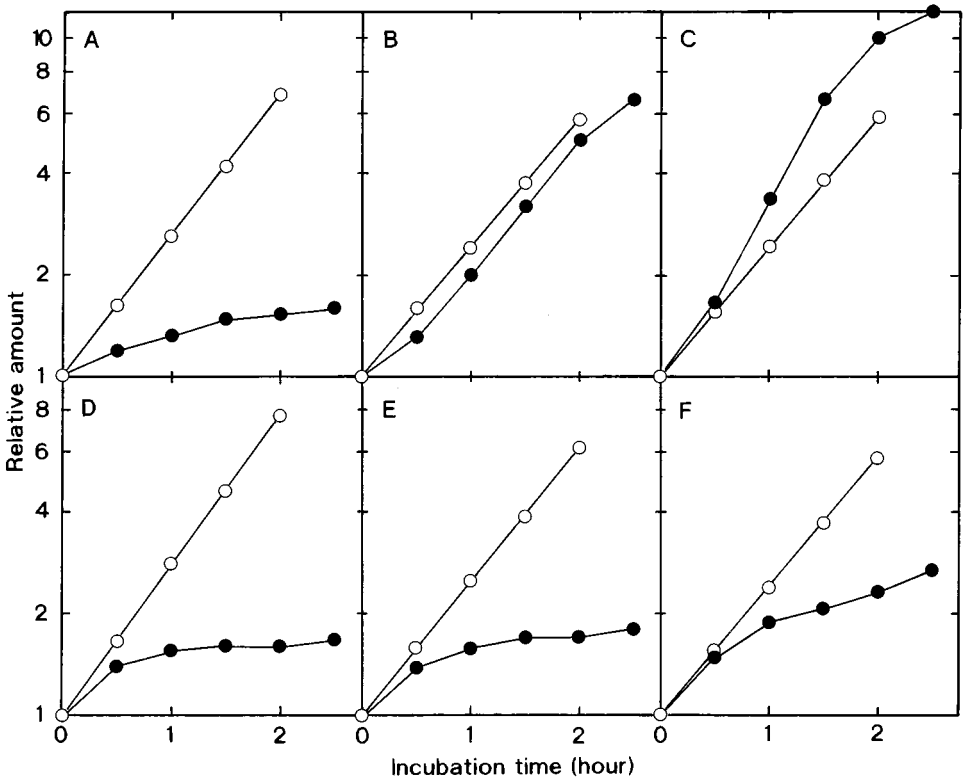

FIG. 2. DNA synthesis in the *dnaR* and *dnaA* suppressor mutants. The *dnaR130* and *dnaA167* mutants and those with the *rpoB11* or *rpoB22* allele were grown in L broth containing [^{14}C]thymine (0.1 μCi/ml) at 30°C from a cell density of about 4×10^6/ml to a cell density of about 6×10^7/ml and then incubated at 30°C (○) and 42°C (●). A 100-μl portion of each culture was withdrawn at the times indicated to determine the radioactivity of the acid-insoluble material. Relative values with respect to the count at the time of transfer for strains HC5103 (*dnaR130*) (932 cpm) (A), HC5152 (*dnaR130 rpoB11*) (840 cpm) (B), HC5172 (*dnaR130 rpoB22*) (736 cpm) (C), HC5112 (*dnaA167*) (851 cpm) (D), HC5153 (*dnaA167 rpoB11*) (922 cpm) (E), and HC5173 (*dnaA167 rpoB22*) (835 cpm) (F) are presented.

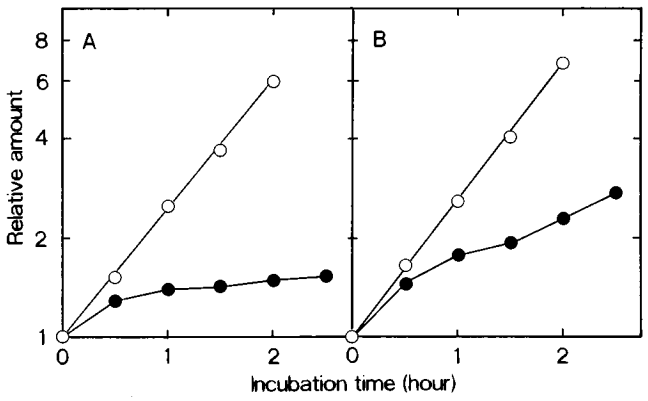

FIG. 3. DNA synthesis in *dnaA* derivatives of the *dnaR* suppressor mutant. DNA synthesis in the *rpoB22 dnaR130* double mutant with the *dnaA5* or *dnaA167* allele was measured at 30°C (○) and 42°C (●) as described in the legend to Fig. 2. Relative values with respect to the count at the time of temperature upshift for the *dnaA5* derivative (764 cpm) (A) and the *dnaA167* derivative (865 cpm) (B) are presented.

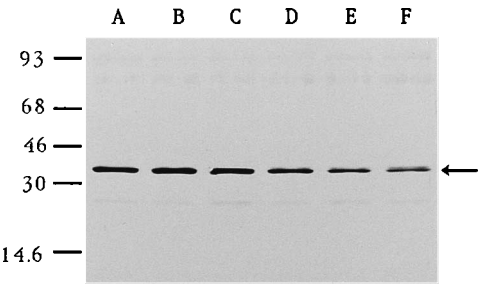

FIG. 4. Amount of the *prs* gene product in the *dnaR* suppressor mutant. The *rpoB22 dnaR130* double mutant and isogenic wild-type cells were inoculated in L broth at a cell density of about 5×10^6/ml and grown at 30 or 42°C to an A_{600} of 0.50 (3×10^8 to 4×10^8 cells per ml). A portion of the mutant culture at the low or high temperature was further incubated, with addition of chloramphenicol at a final concentration of 200 μg/ml, for 60 min at the same temperature. An aliquot of proteins extracted from the cells in 1.0 ml of each culture was subjected to SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-PRPP synthetase antiserum as described in Materials and Methods. The arrow indicates the 34,000-molecular-weight protein corresponding to PRPP synthetase. Positions of molecular weight markers (in thousands) are indicated at the left. The data are for strains HC5101 (*rpoB*$^+$ *dnaR*$^+$) at 30°C (lane A) and 42°C (lane B), HC5172 (*rpoB22 dnaR130*) at 30°C (lane C) and 42°C (lane D), and HC5172 treated with chloramphenicol at 30°C (lane E) and 42°C (lane F).

strains. Their plating efficiencies at the high temperature were less than 10^{-6} of those at the low temperature. DNA synthesis in the *dnaA5 rpoB22 dnaR* triple mutant was completely blocked by temperature upshift after a residual synthesis (Fig. 3A). A similar result was obtained with the *dnaA5 rpoB11 dnaR* triple mutant (data not shown). Thus, the thermoresistant DNA synthesis in the *dnaR* suppressor mutants was rendered thermosensitive by the *dnaA* mutation.

When the *rpoB22 dnaR* double mutant was transduced with the *dnaA46*- or *dnaA167*-linked Tn*10*, no temperature-sensitive strain was found among the Tet^r transductants. To clarify whether the *dnaA* derivatives were temperature resistant, the *rpoB22* allele was introduced into the temperature-sensitive *dnaA46 dnaR* or *dnaA167 dnaR* double mutant by means of cotransduction with *argE*::Tn*10*. Temperature-resistant Rif^r strains were found among the Tet^r transductants of the *dnaA167 dnaR* double mutant. The *rpoB22* derivative of the double mutant showed inefficient DNA synthesis after temperature upshift (Fig. 3B), but the plating efficiency at the high temperature was about 0.8 of that at the low temperature. Thus, the *rpoB22* allele was able to suppress simultaneously both the *dnaA167* and *dnaR* mutations.

In contrast, when the *dnaA46 dnaR* double mutant was transduced with the *rpoB22*-linked Tn*10* at the low temperature, neither Rif^r nor temperature-resistant strains were found. The possibility that the *rpoB* derivative could grow at the high but not the low temperature was excluded by the result that transduction of the temperature-resistant *rpoB22 dnaR* double mutant with the *dnaA46*-linked Tn*10* at the high temperature resulted in no cold-sensitive strains. These results showed that the *rpoB22 dnaA46 dnaR* triple mutant was inviable at the low and high temperatures. Because both the *rpoB22 dnaA46* and *rpoB22 dnaR* double mutants were temperature resistant, the inviability of the triple mutant showed that the *dnaA*- and *dnaR*-suppressing abilities of the *rpoB* allele were dependent on the *dnaR*$^+$ and *dnaA*$^+$ functions, respectively.

Effect of the *rpoB22* mutation on expression of the *prs* gene. Because the temperature sensitivity of the *dnaR* mutant was reversed by an elevated dose of the *prs* gene with the *dnaR* mutation (17), the *rpoB*-mediated suppression of the *dnaR* defect might be due to excess formation of the *dnaR* mutant product by virtue of mutationally altered RNA polymerase. To examine this possibility, the amount of the *prs* product in the *rpoB22 dnaR* double mutant was measured by immunoblotting of the cellular proteins with anti-PRPP synthetase antiserum. The amount of the product in the mutant cells grown at 30°C was comparable to that in isogenic wild-type cells at the low temperature (Fig. 4, lanes A and C) and in the *dnaR* single mutant at the low temperature (data not shown). The amount in the double mutant cells grown at 42°C was slightly smaller than that in the same cells grown at 30°C (Fig. 4, lanes C and D) or that in the wild-type cells at the low and high temperatures (Fig. 4, lanes A and B). When the double mutant at the low or high temperature was further incubated in the presence of chloramphenicol for 60 min at the same temperature, the amount of the *prs* product was significantly reduced, but the degree of the reduction was not varied by the incubation temperature (Fig. 4, lanes E and F). Thus, the mutant product was stable at the high temperature as well as at the low temperature. These results indicated that the expression of the *prs* gene encoding the *dnaR* product was not significantly affected by the *rpoB* mutation.

DISCUSSION

The thermosensitivity of the *dnaR* mutant in initiation of DNA replication is changed to thermoresistance by a spontaneous Rif^r mutation in the *rpoB* gene. Some of the *dnaR*-suppressing *rpoB* alleles suppress the defect of certain *dnaA* mutants in initiation of replication. Some *dnaA*-suppressing *rpoB* alleles obtained spontaneously from *dnaA* mutants also suppress the *dnaR* defect. Thus, a single mutation in the *rpoB* gene can suppress not only the *dnaA* defect but also the *dnaR* defect. This result implies that the *dnaR* product functions at the *dnaA*-dependent step in the initiation of replication. In fact, the *dnaR* defect can be suppressed by certain *dnaA* mutations (18).

Suppression of the *dnaR* defect by the *rpoB11* and *rpoB22* alleles was *dnaA* dependent. Therefore, the restored replication must be initiated from *oriC* and not from other known origins, which operate independently of *dnaA* function. Because the *rpoB22* allele suppresses only certain *dnaA* mutations, the suppression requires the mutant *dnaA* product to be functional in response to the *rpoB* mutation. Allele-specific

suppression between some *dnaA* and *rpoB* mutations has been previously reported (4, 6, 19). The *dnaA* dependency of the *rpoB*-mediated suppression of the *dnaA* or *dnaR* defect shows that the *rpoB* mutation leads to initiation of replication at the normal origin in the denatured state of the *dnaA* or *dnaR* product.

The *rpoB22* allele can suppress simultaneously the *dnaA167* and *dnaR* mutations but can suppress the *dnaA46* and *dnaR* mutations only separately. Therefore, the *rpoB*-mediated suppression of the *dnaA* or *dnaR* defect depends on the functions of both the *dnaA* and *dnaR* products. The *rpoB22* mutation abolishes viability of the *dnaA46 dnaR* double mutant despite conferring thermoresistance to the *dnaA* and *dnaR* single mutants. This result implies cooperation of the *dnaA* and *dnaR* products, which is consistent with the previous finding that the *dnaR* mutant product becomes functional in the presence of certain *dnaA* mutant products (18). It has been noted that some *dnaA*-suppressing *rpoB* alleles are lethal to other *dnaA* mutants (4, 6, 19).

One of the possible mechanisms for the *rpoB*-mediated suppression of the *dnaR* defect is overproduction of the *dnaR* mutant product, which could reverse the *dnaR* defect. However, the intracellular amount of the product in the *dnaR* mutant is not increased by the *rpoB22* mutation. An alternative possibility is that the suppression is caused by induced or enhanced expression of some gene(s) whose product could stabilize the *dnaR* mutant product. However, this possibility seems unlikely because the *dnaR* mutant with the *rpoB22* allele is deprived of thermoresistance by the *dnaA46* mutation, which can be suppressed by the *rpoB* allele. Similarly, the fact that the *rpoB dnaA* double mutant loses thermoresistance in the presence of the *dnaR* mutation is not favorable to the possibility that the suppression of the *dnaA* defect is caused by transcriptional changes in *trans*-acting genes, including *dnaA*. Therefore, it is likely that the *rpoB*-mediated suppression of the *dnaA* or *dnaR* defect takes place at a transcriptional step required in *cis* for the initiation of replication from *oriC*.

It has been shown that some Rif^r mutations in the *rpoB* gene lead to enhanced or diminished transcription termination at the attenuator site of the *trp* operon (27). Thus, the *rpoB* mutations suppressing the *dnaA* or *dnaR* defect may alter RNA polymerase so that it can transcribe the *oriC* region or its vicinity to allow the *dnaA* or *dnaR* mutant product to function. Alternatively, the mutant RNA polymerase may have been altered so as to be adaptable to the *dnaA* or *dnaR* mutant product and thus become able to perform the transcription obligatory for the initiation of replication. The study of *oriC* plasmid replication in vitro by Baker and Kornberg (7) has revealed that transcription by RNA polymerase preceding the initiation of replication from *oriC* assists DnaA protein to open the DNA duplex and that the reaction does not need interaction between the two proteins. However, the mode of transcriptional activation for initiation of replication may differ for the *oriC* plasmid and the bacterial chromosome, because the plasmid is capable of replicating in *dnaR*-deficient cells (16). Transcriptional activation of the chromosomal replication origin may need the *dnaR* product to interact with the *dnaA* product.

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