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

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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(*sftA*::*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 *sfA* 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-*PstI* fragment also containing the *nrdA* and *nrdB* genes.

The *nrdA* and *nrdB* genes encode the $\alpha\alpha'$ and β polypeptide chains, which constitute the B1 ($\alpha\alpha'$) 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 BamHI-PstI 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 $\lambda p(sfiA::lac)$ -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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E. coli K-12 JM101F' traD36 proAB lacP2 $\Delta ZMJSIsupE \Delta (lac-proAB)$ thi F' araD139 $\Delta (argF^1 cac)U69$ rpsL30 [JbB530] pisF25 deoC1 rbsR relA1M. Meijer I. B. Holland via D. Sinden (6)LMC500MC4100 (ysAPiter (100 rpsL30 [JbB530] pisF25 deoC1 rbsR relA1N. Meijer I. B. Holland via D. Sinden (6)LMC501LMC500 [eu::Tn5 LMC510 LMC500 recA56 srlC300::Tn10LMC500 [eu::Tn5 F pbp2158(Ts) thr leu proA his thi argE lac Y galK xyl mtl ara tsx rpsL supE LMC514 LMC500 recA56 srlC300::Tn10LMC502 LMC514 srlC+ F pbp2158(Ts) thr leu proA his thi argE lac Y galK xyl mtl ara tsx rpsL supE ILMC506 argF-lacu)U6 prsL30 relA1 his-1 argH1 thi-1 rpsL9 lac Y1 xyl-7 mtl-2 malA1 U.SchwarzN. KoS14 J. R. Walker (1) PI(LMC500) × MC514 J. R. Walker (1) PI(LMC500 recA56 srlC300::Tn10 LMC506 pred his-1 argH1 thi-1 rpsL9 lac Y1 xyl-7 mtl-2 malA1 J. FuchsN. Schwarz J. Fuchs J. R. Walker (1) PI(LMC500 pred his-1 argH1 thi-1 rpsL9 lac Y1 xyl-7 mtl-2 malA1 J. Fuchs J. FuchsN. Schwarz J. Fuchs J. FuchsJL39F' gyrA nrdA hisd supE44 supE38 lac Y1 or $\Delta (lac ZY)$ 6 galK2 galT22 melB1 rpR λ^{-1} J. Fuchs LMC576 LMC500 mrdA(TS) gyrA LMC576 LMC500 high gifA::lac) cl ind] LMC576 LMC500 high gifA::lac) cl ind] LMC576 LMC500 high gifA::lac) cl ind] LMC576 LMC500 high gifA::lac) cl ind] PLC3-46 imm ^{CodeEI} nrdB+ glpt+ glp	Strain	Genotype	Source (reference)
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$ \begin{array}{llllllllllllllllllllllllllllllllllll$	MC4100	F^- araD139 $\Delta(argF-lac)U169$ rpsL150 f1bB5301 ptsF25 deoC1 rbsR relA1	I. B. Holland via D.
$ \begin{array}{llllllllllllllllllllllllllllllllllll$			Sinden (6)
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	LMC500	MC4100 lysA	P1 (JE5510) \times MC4100;
$ \begin{array}{llllllllllllllllllllllllllllllllllll$			penicillin enrichment
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	JE5510	F ⁻ lpp-5508 man-1 pps dapA lysA his-1 argH1 thi-1 rpsL9 lacY1 xyl-/ mtl-2 malA1	U. Schwarz
$\begin{array}{llllllllllllllllllllllllllllllllllll$	JF427	F gyrA nrdB1 araD139 D(ara-leu)/69/ DlacX/4 galU galK hsdR rpsL	J. Fuchs
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	JF1158	F gyrA nrdA hsdS supE44 supF38 lacy1 or Δ (lac12y)6 galK2 gal122 melB1 trpK λ	J. Fuchs
MF184FInval init-1 leugo lini-1 argH inition inp-1 laboratory stock (3) fluid 2 supE44 for mel (fsB84(Ts))J. R. Walker (21)LMC690MFT84 zei-724::Tn10 $\Delta(g pT-g pA)593$? LMC500 nrddI(Ts) gyrA LMC576 LMC500 nrddI(Ts) gyrA LMC500 nrddI(Ts) gyrA LMC576 LMC500 frdsB4(Ts) zei-724::Tn10 $\Delta(g pT-g pA)593$? LMC576 LMC576 LMC500 frdsB4(Ts) zei-724::Tn10 $\Delta(g pT-g pA)593$? LMC590 LMC576 LMC590 LMC576 LMC576 LMC591 LMC574[λ p(sfiA::lac) cl ind] LMC574[λ p(sfiA::lac) cl ind] LMC576 LMC593 LMC500[λ p(sfiA::lac) cl ind] LMC576 LMC500[λ p(sfiA::lac) cl ind] LMC576 LMC500 LMC576 LMC593 LMC500[λ p(sfiA::lac) cl ind] LMC500[λ p(sfiA::lac) cl ind] LMC576 LMC500Laboratory stock N. Kleckner (30) λ cl ind p(sfiA::lac)cl ind glp2 rc' rndB+ glpt+ glpQ+ Plasmids pLC3-46R. D'Ari (16) λ pUC18 pUC18 pUC18 pUC18 pUC18 pUC18 pPS22r. rdB+ glpt+ glpQ+ slpQ+Phabagen Collection, Utrecht Laboratory stock (3) 34 4	DL39	F araD139 \(\argF-\argLac)U109 rpsL150 relA1 thiA gipR gyrA \(\argLgip1-gipA)595 zel-/24:: 1n10	W. BOOS (22)
JulA2 sup244 fic met fiste 4(18)LMC 500MFT84 zei-724:: Tn10 $\Delta(g p-g pA)593$?P1(DL39) × MFT84LMC574LMC500 nrdBI(Ts) gyrAP1(JF427) × LMC500LMC575LMC500 nrdB(Ts) zei-724:: Tn10 $\Delta(g pT-g pA)593$?P1(ILMC690) ×LMC576LMC500 fisB84(Ts) zei-724:: Tn10 $\Delta(g pT-g pA)593$?P1(ILMC690) ×LMC576LMC576LMC575[λ p(sfiA::lac) cl ind] λ p(sfiA::lac) cl ind ×LMC591LMC574[λ p(sfiA::lac) cl ind] λ p(sfiA::lac) cl ind ×LMC592LMC576[λ p(sfiA::lac) cl ind] λ p(sfiA::lac) cl ind ×LMC593LMC500[λ p(sfiA::lac) cl ind] λ p(sfiA::lac) cl ind ×LMC503LMC500[λ p(sfiA::lac) cl ind] λ p(sfiA::lac) cl ind ×LMC593LMC500[λ p(sfiA::lac) cl ind] λ p(sfiA::lac) cl ind ×LMC500 λ cl ind λ p(sfiA::lac) cl ind × λ cl indp(sfiA::lac)N λ cl indR. D'Ari (16) λ cl indplC3-46imm ^{ColE1} nrdB ⁺ g pt ⁺ g pQ ⁺ Phabagen Collection, UtrechtpBR322Ap' Tc'J4pUC18Ap'pUC18Ap'pUC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18Ap'pVC18 </td <td>MF184</td> <td>F thyAI thr-1 leuBo thi-1 argH1 hisG1 trp-1 lacY1 gal-6 mit-2 xyl-/ matA1 k ara-13 rpsL9</td> <td>J. R. walker (27)</td>	MF184	F thyAI thr-1 leuBo thi-1 argH1 hisG1 trp-1 lacY1 gal-6 mit-2 xyl-/ matA1 k ara-13 rpsL9	J. R. walker (27)
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pUC18 Ap^r 34pPS2 $Tc^r nrdA^+ nrdB^+$ J. Fuchs (25)	pBR322	Ap ^r Tc ^r	Laboratory stock (3)
pPS2 $Tc^r nrdA^+ nrdB^+$ J. Fuchs (25)	pUC18	Ap ^r	34
	pPS2	$Tc^r nrdA^+ nrdB^+$	J. Fuchs (25)

TABLE	1.	Bacterial	strains.	phage.	and	plasmids
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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 μ g of thiamine per ml, 0.4% glucose, and 50 μ g of amino acids per ml. If necessary, tetracycline (12.5 μ g/ml), ampicillin (100 μ g/ml), kanamycin (50 μ g/ml), and nalidixic acid (40 μ g/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- μ m-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; \Box , 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^c 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. λ 1105-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. $\lambda p(sfiA::lac)$ 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 Trisborate-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/($A_{450} \times 0.227$). 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% OsO₄ 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 PstI 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 PstI 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 PstI fragment of pLC3-46. Only a plasmid containing the 5.3-kb PstI 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 PstI and BglII, and the two resulting PstI-BglII 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 (\bigcirc) and cell number (\square) were determined at intervals. Mean cell mass (\triangle) is expressed in arbitrary units calculated by dividing the absorbance by the cell number.

ments were ligated in the PstI-BamHI sites of pUC18 (34). Plasmid pMC7 which contains a 2.8-kb PstI-BamHI fragment did complement the ftsB mutation in MFT84. pMC5 was also cleaved with PstI and KpnI, and the two resulting PstI-KpnI fragments were ligated into the PstI-KpnI sites of pUC18 to construct pMC9 and pMC11. Both plasmids were unable to complement the *ftsB* mutation. This suggests that KpnI 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 KpnI 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 SphI 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 *NruI* 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 *PvuII* fragment of pMC9, ligated in the *SmaI* 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 PstI fragment as pPS2, but in the opposite orientation and in addition to a 1.1-kb PstI 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 $\lambda p(sfiA::lac) cI$ ind strains grown in TY broth. The specific activities of β galactosidase in strains LMC590 nrdA (\bullet), LMC591 nrdB (Δ), LMC592 ftsB (\Box), and wild-type LMC593 (\bigcirc) 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 nrdBand 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).

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