JOURNAL OF BACTERIOLOGY, Jan. 1987, p. 19–25
0021-9193/87/010019-07$02.00/0

Vol. 169, No. 1

Genetic and Morphological Characterization of *ftsB* and *nrdB* Mutants of *Escherichia coli*

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Received 27 June 1986/Accepted 29 September 1986

The *ftsB* gene of *Escherichia coli* is believed to be involved in cell division. In this report, we show that plasmids containing the *nrdB* gene could complement the *ftsB* mutation, suggesting that *ftsB* is an allele of *nrdB*. We compared changes in the cell shape of isogenic *nrdA*, *nrdB*, *ftsB*, and *pbpB* strains at permissive and restrictive temperatures. Although in rich medium all strains produced filaments at the restrictive temperature, in minimal medium only a 50 to 100% increase in mean cell mass occurred in the *nrdA*, *nrdB*, and *ftsB* strains. The typical *pbpB* cell division mutant also formed long filaments at low growth rates. Visualization of nucleoid structure by fluorescence microscopy demonstrated that nucleoid segregation was affected by *nrdA*, *nrdB*, and *ftsB* mutations at the restrictive temperature. Measurements of β-galactosidase activity in λ p(*sfiA*::*lac*) lysogenic *nrdA*, *nrdB*, and *ftsB* mutants in rich medium at the restrictive temperature showed that filamentation in the *nrdA* mutant was caused by *sfiA* (*sulA*) induction, while filamentation in *nrdB* and *ftsB* mutants was *sfiA* independent, suggesting an SOS-independent inhibition of cell division.

The *ftsB* mutation in *Escherichia coli* K-12 was first described by Ricard and Hirota (27), who analyzed strain MFT84, a thermosensitive *ftsB* cell division mutant that forms multinucleated filaments at the restrictive temperature. At 41°C, rates of synthesis of mass, DNA, RNA, and peptidoglycan in MFT84 were exponential. Also, the *recA* mutation did not suppress filamentation, and a λ-lysogenic *ftsB* strain did not show prophage induction while producing filaments. These results suggest that the filamentation is not an effect of the SOS response caused by an inhibition of DNA replication, but is a result of a specific block in septum formation. On the other hand, Ricard and Hirota reported that cells lacking DNA were formed and that nucleoid segregation was somewhat disturbed (27).

The *ftsB* mutation has been mapped at 48 min on the *E. coli* chromosome and shown to be complemented by plasmids from the Clarke and Carbon collection containing this region (7). Yamada et al. (33) analyzed these plasmids and mapped *ftsB* near *glpT* on a 5-kilobase (kb) *Eco*RI-*Pst*I fragment also containing the *nrdA* and *nrdB* genes.

The *nrdA* and *nrdB* genes encode the αα′ and β polypeptide chains, which constitute the B1 (αα′) and B2 (β_2) subunits of the enzyme ribonucleoside-diphosphate reductase (EC 1.17.4.1) (28). This enzyme reduces ribonucleotides to deoxyribonucleotides and performs the first essential step in the synthesis of DNA precursors in *E. coli*. Several nucleoside triphosphates act as allosteric effectors, which regulate enzyme activity and substrate specificity by binding to the allosteric sites of the B1 subunit. Synthesis of ribonucleoside-diphosphate reductase is also subject to regulation on the transcription level (9), whereas stimulation of mRNA synthesis during the inhibition of DNA replication requires protein synthesis (9, 13).

Until recently, only one chromosomal mutant in each gene had been described; by random mutagenesis several mutations have now been introduced in the *nrd* genes cloned on a multicopy plasmid (26). The *nrdA* mutant was originally isolated as a *dnaF* mutant showing temperature-sensitive DNA synthesis (12, 31). The *nrdB* mutant was isolated as a temperature-sensitive deoxyuridine auxotroph (11). Temperature sensitivity of the *nrdB* mutant is not the effect of a thermolabile B2 subunit of ribonucleoside-diphosphate reductase but the result of an increased deoxyribonucleotide demand associated with decreased enzyme activity and increased growth rates (10). The addition of deoxyuridine restores growth at 37 and 42°C (10). A characteristic of the *nrd* mutant is its sensitivity to hydroxyurea, which inhibits DNA synthesis by inactivation of the B2 subunit of ribonucleoside-diphosphate reductase (28). Because the hydroxyurea sensitivity of *ftsB* strain MFT84 is not different from that of its parental strain, PA3092, Yamada et al. (33) concluded that the *ftsB* gene is not an allele of the *nrdA* and *nrdB* genes and that a possible location of *ftsB* would be between *nrdB* and *glpT*.

Carlson et al. (5) sequenced the 8,557-base-pair *Bam*HI-*Pst*I fragment containing the *nrdA* and *nrdB* genes and, based on the data of Yamada et al. (33), also sequenced the *ftsB* gene. This sequence contained an open reading frame which could be *ftsB* in both strands downstream of the *nrd* operon.

Temperature shift experiments with isogenic *nrdA*, *nrdB*, and *ftsB* mutants cultured in minimal and rich media showed that in all mutants filamentation seemed to be suppressed at low growth rates, whereas at high growth rates all strains produced filaments and showed aberrant nucleoid structures and segregation at the restrictive temperature.

Treatments affecting DNA synthesis are known to induce the SOS response, which leads to an increased expression of the cell division inhibitor *sfiA* (*sulA*) (16). Since the effect of the restrictive temperature on nucleoid segregation seemed to vary among the mutants, we measured the induction of *sfiA* expression in λ p(*sfiA*::*lac*)-lysogenic *nrdA*, *nrdB*, and *ftsB* mutants to see whether there is any difference in induction of the SOS response.

In the present work, we found by complementation studies that *ftsB* is an allele of *nrdB*. Further evidence for this is presented in an accompanying paper (20) in which the

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TABLE 1. Bacterial strains, phage, and plasmids

Strain	Genotype	Source (reference)
E. coli K-12		
JM101	F′ *traD36 proAB lacI*^{q}*ZΔZM15/supE* Δ(*lac-proAB*) *thi*	M. Meijer
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 flbB5301 ptsF25 deoC1 rbsR relA1*	I. B. Holland via D. Sinden (6)
LMC500	MC4100 *lysA*	P1 (JE5510) × MC4100; penicillin enrichment
LMC502	LMC500 *leu*::Tn*5*	P1(6976) × LMC500
LMC510	LMC502 *leu*$^+$ *pbpB2158*(Ts)	P1(LMC360) × LMC502
LMC514	LMC500 *recA56 srlC300*::Tn*10*	P1(LMC133) × LMC500
LMC522	LMC514 *srlC*$^+$	P1(LMC500) × MC514
AX655	F$^-$ *pbpB2158*(Ts) *thr leu proA his thi argE lacY galK xyl mtl ara tsx rpsL supE*	J. R. Walker (1)
LMC360	AX655 *leu*$^+$	P1(LMC500) × AX655
6976	F$^-$ *leu*::Tn*5 pro endA hsdR thi*	J. R. Walker
JE5510	F$^-$ *lpp-5508 man-1 pps dapA lysA his-1 argH1 thi-1 rpsL9 lacY1 xyl-7 mtl-2 malA1*	U. Schwarz
JF427	F$^-$ *gyrA nrdB1 araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK hsdR rpsL*	J. Fuchs
JF1158	F$^-$ *gyrA nrdA hsdS supE44 supF58 lacY1* or Δ(*lacIZY*)*6 galK2 galT22 metB1 trpR* λ$^-$	J. Fuchs
DL39	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 thiA glpR gyrA* Δ(*glpT-glpA*)*593 zei-724*::Tn*10*	W. Boos (22)
MFT84	F$^-$ *thyA1 thr-1 leuB6 thi-1 argH1 hisG1 trp-1 lacY1 gal-6 mtl-2 xyl-7 malA1* λ$^-$ *ara-13 rpsL9 fhuA2 supE44 fic mel ftsB84*(Ts)	J. R. Walker (27)
LMC690	MFT84 *zei-724*::Tn*10* Δ(*glpT-glpA*)*593*?	P1(DL39) × MFT84
LMC574	LMC500 *nrdB1*(Ts) *gyrA*	P1(JF427) × LMC500
LMC575	LMC500 *nrdA*(Ts) *gyrA*	P1(JF1158) × LMC500
LMC576	LMC500 *ftsB84*(Ts) *zei-724*::Tn*10* Δ(*glpT-glpA*)*593*?	P1(LMC690) × LMC500
LMC590	LMC575[λ p(*sfiA*::*lac*) cI *ind*]	λ p(*sfiA*::*lac*) cI *ind* × LMC575
LMC591	LMC574[λ p(*sfiA*::*lac*) cI *ind*]	λ p(*sfiA*::*lac*) cI *ind* × LMC574
LMC592	LMC576[λ p(*sfiA*::*lac*) cI *ind*]	λ p(*sfiA*::*lac*) cI *ind* × LMC576
LMC593	LMC500[λ p(*sfiA*::*lac*) cI *ind*]	λ p(*sfiA*::*lac*) cI *ind* × LMC500
Phage		
P1 *vir*		Laboratory stock
λ1105	λ (Km^r p_{tac}-transposase) *b522* cI857 *P*am80 *nin5*	N. Kleckner (30)
λ p(*sfiA*::*lac*)	cI *ind*	R. D'Ari (16)
Plasmids		
pLC3-46	*imm*^{ColE1} *nrdB*$^+$ *glpt*$^+$ *glpQ*$^+$	Phabagen Collection, Utrecht
pBR322	Ap^r Tc^r	Laboratory stock (3)
pUC18	Ap^r	34
pPS2	Tc^r *nrdA*$^+$ *nrdB*$^+$	J. Fuchs (25)

enzyme activity of ribonucleoside-diphosphate reductase in *ftsB* mutant MFT84 is shown to be decreased.

MATERIALS AND METHODS

Bacterial strains, phages and plasmids. All strains, bacteriophages, and plasmids used in this study are listed in Table 1.

Media and growth conditions. Broth containing 1% tryptone (Difco Laboratories), 0.5% yeast extract (Difco), and 0.5% NaCl was used as rich medium (TY). TY agar consisted of TY broth supplemented with 1.5% agar (Difco). Minimal medium (15) was supplemented with 1 μg of thiamine per ml, 0.4% glucose, and 50 μg of amino acids per ml. If necessary, tetracycline (12.5 μg/ml), ampicillin (100 μg/ml), kanamycin (50 μg/ml), and nalidixic acid (40 μg/ml) were added. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG) (Boehringer Mannheim Biochemicals) were added to final concentrations of 0.004% and 0.1 mM, respectively. For temperature shift experiments, cultures growing in the steady state at 28°C in a water bath shaker were diluted 5- to 10-fold in prewarmed medium at 42°C. Absorbance was measured with a 300 T-1 spectrophotometer (Gilford Instrument Laboratories, Inc.). Cell numbers were determined by using a Coulter Counter (Coulter Electronics, Inc.) with a 30-μm-diameter orifice.

Genetical techniques. P1 *vir*-mediated transduction was carried out as described by Miller (24). Plasmid transformations were carried out by using the $CaCl_2$ method of Cohen et al. (8). Transformed cells were incubated in TY broth for 1 h to induce expression of antibiotic resistance before they were plated on selective media. Complementation of temperature sensitivity was determined by streaking single

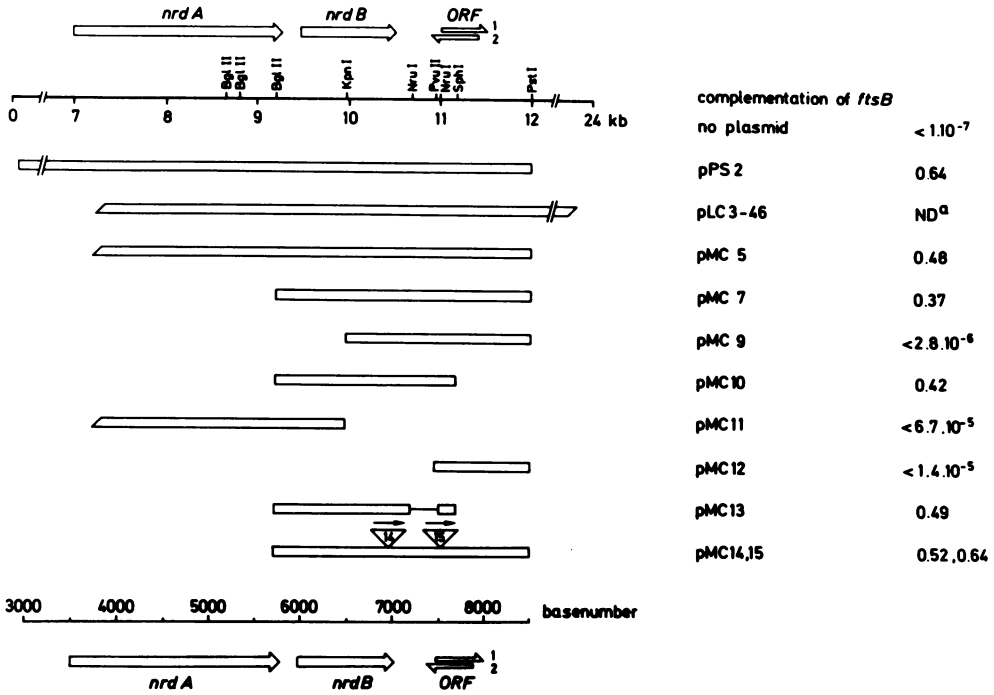

FIG. 1. Physical map and complementation analysis of the *nrdA-nrdB-orf* region of the *E. coli* chromosome. The locations of the genes and the restriction endonuclease sites are based on the nucleotide sequence described by Carlson et al. (5). Base numbers taken from this sequence are indicated. Open arrows, Dimensions of *nrdA*, *nrdB*, and the two possible ORFs, ORF1 and ORF2, and their directions of transcription; ▭, chromosomal fragment of a hybrid plasmid (a sloped edge indicates the approximate position of the connection between chromosomal DNA and ColE1 DNA); ▽ (numbered 14 and 15), mini-Km transposon insertions in pMC14 and pMC15, respectively (the arrows above the triangles show the direction of transcription of the Km^r gene of the transposon). The complementation of *ftsB* strain MFT84 by hybrid plasmids was derived from the efficiency of plating on TY plates without salt at 42°C.

colonies on TY plates without NaCl at 42°C and on TY plates with 0.5% NaCl at 30°C. λ1105-mediated transposon mutagenesis was performed by the "λ hop" procedure of Way et al. (30). Transposase was induced by adding IPTG to a 0.1 mM final concentration. λ p(*sfiA*::*lac*) lysogens were constructed as described by Huisman and D'Ari (17).

Plasmid isolation and analysis. Large quantities of plasmid DNA were isolated by a modification (23) of the cleared-lysate method of Birnboim and Doly (2), followed by centrifugation to equilibrium in CsCl-ethidium bromide density gradients. Small amounts of plasmid DNA were isolated by the modified cleared-lysate method (23) or the method of Holmes and Quigley (14). Plasmids and restriction fragments were analyzed on 0.8% agarose gels, using a Tris-borate–EDTA buffer (23). Restriction endonucleases, T4 ligase, and alkaline phosphatase were obtained from Boehringer Mannheim Biochemicals and used as recommended by that supplier.

β-galactosidase assay. Induction of the SOS response in λ p(*sfiA*::*lac*) lysogens during the temperature shift experiments was measured by the β-galactosidase assay described by Miller (24). One unit (U) of β-galactosidase is the amount of enzyme needed to produce 1 nmol of orthonitrophenol per min at 28°C (pH 7.0). Samples (1 ml each) from TY cultures were chilled on ice and centrifuged. The supernatant was removed, and the pellets were suspended in Vogel-Bonner medium (29) before assaying β-galactosidase activity. Specific activities measured in activity units (U) were calculated as described by Miller (24). β-Galactosidase activity, expressed in U per milligram (dry weight), was calculated as follows: U/mg = U/(A_{450} × 0.227). In this formula, an A_{450} of 1 corresponds to 227 μg (dry weight)/ml (19).

Fluorescence microscopy. Samples taken during temperature shift experiments were prepared for fluorescence microscopy by fixation with 0.1% OsO_4 and subsequent centrifugation. The pellet was suspended in 100 μl of Veronal (E. Merck AG)-acetate buffer (pH 6). Fluorochrome 33342 (Hoechst-Roussel Pharmaceuticals Inc.; 2 mM) (21), which binds specifically to DNA, was added to a final concentration of 20 μM. The fluorochrome was irradiated at 340 to 380 nm with a type A filter in a Dialux 22 EB fluorescence microscope (Leitz-Wetzlar). The fluorescence of the nucleoids was photographed with TriX pan films (Eastman Kodak Co.), which were developed with Diafine developer (Acufine Inc.).

RESULTS

Construction of hybrid plasmids and complementation analysis. Yamada et al. (33) reported that the Clarke and Carbon plasmid pLC3-46 (7) complements the *ftsB* mutation in MFT84. For further characterization of the *ftsB* gene, subclones of pLC3-46 were made. Restriction endonuclease *Pst*I cleaved pLC3-46 into five fragments of 7.3, 5.9, 5.3, 4.6 and 1.1 kb. These fragments were ligated into the *Pst*I site of pBR322 (3). *recA* strain LMC522 was transformed with the ligation mixture to Tc^r. Plasmid DNA was isolated from Ap^s Tc^r clones and analyzed. MFT84 was transformed with five plasmids each containing a different *Pst*I fragment of pLC3-46. Only a plasmid containing the 5.3-kb *Pst*I fragment complemented the *ftsB* mutation. The inserts of this plasmid and its derivatives and the plating efficiencies of MFT84 containing them are depicted in Fig. 1. pMC5 was cleaved with *Pst*I and *Bgl*II, and the two resulting *Pst*I-*Bgl*II frag-

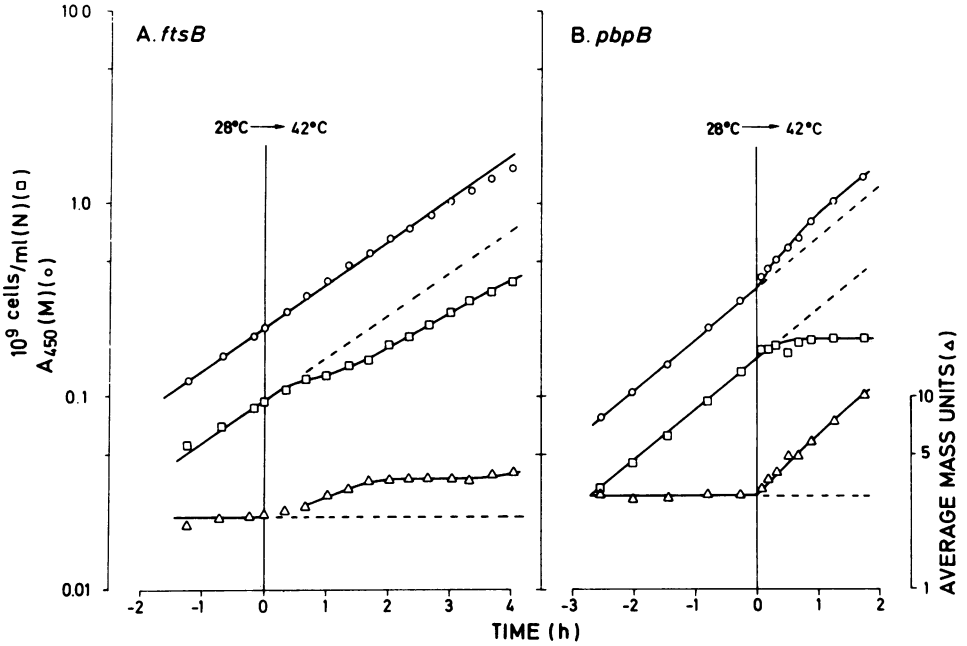

FIG. 2. Comparison of the effect of a temperature shift on cell division in two isogenic strains, *ftsB* mutant LMC576 (A) and *pbpB* mutant LMC510 (B), grown in minimal medium. At time zero, cultures growing at steady state were shifted from 28 to 42°C. Absorbance (○) and cell number (□) were determined at intervals. Mean cell mass (△) is expressed in arbitrary units calculated by dividing the absorbance by the cell number.

ments were ligated in the *Pst*I-*Bam*HI sites of pUC18 (34). Plasmid pMC7 which contains a 2.8-kb *Pst*I-*Bam*HI fragment did complement the *ftsB* mutation in MFT84. pMC5 was also cleaved with *Pst*I and *Kpn*I, and the two resulting *Pst*I-*Kpn*I fragments were ligated into the *Pst*I-*Kpn*I sites of pUC18 to construct pMC9 and pMC11. Both plasmids were unable to complement the *ftsB* mutation. This suggests that *Kpn*I cleaves within the *ftsB* gene. Further cloning of the *ftsB* gene was facilitated by the DNA sequence of Carlson et al. (5), according to which the *Kpn*I site is in the middle of the *nrdB* gene. Two possible open reading frames (ORFs) downstream of the *nrd* operon were detected by computer analysis of this sequence. ORF1 was found to be encoded between positions 7531 and 8025 in the sequence determined by Carlson et al. (5), while ORF2 was found to be encoded by the complementary strand between positions 7954 and 7451 (Fig. 1).

The fact that the ORFs on pMC9 did not complement the *ftsB* mutation made it unlikely that one of the ORFs is the *ftsB* gene. Additional evidence was obtained with pMC10, which was constructed by deletion of the 820-base-pair *Sph*I fragment of pMC7. It contained the whole *nrdB* gene and only a small part of the ORFs. Nevertheless, pMC10 did complement the *ftsB* mutation.

To be sure that the *ftsB* mutation was in the *nrdB* gene, we made a 298-base-pair *Nru*I deletion in pMC10. The resulting plasmid, pMC13, still complemented the *ftsB* mutation, but did not contain the presumable promoter of ORF1 with the same direction of transcription as that of the *nrd* operon. A *Pvu*II fragment of pMC9, ligated in the *Sma*I site of pUC18 to construct pMC12, contained only the ORFs, but was unable to complement the *ftsB* mutation in MFT84. From these results, we concluded that plasmids containing the *nrdB*$^+$ sequence complemented *ftsB* and that the *ftsB* mutation is in *nrdB*.

We also constructed pMC7 derivatives containing mini-Km transposon insertions. The transposons were mapped by use of the internal *Hin*dIII and *Cla*I restriction sites and other appropriate sites. The transposon insertion in pMC14 was approximately at position 6980 of the nucleotide sequence of Carlson et al. (5) at the end of the *nrdB* gene. Apparently, a transposon insertion close to the end of the *nrdB* coding sequence at position 7137 had no effect on the function of the β component of ribonucleoside-diphosphate reductase, as determined by complementation. The transposon insertion in pMC15 disrupted both of the ORFs downstream of the *nrd* operon approximately at position 7580 (Fig. 1). All plasmids still complemented the *ftsB* mutation in MFT84, showing again that the *ftsB* mutation was not situated in the ORFs.

Differences in the efficiency of plating between *nrdB* and *ftsB* strains containing plasmids. Complementation of *nrdA*, *nrdB*, and *ftsB* mutations by hybrid plasmids was quantitated by determining the efficiency of plating on TY plates without salt at 42°C. The complementation of JF1158 (*nrdA*), JF427 (*nrdB*), and MFT84 by plasmid pPS2 containing the *nrdA* and *nrdB* genes resulted in efficiencies of plating at 42°C of 0.88, 1.5×10^{-2}, and 0.64, respectively. This suggests that, in contrast to the *ftsB* mutation, the *nrdB* mutation was not completely complemented by plasmid pPS2. More evidence for this observation was reported by Platz and Sjöberg (25), who determined the plating efficiency of the *nrdA-nrdB* double mutant KK535 containing pPS2 or pPS1. Plasmid pPS1 contains the same chromosomal *Pst*I fragment as pPS2, but in the opposite orientation and in addition to a 1.1-kb *Pst*I fragment of the ColE1 part of pJC808. Strain KK535(pPS2) plated with 0.3-fold the efficiency of KK535(pPS1) at 40°C. Our efficiency of plating values suggest that this was a result of the *nrdB* mutation. We found a similar plating efficiency with strain JF427 containing pMC7. In this respect, the *nrdB* mutation differed from the *ftsB* mutation. Perhaps the *ftsB* mutation caused the synthesis of

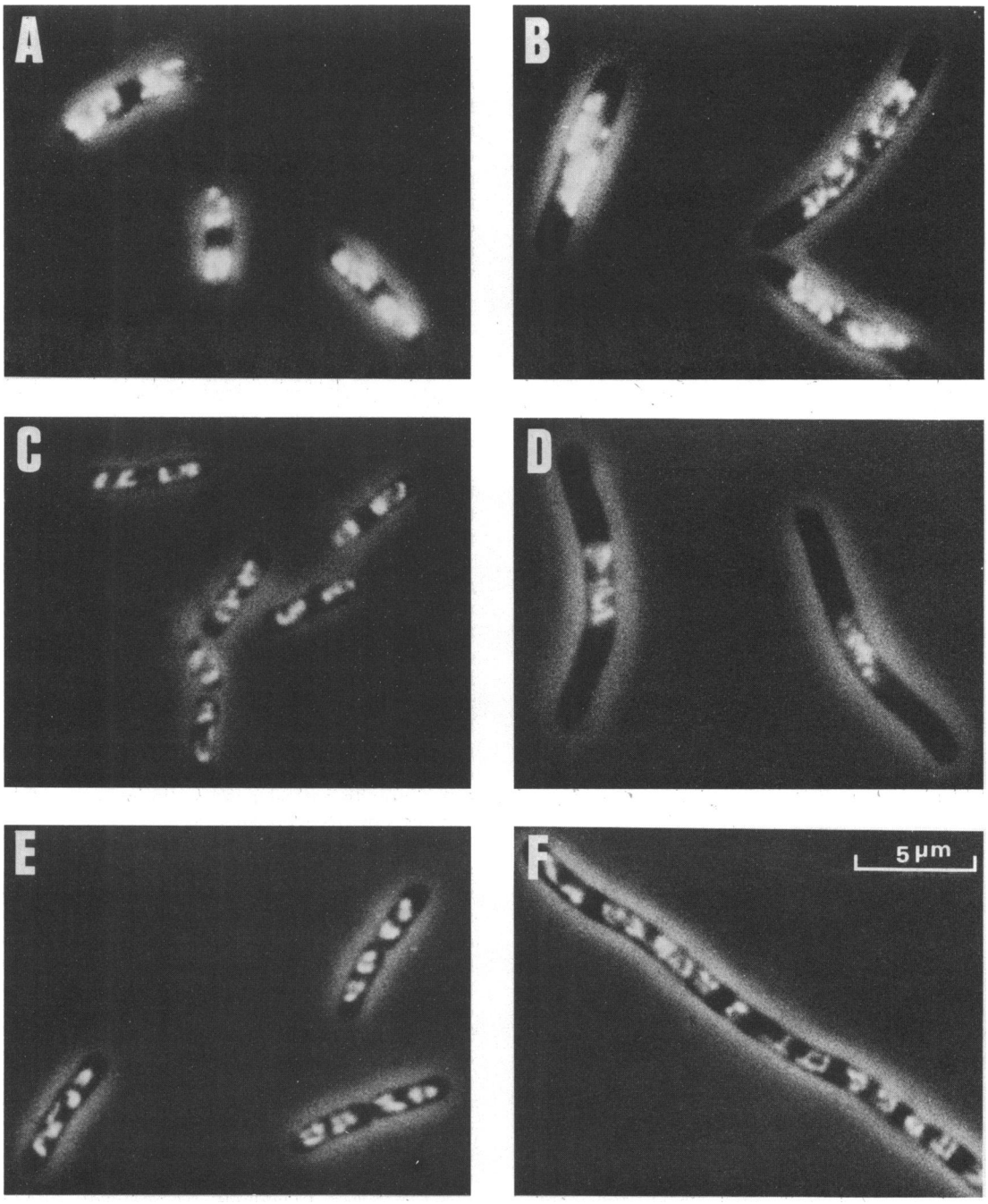

FIG. 3. Nucleoid structure and segregation visualized by fluorescence microscopy in isogenic strains grown in TY broth. (A) *nrdA* mutant LMC575 at 28°C and (B) after shifting to 42°C for 100 min; (C) *ftsB* mutant LMC576 at 28°C and (D) after shifting to 42°C for 100 min; (E) *pbpB* mutant LMC510 at 28°C and (F) after shifting to 42°C for 50 min.

a thermolabile B2 subunit, which dissociated from the B1 subunit at 42°C. Fuchs and Kalström (10) reported that the B2 subunit in the *nrdB* mutant was not thermolabile. If the affinity of the mutant B2 subunit for the B1 subunit had increased, complementation by wild-type B2 subunits would have been impaired.

Temperature shift experiments of isogenic *nrdA*, *nrdB*, *ftsB*, and *pbpB* mutants. Since the *nrdA* and *nrdB* genes are involved in the synthesis of DNA precursors, we decided to compare isogenic *nrdA*, *nrdB*, and *ftsB* mutants with a typical *pbpB* cell division mutant in temperature shift experiments. In minimal medium, only the *pbpB* mutant formed long filaments and showed an increased growth rate, while the *nrdA*, *nrdB*, and *ftsB* mutants increased their mean cell mass by 50 to 100% without an increase in growth rate at the restrictive temperature (Fig. 2A). The *pbpB* mutant did show 9% residual division during the first 20 min at 42°C (Fig. 2B). By contrast, cell number continued to increase for the *nrdA*, *nrdB*, and *ftsB* mutants. These results indicate that the defect in cell division of the *nrdA*, *nrdB*, and *ftsB* mutants was partially suppressed at a low growth rate, while expression of the *pbpB* mutation also occurred at low growth rates.

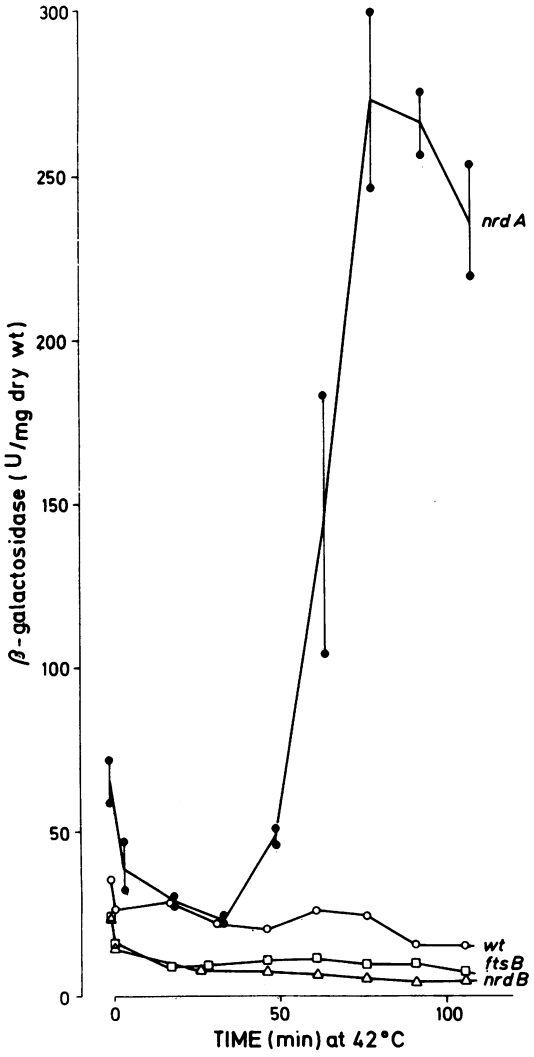

FIG. 4. Induction of cell division inhibitor *sfiA* in λ p(*sfiA*::*lac*) cI *ind* strains grown in TY broth. The specific activities of β-galactosidase in strains LMC590 *nrdA* (●), LMC591 *nrdB* (△), LMC592 *ftsB* (□), and wild-type LMC593 (○) were assayed before and at different times after a shift to the nonpermissive temperature.

In TY broth all mutants produced filaments. At high growth rates, the *nrdA*, *nrdB*, and *ftsB* mutations were fully expressed and led to an inhibition of cell division. Because the *nrd* operon encodes the enzyme ribonucleoside-diphosphate reductase, which is involved in the synthesis of DNA precursors, we decided to look at the nucleoid segregation and structure of the mutants by fluorescence microscopy. The effects of the temperature shift on nucleoid segregation in the *nrdA*, *ftsB*, and *pbpB* mutants are shown in Fig. 3. At the restrictive temperature the nucleoid segregation and structure remained the same in the *pbpB* mutant, but they were clearly altered in the *nrdA*, *nrdB*, and *ftsB* mutants. The *nrdB* strain showed nucleoid aggregates similar to those of the *ftsB* mutant (data not shown). Nucleoid segregation in the *nrdA* mutant was clearly more affected than that in the *ftsB* or *nrdB* mutant.

Induction of cell division inhibitor *sfiA* in isogenic *nrdA*, *nrdB*, and *ftsB* mutants. Presumably the effect on nucleoid segregation in *nrdA*, *nrdB*, and *ftsB* mutants was caused by a more or less partial inhibition of DNA synthesis (27). In cases in which DNA synthesis was inhibited, the SOS response was usually induced. Cell division was then inhibited by the induction of *sfiA* expression. To see whether the filamentation in *nrdA*, *nrdB*, and *ftsB* mutants at the restrictive temperature was the effect of *sfiA* expression, we measured the induction of β-galactosidase activity in λ p(*sfiA*::*lac*) lysogens in a temperature shift experiment. Our results showed that only the *nrdA* mutant induced *sfiA* expression at 42°C and, thus, that filaments were caused by induction of the SOS response (Fig. 4). The *nrdB*, *ftsB*, and wild-type strains showed no induction of the SOS response.

DISCUSSION

We have shown (Fig. 1) that plasmids containing the wild-type *nrdB* gene complemented the *ftsB* mutation in MFT84, which was originally defined as a cell division mutant (27). This suggests that *ftsB* is an allele of *nrdB*.

The *nrdB* gene is part of the *nrd* operon which is involved in the synthesis of the enzyme ribonucleoside-diphosphate reductase, an enzyme essential for the synthesis of DNA precursors (28).

We compared the *nrdA*, *nrdB*, and *ftsB* mutants with a *pbpB* mutant, which behaved like a typical cell division mutant at the restrictive temperature (Fig. 2 and 3). Growth and division of the *nrdA*, *nrdB*, and *ftsB* mutants did not differ significantly. At 42°C in rich medium, filaments were formed, but in minimal medium cell division continued after a brief lag phase (Fig. 2). We assigned this difference between the *pbpB* mutant and the *nrdA*, *nrdB*, and *ftsB* mutants to the fact that cell division inhibition is independent of the growth rate in a typical cell division mutant, but seems to be growth rate dependent in mutants affected in the synthesis of DNA precursors.

At low growth rates, residual ribonucleoside-diphosphate reductase activity is presumably sufficient to sustain DNA synthesis, although the speed of the replication forks may slow down, thereby increasing the elongation time (C period) of the DNA replication (35). Thus, the effect of a temperature shift in *nrdA*, *nrdB*, and *ftsB* mutants is perhaps physiologically comparable to a lowering of the thymine concentration in the growth medium of thymine auxotrophs. Zaritsky and Pritchard determined the effect of a step-down experiment on cell size (35). When the C period increases, the cell will postpone cell division until after the termination of DNA replication. Cell division then takes place at an increased mean cell mass. Meanwhile, a new round of replication is initiated leading to multiple-fork replication, under conditions that normally allow only one replication fork to proceed. Eventually, a new steady state is reached at a higher mean cell mass. A transition to multiple-fork replication at lower speeds of the replication forks after a temperature shift in strain MFT84 could explain why no effect on the rate of DNA synthesis was found by Ricard and Hirota (27).

At a high growth rate, the speed of the replication forks may be slowed down, similar to the situation in minimal medium, thereby increasing the C period and mean cell mass. This time, however, the cells do not reach a new steady state. They could be trapped in the transition to a new steady state, since the diminishing ratio of genomes per mass unit could lead to a decrease in growth rate, which is usually observed in these cultures (data not shown).

If DNA replication is severely inhibited, the SOS response is induced. However, only the *nrdA* mutant showed

filamentation caused by *sfiA* induction, whereas the *nrdB* and *ftsB* mutants showed *sfiA*-independent filamentation (Fig. 4). Under normal circumstances, cell division may be prevented by the physical presence of DNA (32). After nucleoid segregation this inhibition is removed. Filaments of *nrdB* and *ftsB* mutants prepared for fluorescence microscopy showed large nucleoid aggregates (Fig. 3B). Although these sometimes appeared to have separated, the cells were still unable to divide. Filamentation of *nrdB* and *ftsB* mutants must therefore have resulted from a still hypothetical cell division inhibitor, independent of the SOS response (cf. references 4 and 18).

ACKNOWLEDGMENTS

This work was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research.

We thank J. R. Walker for inspiring us in this work, R. D'Ari for suggesting the SOS induction experiment, and J. Fuchs for communicating results before publication. We also thank R. D'Ari, W. Boos, J. Fuchs, N. Kleckner, M. Meijer, the Phabagen Collection (Utrecht, The Netherlands), U. Schwarz, D. Sinden, and J. R. Walker for providing bacterial strains, phages, and plasmids; J. Leutscher for the drawings; J. Sikking for the photographs; C. van Wijngaarden for preparing the manuscript; E. van Spronsen for help with the computer analysis; E. Pas and I. Erdös for technical assistance; and A. R. Stuitje for helpful discussions.

LITERATURE CITED

1. **Allen, J. S., C. C. Philip, R. A. Gustafson, R. G. Allen, and J. R. Walker.** 1974. Regulation of bacterial cell division: genetic and phenotypic analysis of temperature-sensitive, multi-nucleate, filament-forming mutants of *Escherichia coli*. J. Bacteriol. **117**:978–986.
2. **Birnboim, H. C., and J. Doly.** 1979. Rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Burton, P., and I. B. Holland.** 1983. Two pathways of division inhibition in UV-irradiated *E. coli*. Mol. Gen. Genet. **190**: 128–132.
5. **Carlson, J., J. A. Fuchs, and J. Messing.** 1984. Primary structure of the *Escherichia coli* ribonucleoside diphosphate reductase operon. Proc. Natl. Acad. Sci. USA **81**:4294–4297.
6. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–555.
7. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *Escherichia coli* genome. Cell **9**:91–99.
8. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
9. **Filpula, D., and J. A. Fuchs.** 1977. Regulation of ribonucleoside diphosphate reductase synthesis in *Escherichia coli*: increased enzyme synthesis as a result of inhibition of deoxyribonucleic acid synthesis. J. Bacteriol. **130**:107–113.
10. **Fuchs, J., and H. O. Kalström.** 1973. A mutant of *Escherichia coli* defective in ribonucleoside diphosphate reductase. 2. Characterization of the enzymatic defect. Eur. J. Biochem. **32**:457–462.
11. **Fuchs, J., and J. Neuhard.** 1973. A mutant of *Escherichia coli* defective in ribonucleoside diphosphate reductase. 1. Isolation of the mutant as a deoxyuridine auxotroph. Eur. J. Biochem. **32**:451–456.
12. **Fuchs, J. A., H. O. Karlström, H. R. Warner, and P. Reichard.** 1972. Defective gene product in *dnaF* mutant of *Escherichia coli*. Nature (London) New Biol. **238**:69–71.
13. **Hanke, P. D., and J. A. Fuchs.** 1984. Requirement of protein synthesis for the induction of ribonucleoside diphosphate reductase mRNA in *Escherichia coli*. Mol. Gen. Genet. **193**: 327–331.
14. **Holmes, D. S, and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**: 193–197.
15. **Howard-Flanders, P., E. Simson, and T. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli*. Genetics **49**:237–246.
16. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
17. **Huisman, O., and R. D'Ari.** 1983. Effect of suppressors of SOS-mediated filamentation on *sfiA* operon expression in *Escherichia coli*. J. Bacteriol. **153**:169–175.
18. **Jaffé, A., R. D'Ari, and V. Norris.** 1986. SOS-independent coupling between DNA replication and cell division in *Escherichia coli*. J. Bacteriol. **165**:66–71.
19. **Koch, A. L.** 1981. Growth measurement, p. 179–207. *In* P. Gerhardt, R. Murray, R. Costilow, E. Nester, W. Wood, N. Krieg, and G. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
20. **Kren, B., and J. A. Fuchs.** 1987. Characterization of *ftsB* as an allele of *nrdB* in *Escherichia coli*. J. Bacteriol. **169**:14–18.
21. **Latt, S. A., and G. Statten.** 1976. Spectral studies on 33258 Hoechst and related bisbenzimidazole dyes useful for fluorescent detection of DNA synthesis. J. Histochem. Cytochem. **24**:24–32.
22. **Ludtke, D., T. J. Larson, C. Beck, and W. Boos.** 1982. Only one gene is required for the *glpT*-dependent transport of sn-glycerol-3-phosphate in *Escherichia coli*. Mol. Gen. Genet. **186**:540–547.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Platz, A., and B. M. Sjöberg.** 1980. Construction and characterization of hybrid plasmids containing the *Escherichia coli nrd* region. J. Bacteriol. **143**:561–568.
26. **Platz, A., and B. M. Sjöberg.** 1984. Mutationally altered ribonucleotide reductase from *Escherichia coli*: characterization of mutations isolated on multicopy plasmids. J. Bacteriol. **160**: 1010–1016.
27. **Ricard, M., and Y. Hirota.** 1973. Process of cellular division in *Escherichia coli*: physiological study on thermosensitive mutants defective in cell division. J. Bacteriol. **116**:314–322.
28. **Thelander, L., and P. Reichard.** 1979. Reduction of ribonucleotides. Annu. Rev. Biochem. **48**:133–158.
29. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *E. coli*: partial purification and some properties. J. Biol. Chem. **218**: 97–106.
30. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32**:369–379.
31. **Wechsler, J. A., and J. D. Gross.** 1971. *Escherichia coli* mutants temperature sensitive for DNA synthesis. Mol. Gen. Genet. **113**:273–284.
32. **Woldringh, C. L., J. A. C. Valkenburg, E. Pas, P. E. M. Taschner, P. Huls, and F. B. Wientjes.** 1985. Physiological and geometrical conditions for cell division in *Escherichia coli*. Ann. Inst. Pasteur (Paris) **136A**:131–138.
33. **Yamada, M., Y. Takeda, K. Okamoto, and Y. Hirota.** 1982. Physical map of the *nrdA-nrdB-ftsB-glpT* region of the chromosomal DNA of *Escherichia coli*. Gene **18**:309–318.
34. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
35. **Zaritsky, A., and R. H. Pritchard.** 1973. Changes in cell size and shape associated with changes in the replication time of the chromosome of *Escherichia coli*. J. Bacteriol. **114**:824–837.