Missense mutations in the 3' end of the *Escherichia coli* *dnaG* gene do not abolish primase activity but do confer the chromosome-segregation-defective (*par*) phenotype

James Versalovic¹† and James R. Lupski^{1,2}

Author for correspondence: James R. Lupski. Tel: +1 713 798 6530. Fax: +1 713 798 5073.
e-mail: jlupski@bcm.tmc.edu

Department of Molecular and Human Genetics¹, and Department of Pediatrics², Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Isogenic *dnaG* strains of *Escherichia coli* with the *parB* and *dnaG2903* alleles in the MG1655 chromosomal background displayed the classic *par* phenotype at the nonpermissive temperature of 42 °C. These strains synthesized DNA at 42 °C, but remained chromosome segregation defective as determined by cytology. A strain with the *dnaG2903* allele was tested for its ability to support DNA replication of a primase-dependent G4ori_c-containing M13 phage derivative by quantitative competitive PCR (QC-PCR). The *dnaG2903* strain converted the single-stranded DNA into double-stranded replicative form DNA at 42 °C. These results indicate that DnaG2903 retains primase activity at the restrictive temperature. Nucleoids remained unsegregated in the central region of cell filaments at 42 °C. The observed suppression of cell filamentation in *dnaG* *sfiA* or *dnaG* *lexA* double mutants suggests that the SOS response is induced at the restrictive temperature in *parB* and *dnaG2903* strains but fails to account entirely for the cell filamentation phenotype. *ParB* and DnaG2903 presumably can synthesize primer RNA for DNA replication, but may be defective in their interactions with DNA replication proteins, cell cycle regulatory factors, or the chromosome segregation apparatus itself.

Keywords: chromosome, DNA replication, partitioning, segregation, SOS response

INTRODUCTION

DNA polymerases require the presence of a free 3' hydroxyl primer terminus for the initiation of DNA synthesis (Kornberg & Baker, 1992). An important mechanism for the formation of primer termini is the synthesis of RNA oligonucleotide primers (pRNA) by either RNA polymerases or enzymes termed 'primases' (Rowen & Kornberg, 1978). The DnaG primase was purified from *Escherichia coli* (Bouche *et al.*, 1978; Rowen & Kornberg, 1978) and shown to synthesize the pRNA of both the leading (van der Ende *et al.*, 1985) and lagging (Lark, 1972) strands during chromosomal replication.

Conditional-lethal mutations in *dnaG* were isolated by chemical mutagenesis and screening for temperature-sensitive defects in DNA replication (Carl, 1970; Wechsler & Gross, 1971; Gross, 1972; Marinus & Adelberg, 1970). *E. coli* strains with mutations in the 5' half of *dnaG* lacked the ability to synthesize DNA at the restrictive temperature (Grompe *et al.*, 1991). The mutant alleles, *dnaG3* (G247D), *dnaG308* (P207L and Q252L) and *dnaG399* (G247D), were previously characterized and contained nonconservative substitutions (Grompe *et al.*, 1991). These mutations clustered within a region that is highly conserved among bacterial primases (Versalovic & Lupski, 1993), contains a motif shared with RNA polymerases (Versalovic & Lupski, 1993), and includes the catalytic site of primase (Mustaev & Godson, 1995). One of three lysines at positions 211, 229 or 241 of primase lies within the catalytic site as determined by cross-linked, radio-labelled nucleotide substrates (Mustaev & Godson, 1995).

† **Present address:** Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA

Abbreviation: QC-PCR, quantitative competitive polymerase chain reaction.

Two conditional-lethal mutations, *dnaG2903* and *parB*, were located at the divergent 3' end of *dnaG* and were isolated by different screening methods (Grompe *et al.*, 1991). The *dnaG2903* mutation, initially designated as the '*dnaP*' allele, was isolated by screening temperature-sensitive strains for phenylethyl alcohol resistance (Wada & Yura, 1974; Murakami *et al.*, 1985). Phenylethyl alcohol primarily inhibits phospholipid biosynthesis and was thought to interfere with chromosome-membrane interactions (Silver & Wendt, 1967). The *parB* mutation was contained in one of the original *par* strains isolated by Hirota *et al.* (1970) that continue synthesizing DNA at the restrictive temperature but fail to partition daughter chromosomes within cell filaments. The mutation in the *parB* strain mapped to the *dnaG* locus (Norris *et al.*, 1986) and has been implicated in chromosomal segregation by cytological studies (Grompe *et al.*, 1991). The exact role of DnaG in the chromosome segregation process is not known. Therefore, it is not clear whether the defects in chromosome partitioning represent a secondary effect of perturbed DNA replication or indicate a second cell cycle function for DnaG in chromosome segregation.

In an attempt to clarify the functional roles of DnaG in the *E. coli* cell, a rigorous phenotypic analysis was performed with newly constructed isogenic *parB* and *dnaG2903* strains. Steady-state labelling assays were used to evaluate total DNA synthesis, and a recombinant M13 derivative with the *G4ori_c* was used to study primase function *in vivo* by competitive PCR. Chromosome segregation phenotypes were examined simultaneously by fluorescence-phase-contrast microscopy. Finally, induction of the SOS response was measured indirectly by cytology of *dnaG* double mutants with defects in *lexA* or the SOS-inducible *sfiA* (*sulA*) gene. Our results demonstrate that mutations in the carboxy terminus of primase do not appear to inhibit its ability to synthesize pRNA. Moreover, such mutations confer a *par* phenotype at the restrictive temperature, not entirely explained by the SOS response.

METHODS

Strain constructions. Isogenic strains were constructed by P1-mediated transduction (Weinstock, 1991) (Table 1). Two different strategies were used to link dominant antimicrobial resistance markers to the *dnaG* alleles, *dnaG2903* and *parB*. The first strategy involves a defective Tn10 element containing a chloramphenicol resistance marker. Two plasmids were used to deliver the defective Tn10 element. One plasmid, pMS421 (Churchward *et al.*, 1984; Heath *et al.*, 1992), contains *lacI^q* (Calos, 1978), and a spectinomycin resistance gene. The second plasmid, pJD12 (Heath *et al.*, 1992), contains β -lactamase, a defective mini-Tn10 that includes the chloramphenicol (Cm) acetyltransferase gene (Elliott & Roth, 1988), and the transposase gene outside the mini-Tn10 element. MG1655 cells were transformed with pMS421 and pJD12 and plated on Luria-Bertani (LB) medium (Miller, 1972) with Timentin (ticarcillin/clavulanate; SmithKline Beecham) (80 $\mu\text{g ml}^{-1}$) and spectinomycin (Sp) (50 $\mu\text{g ml}^{-1}$). Cells were cultivated overnight in LB broth with Sp and Cm (34 $\mu\text{g ml}^{-1}$). They were then diluted 1:50 in LB medium with Sp, Cm, and

the inducer, IPTG (1 mM), and incubated for 4 h with agitation at 37 °C. Cells were infected with P1vir, transduced into KN1378 (temperature-sensitive) cells, and plated on LB agar with Cm at 42 °C. Temperature-resistant, chloramphenicol-resistant (Cm^r) clones (wild-type *dnaG* linked to Cm^r) were infected with P1vir, transduced into GC2530 (temperature-sensitive) recipient cells, and plated on LB-Cm at 30 °C. Individual colonies were picked and streaked at 30 °C and 42 °C to screen for temperature-sensitive clones. Temperature-sensitive, Cm^r (*parB* linked with Cm^r) clones were reinfected with P1vir and transduced into recipient MG1655 cells. Temperature-sensitive, Cm^r clones were examined for the presence of the *parB* allele by restriction digestion of PCR-amplified *dnaG* fragments (Grompe *et al.*, 1991).

The second strategy involved the use of a recombinant λ :Tn5 derivative containing a kanamycin (Km) resistance gene (de Bruijn & Rossbach, 1994). One millilitre aliquots of the MG1655 cells were infected with 100 μl of λ :Tn5 lysate ($\sim 10^{10}$ c.f.u. ml⁻¹; provided by Robert A. Britton). Phages and cells were mixed gently and the mixture was maintained at room temperature for 2 h. Two hundred microlitre aliquots of these mixtures were plated on LB agar with Km (50 $\mu\text{g ml}^{-1}$). More than three hundred colonies were pooled into 5 ml LB medium, diluted 1:5 in LB, and infected with P1vir. Lysates were transduced into KN1378 (temperature-sensitive) recipient cells and transductants were plated on LB agar with Km (50 $\mu\text{g ml}^{-1}$) at 42 °C. Temperature-resistant, Km^r clones (wild-type *dnaG* linked to Km^r) were infected with P1vir and transduced into KN1378 or GC2530 recipient cells. Individual colonies were picked and streaked at 30 °C and 42 °C to screen for temperature-sensitive clones. Temperature-sensitive, kanamycin-resistant (Km^r) clones (*dnaG2903* or *parB* linked to Km^r) were infected with P1vir and transduced into MG1655 recipient cells. Temperature-sensitive, Km^r clones were examined for the presence of the *parB* and *dnaG2903* mutations by solid-phase-based dideoxy DNA sequencing of PCR-amplified *dnaG* segments (Roa *et al.*, 1993). The presence of the *parB* mutation was also confirmed by restriction digestion of a PCR-amplified *dnaG* fragment (Grompe *et al.*, 1991).

Mutant strains containing the *sfiA* null allele (*sfiA*:Tn5), *lexA3*, or *parC281* in the MG1655 chromosomal background were constructed as described above by P1-mediated transduction and selective cultivation (Table 1). *E. coli* strains containing the *sfiA*:Tn5 allele were verified by fluorescence-phase-contrast (fluo-phase) microscopy for the expected cytology (i.e. suppression of filamentation at the non-permissive temperature). Strains with the *lexA3* allele were verified by screening for UV sensitivity. *E. coli* clones (JV46) containing *parC281* were examined for their temperature-sensitive phenotype and appearance by fluo-phase microscopy. Similarly a strain with the *dnaG2903* allele in the W1485 chromosomal background was also created. *E. coli* W1485 derivatives containing either the *dnaG3*, the *dnaG308* or the *parB* allele were not successfully constructed following multiple attempts.

Mass doubling time. Mass doubling times were measured in a microtitre plate assay adapted from a method used to measure β -galactosidase activity (Bianco & Weinstock, 1994). Overnight cultures were diluted 1:50 in fresh LB medium and incubated at 32 °C or 37 °C to OD₆₀₀ 0.600. Cells were placed on ice until the assay was started. Each well of a 96-well microtitre plate (Falcon 3072) contained 240 μl LB medium. To each of seven wells, 10 μl of each cell suspension was added. The microtitre plates were placed on a shaker and

Table 1. *E. coli* strains

Strain	Relevant genotype	Derivation/source/reference
MG1655	F ⁻ prototroph	G. Weinstock
W1485	F ⁺ MG1655	R. Maurer
KY1420	<i>dnaG9</i> (Am) <i>ilv thr metE trpE9829</i> (Am) <i>tyr</i> (Am) <i>thy supF</i> (Ts6)	Y. Nakamura (Nakamura, 1984)
KN1378	<i>dnaG2903</i> in W3110; Ts	T. Katayama (Katayama <i>et al.</i> , 1989)
GC2530	<i>parB thr-1 leu-6 proA2 his-4 argE3 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 sup-37 rpsL31</i> ; Ts	R. D'Ari (Norris <i>et al.</i> , 1986)
JV53	<i>parB</i> linked to mini-Tn10 (Cm ^r) in MG1655; Ts	This work
JV8	<i>parB</i> linked to Tn5 (Km ^r) in MG1655; Ts	This work
JV10	<i>dnaG2903</i> linked to Tn5 (Km ^r) in MG1655; Ts	This work
JV45	<i>dnaG2903</i> linked to Tn5 (Km ^r) in W1485; Ts	This work
SE2112	<i>parC281</i> (<i>S. typhimurium</i>) <i>zge-2393::Tn10, mutL211::Tn5</i> in <i>E. coli</i> AB1157; Ts	M. Schmid (Adams <i>et al.</i> , 1992)
JV46	<i>parC281</i> (<i>S. typhimurium</i>) linked to Tn10 in MG1655; Ts	This work
GC4540	<i>sfiA::Tn5</i> (Km ^r)	R. Moses
JV20	<i>sfiA::Tn5</i> (Km ^r) in JV53; Ts	This work
JV48	<i>sfiA::Tn5</i> (Km ^r) in MG1655	This work
GE13	<i>lexA3</i> linked to Tn10 (Tc ^r)	G. Weinstock
JV13	<i>lexA3</i> linked to Tn10 (Tc ^r) in MG1655	This work
JV11	<i>lexA3</i> linked to Tn10 (Tc ^r) in JV10; Ts	This work
JV14	<i>lexA3</i> linked to Tn10 (Tc ^r) in JV8; Ts	This work

incubated at 30 °C and 42 °C. The OD₆₀₀ values were measured in a microtitre plate reader connected serially with a Macintosh computer. The numerical data were collected, and mean values were calculated for each sample. Plots were generated and points in the linear range were chosen for doubling time calculations. The formula used to calculate each doubling time, g , was as follows: $g = t/n$, where $n = [(\log_{10} T_2) - (\log_{10} T_1)]/0.301$. T_2 and T_1 represent the OD₆₀₀ measurements at time 1 and time 2, respectively, while t is the elapsed time (min) between the T_2 and T_1 measurements.

Cell viability. Cell viability was measured by plate transfer experiments. Cells were grown for 12–14 h at 30 °C in LB broth. Cells were diluted 1:50 in fresh LB medium and cultivated until OD₆₀₀ equalled 1.00. Cells were diluted 10⁻⁶ in LB medium and added to LB agar plates. Individual plates were placed at restrictive temperature, 42 °C, and incubated for various periods. At the designated time points, plates were transferred to 30 °C and incubated for 12–14 h. Percentage cell viability was calculated by comparison with plates incubated only at 30 °C.

DNA synthesis studies. Steady-state labelling assays were performed to measure total DNA synthesis at permissive and restrictive temperatures. The assay was adapted from a protocol obtained from Molly Schmid (Schmid, 1990). Cells were cultivated for 12–14 h in LB medium without selection. They were then diluted 1:200 in 10 ml LB medium and incubated at 30 °C with shaking to OD₆₀₀ 0.1. Cells were diluted again 1:10 in 10 ml prewarmed LB prior to addition of 30 µCi [*methyl*-³H]thymidine (81.1 Ci mmol⁻¹, 3000 GBq mmol⁻¹; Dupont/NEN), then incubated with agitation and sampled at different time points. Samples of 0.25 ml of culture were withdrawn, added to 1 ml ice-cold 10% trichloroacetic acid (TCA) (Sigma), and precipitated on ice for a minimum of 30 min. Precipitates were collected by vacuum filtration through GF/A filters (Whatman). Filters were washed once each with 3 ml cold 10% TCA and 95% ethanol, dried for at least 4 h with a heat lamp, and placed in 5 ml 3a20

scintillation fluid (Research Products International). The radioactivity of each sample was measured by counting for 10 min in a Beckman LS 3801 scintillation counter.

Priming at G4ori_c. Primase activity at G4ori_c was measured by using M13 derivatives obtained from G. Nigel Godson (NYU Medical Center). The recombinant M13 derivative, M13ΔE101/G4, has the 278 bp *EcoRI* fragment containing the wild-type G4ori_c inserted into the filamentous phage vector M13ΔE101 (Sakai & Godson, 1985; Hiasa *et al.*, 1989). The MG1655 F⁺ host strain, W1485, and its *dnaG2903* (JV45) derivative were incubated overnight at 30 °C in tryptone/yeast extract (TYE) medium (Miller, 1972). Cells were diluted 1:50 in glucose/Casamino acids medium (Ray & Schekmann, 1969) and cultivated at 30 °C to OD₆₀₀ 0.800. Cells were diluted 1:2 by transferring 0.5 ml cells into a tube containing 0.5 ml glucose/Casamino acids medium. These tubes were incubated for 80 min at either 30 °C or 42 °C. Rifampicin (200 µg ml⁻¹) (Boehringer Mannheim) was added to the cells, and the suspensions were incubated at 30 °C for 5 min before addition of phage. Cells were then infected at an m.o.i. of 10⁻³ with f1R199 (Sakai & Godson, 1985; Zinder & Boeke, 1982) or M13ΔE101/G4 for 2 h at 30 °C or 42 °C with shaking. Negative control reactions included assays with host cells only, phage lysates only, and f1R199 (contains rifampicin-sensitive native f1 origin instead of G4ori_c) with rifampicin. Positive control reactions included purified φX54 or φX56 replicative form (RF) DNA (see below) and f1R199 without rifampicin. To stop the reaction, cells were transferred immediately to -80 °C and frozen for at least 30 min. Cells were thawed at room temperature, lysed with two or three drops of toluene, and vortexed vigorously for 30 s. The tubes were placed on ice, and 1 µl aliquots of lysates were added to PCR reactions.

PCR reactions (25 µl volumes) included 25 pmol of each primer, 1.25 mM dNTPs (Pharmacia), 2.5 U AmpliTaq (Perkin Elmer) in a buffer containing 10% DMSO (Kogan *et al.*, 1987). Oligonucleotide primers were synthesized in the

Nucleic Acids Core Facility of the Department of Molecular and Human Genetics at Baylor College of Medicine. Oligonucleotide primer sequences are as follows: M13-1 (complementary to M13 gene IV sequence), 5'-GAGGAAAGCAC-GTTGTACGTGC-3'; and M13-2 (complementary to M13 gene II sequence), 5'-AAACAAGAGAATCGATGAACGG-3'. PCR conditions included an initial denaturation (95 °C, 7 min) followed by 35 cycles of denaturation (90 °C, 30 s), annealing (48 °C, 1 min), extension (72 °C, 3 min) and a final extension (72 °C, 6 min). After the PCR, samples were immediately frozen at -20 °C prior to electrophoresis. Ten microlitres of each PCR reaction were analysed by agarose gel electrophoresis (1% agarose, 1× TAE, 0.5 µg ethidium bromide ml⁻¹).

Quantitative competitive PCR (QC-PCR) (Piatak *et al.*, 1993) was performed by adding defined amounts of a competitor template to each PCR reaction prior to addition of phage-infected cell lysates. The competitor templates were the double-stranded replicative forms of the f1R199 derivatives, ϕ X54 and ϕ X56 (Sakai & Godson, 1985). ϕ X54 and ϕ X56 contain 25 bp and 50 bp insertions, respectively, within stem-loop II of the *G4ori* and convert ssDNA to RF DNA in the presence of rifampicin (Sakai & Godson, 1985). These templates were purified from 200 ml infected cells in TYE media with silica-gel-based anion-exchange columns (Qiagen). DNA was isolated according to the manufacturer's instructions (Qiagen). The competitor DNAs were resuspended in Tris/EDTA (10 mM Tris, 1 mM EDTA, pH 8.0) and quantified by A_{260} measurements. Various amounts of competitor were added to each reaction to determine the relative amounts of starting M13 Δ E101/G4 replicative form DNA in each assay.

Combination fluorescence-phase-contrast microscopy. Cells were prepared for combination 'fluo-phase' microscopy by a method adapted from Hiraga *et al.* (1989). Cells were grown for 12–14 h at 30 °C, diluted 1:20, and grown for 1–2 h at 30 °C in LB medium to OD₆₀₀ 0.600. Samples (1 ml) of each cell suspension were transferred to 1.5 ml polypropylene tubes and incubated at 30 °C or 42 °C with gentle agitation. Cells were collected and prepared for microscopy as described previously (Grompe *et al.*, 1991). Fluorescence microscopy was performed with a filter unit (Zeiss) that allowed transmission of emitted light with wavelengths greater than 400 nm. Photographs were taken with Ektachrome 400 colour slide film (Kodak).

Evaluation of SOS induction by cell length measurements. Cells were prepared as described above for 'fluo-phase' microscopy. Photography was performed with black-and-white Tri-X (Kodak) film. Photographic prints (5 inches × 7 inches) were developed and used for manual measurements of cell lengths by a ruler. Cell lengths were measured in millimetres along the longest axis of each cell. At least 20 cells were measured for each strain and assay. Mean cell lengths and standard deviations were calculated.

RESULTS

Total DNA synthesis of *E. coli* strains containing the *dnaG2903* and *parB* alleles

Total DNA synthesis was measured by steady-state labelling of cells in liquid culture with [³H]thymidine. The *dnaG* alleles, *parB* and *dnaG2903*, were introduced by P1-mediated transduction into the MG1655 chromosome to construct the isogenic strains JV8 and

Fig. 1. DNA synthesis at restrictive temperature in MG1655 and isogenic *parB* and *dnaG2903* strains. Incorporation of [³H]thymidine in steady-state labelling experiments was used to evaluate total DNA synthesis *in vivo* at restrictive temperatures (42 °C). The isogenic strains, MG1655 (wild-type parent), JV8 (*parB* derivative) and JV10 (*dnaG2903* derivative), are depicted with solid circles, solid squares, and open circles, respectively. Additionally, the control strain KY1420 is indicated by open squares; it has a *dnaG* amber allele [*dnaG9*(Am)] with a temperature-sensitive amber suppressor tRNA (Nakamura, 1984). The dashed vertical line indicates the time of temperature upshift from 30 °C to 42 °C. The assay was actually started 40 min prior to upshift, with the '-20' time point representing the first sampling after 20 min at 30 °C.

JV10, respectively. These strains and the wild-type parent, MG1655, were used in steady-state labelling assays.

Patterns of incorporation of labelled thymidine were equivalent in MG1655 and its *dnaG* derivatives, JV8 and JV10 (Table 1), during exponential-phase growth at 30 °C for 160 min (data not shown). These results were consistent with equivalent mass doubling times obtained among these three strains (data not shown). Following upshift to restrictive 42 °C, DNA synthesis was maintained in the *parB* and *dnaG2903* strains for 80 min at nearly wild-type levels (Fig. 1). DNA synthesis continued, though slightly inhibited, in the *dnaG2903* strain at the restrictive temperature. The non-isogenic control strain KY1420 contains the temperature-sensitive allele *dnaG9*(Am) (Nakamura, 1984; Grompe *et al.*, 1991) and was DNA replication-defective at 42 °C (Fig. 1). The positive DNA synthesis control was *E. coli*

Fig. 2. QC-PCR assay for *G4ori*_c priming activity in W1485 and its isogenic *dnaG2903* derivative, JV45. The competitor DNA amplification product is indicated by 'comp', and the target DNA amplification product is labelled as 'target'. Lanes 1 and 2 contained f1R199→W1485 without and with rifampicin, respectively. Every lane (except lanes 1, 13, and 14) contained rifampicin-treated reactions. M13ΔE101/G4→W1485 lysates were added to PCR reactions in lanes 3 (30 °C), 4 (42 °C), 5 (42 °C, 21.7 ng ϕ X56 competitor DNA per PCR reaction), 6 (42 °C, 2.17 ng ϕ X56) and 7 (42 °C, 0.217 ng ϕ X56). M13ΔE101/G4→JV45 lysates were added to PCR reactions in lanes 8 (30 °C), 9 (42 °C), 10 (42 °C, 21.7 ng ϕ X56), 11 (42 °C, 2.17 ng ϕ X56) and 12 (42 °C, 0.217 ng ϕ X56). The competitor DNA, ϕ X56, contains a 50 bp insertion in *G4ori*_c and therefore migrates more slowly than the target phage DNA in electrophoresis. Lane 13 contained only purified ϕ X56 RF DNA as template in the PCR. Lane 14 contained a lysate of W1485 cells only (no phage added) as a negative control for the PCR reaction. Lanes marked 'M' contained 1 kb DNA ladder (Gibco/BRL).

JV46 (Table 1), which contains the *Salmonella typhimurium parC281* allele (Adams *et al.*, 1992). The *parC281* allele in its native host yielded a classical *par* phenotype, which includes proficient DNA synthesis and obvious chromosome segregation defects at the restrictive temperature. JV46 continued to synthesize DNA at wild-type levels for at least 80 min at the restrictive temperature and was chromosome segregation-defective by cytology (data not shown), consistent with previous observations (Adams *et al.*, 1992).

Primase activity in isogenic *E. coli dnaG*⁺ and *dnaG2903* strains

The coliphage G4 requires primase for conversion of its ssDNA template to the corresponding double-stranded replicative form (RF) during DNA replication in the host cell (Kornberg & Baker, 1992). However, G4 infects *E. coli* B strains and fails to infect *E. coli* K12 strains. The filamentous phages (M13, f1, fd) do not require primase, utilize the host's RNA polymerase for ss→RF conversion (Kornberg & Baker, 1992), and productively infect *E. coli* K12 strains. The recombinant M13 derivative, M13ΔE101/G4, contains the primase-dependent rifampicin-resistant replication origin, *G4ori*_c, infects *E. coli* K12, and can be used to assay *E. coli* primase activity *in vivo* (Sakai & Godson, 1985; Hiasa *et al.*, 1989).

The current study utilized M13ΔE101/G4 in a PCR-based assay to examine ss→RF conversion in isogenic *dnaG* host cells. The M13/f1 RNA-polymerase-depen-

dent origin of replication is functional only in the absence of rifampicin (Fig. 2, lane 1) and fails to support replication in the presence of rifampicin (Fig. 2, lane 2). These controls confirm that the assay measures DNA replication if rifampicin-resistant, primase-dependent origins are present. To measure the relative amounts of template RF DNA synthesized by primase, the QC-PCR method was applied with different amounts of competitor DNA. After preincubation at 42 °C for 80 min, cells were infected with M13ΔE101/G4 in the presence of rifampicin and cultivated at 42 °C for 2 h. The wild-type parent, W1485 (Fig. 2, lanes 3–7), and its *dnaG2903* derivative, JV45 (Fig. 2, lanes 8–12), yielded similar amounts of target RF DNA of equivalent size by the QC-PCR assay (Fig. 2). The expected amplification product of M13ΔE101/G4 RF DNA appeared with the W1485 (lane 6) and JV45 (lane 11) cell lysates when the same amount of competitor DNA (2.17 ng) was added. In other words, a similar amount of double-stranded RF DNA production is observed in the *dnaG2903* strain (JV45) when compared to the wild-type (W1485) at 42 °C. The cells were infected after preincubation at 42 °C for 80 min. By 80 min at the restrictive temperature, *dnaG2903* strains display severe chromosome segregation and cell division defects (data not shown). Importantly, the *dnaG2903* host strain, even when defective for chromosome segregation, supports ss→RF conversion as evidenced by its ability to produce double-stranded target DNA (Fig. 2, lanes 6 and 11). Such conversion of single-stranded to double-stranded RF DNA indicates that DnaG2903 has retained primase activity.

Fig. 3. Chromosome segregation phenotypes in wild-type MG1655 (a) and isogenic derivatives JV10 (*dnaG2903*) (b) and JV8 (*parB*) (c). Cells were prepared for photography as described in Methods following incubation for 2 h at 42 °C. Magnification $\times 4300$.

Chromosome segregation and cell viability phenotypes of strains with the *dnaG2903* and *parB* alleles

Combination 'fluo-phase' microscopy allowed the analysis of chromosome segregation phenotypes among isogenic *dnaG* strains. Isogenic strains JV8 and JV10 formed chromosome-segregation-defective cell filaments at 42 °C with centrally located nucleoids (Fig. 3b, c). This phenotype contrasts with that of the wild-

type, parent MG1655 cells, which display smaller rod shapes containing distinct nucleoids (Fig. 3a). After 2 h at restrictive 42 °C, more than 60 % of JV10 filamentous cells and more than 80 % of JV8 filamentous cells contained single, unsegregated nucleoids (data not shown). MG1655 rarely displays filamentous cells. After 1 h at 42 °C, fewer than 50 % of JV8 and JV10 cells remained viable, and the *par* phenotype was irreversible (data not shown). Cells of the isogenic *dnaG* strains, JV8 and JV10, steadily increased in length at 42 °C and exceeded 10 μm after 2 h (data not shown). By 2 h at 42 °C, fewer than 5 % of cells remained viable at 42 °C (data not shown). While cell viability dramatically decreased during 2 h at 42 °C, DNA synthesis continued to increase exponentially. Cells continued to synthesize DNA, increased in length, and traversed a physiological boundary beyond which cell viability could not be restored (data not shown).

Induction of the SOS response in strains with the *dnaG2903* and *parB* alleles

To investigate a possible role for SOS genes in the production of the cell filamentation phenotype observed in the *dnaG2903* and *parB* mutants at the restrictive temperature, the induction of the SOS response in *dnaG* mutant strains was studied with isogenic strains containing either the defective *lexA3* allele or a Tn5 insertion in *sfiA*. The *lexA3* allele, which encodes a noncleavable LexA repressor (Mount *et al.*, 1972), was introduced into the isogenic *dnaG* strains. This mutant LexA repressor cannot undergo autoproteolysis in the presence of activated RecA, thereby preventing induction of the SOS response. LexA3 continues to bind to its operator sequences in the chromosome and maintains repression of SOS gene expression. Calculated mean cell lengths of isogenic *dnaG2903* (JV11) and *parB* (JV14) strains with the *lexA3* allele were approximately half the lengths of the isogenic *dnaG* strains with the wild-type *lexA* allele, JV10 and JV8 respectively, after 1 h (Fig. 4a) and 2 h (Fig. 4b) incubations at 42 °C. These results indicated that SOS-dependent cell division inhibition was active in *dnaG* strains at 42 °C. The reduction in cell length remained after 2 h at 42 °C probably because SfiA and other SOS-associated division inhibition mechanisms were blocked in the *lexA3* background. Additionally, mean cell lengths continued to increase in the *dnaG lexA3* double mutants, suggesting that SOS-independent pathways of division inhibition were also active at 42 °C in chromosome-segregation-defective *dnaG* strains.

An *sfiA* null allele abolishes the synthesis of the SOS-inducible cell division inhibitor, SfiA. If the SOS response is normally induced in the presence of the *parB* allele, the inactivation of *sfiA* (*sfiA*::Tn5) should suppress SOS-dependent cell filamentation. Mean cell lengths of isogenic *parB* strains with the wild-type *sfiA* allele and *sfiA*::Tn5 were compared at the restrictive temperature (Fig. 4c, d). The *parB*, *sfiA*::Tn5 derivative, JV20, had a mean cell length that was less

Fig. 4. Evaluation of SOS response by cell length measurements in isogenic *parB* and *dnaG2903* strains. Cell lengths of different isogenic strains were measured following incubation at 42 °C for 1 h (a, *lexA*⁺ or *lexA3*; c, *sfiA*⁺ or *sfiA::Tn5*) or 2 h (b, *lexA*⁺ or *lexA3*; d, *sfiA*⁺ or *sfiA::Tn5*). Hatched bars indicate the presence of the *lexA3* allele (a, b) or the *sfiA::Tn5* derivative (c, d). Standard deviations are indicated by bracketed lines.

than that of the corresponding *parB* strain with a wild-type *sfiA* allele, JV8, after 1 h at the restrictive temperature (Fig. 4c). This result indicated that an *sfiA*-dependent mechanism of division inhibition was induced in the *parB* strain and was, at least partially, responsible for cell filamentation in this strain. However, after 2 h at the restrictive temperature, mean cell lengths were similar for JV20 and JV8 (Fig. 4d), suggesting that other SOS-associated filamentation pathways (e.g. SfiC) were activated during extended periods at 42 °C.

DISCUSSION

Isogenic *E. coli* strains containing the *dnaG* alleles, *parB* and *dnaG2903*, yield classic *par* phenotypes. That is, the cells continue to synthesize DNA at the restrictive temperature but fail to segregate their chromosomes. Since primase is essential for *E. coli* chromosomal replication, steady-state labelling experiments with [³H]thymidine suggest that the mutant primases, ParB and DnaG2903, can synthesize primer RNA during DNA replication at the restrictive temperature. A *dnaG2903* strain can support ss → RF DNA conversion

at the primase-dependent *G4ori*_c at 42 °C, indicating that DnaG2903 can synthesize primer RNA at the restrictive temperature. Either defects in DNA replication fork progression or the obviously aberrant chromosome segregation pattern induce the SOS response in the isogenic *dnaG2903* and *parB* strains. These experiments indicate that an intact DnaG carboxy terminus is not essential for primase activity. In light of a recently published study (Tougu *et al.*, 1994), the results suggest that an intact carboxy terminus of primase is important for interactions with other DNA replication proteins (e.g. DnaB), cell cycle regulatory factors, or the chromosome segregation apparatus (reviewed by Britton & Lupski, 1997).

The amino-terminal halves of bacterial primases are conserved by primary sequence and contain motifs with possible functional importance. Primase molecules that contained the amino-terminal 398 amino acids retained primase activity (Sun *et al.*, 1994). A zinc-finger-like motif (Ilyina *et al.*, 1992; Mendelman *et al.*, 1993; Versalovic & Lupski, 1993) and the RNAP-basic motif (Versalovic & Lupski, 1993), shared only with RNA polymerase β -like subunits, are located in the amino-terminal halves of bacterial primases. Amino acid residues in the zinc finger and adjacent to the RNAP-basic motifs comprise the catalytic centre of primase as determined by cross-linking experiments (Mustaev & Godson, 1995). Mutations in *dnaG* alleles *dnaG3*, *danG308* and *dnaG399*, which result in severe DNA replication and cell growth defects (Grompe *et al.*, 1991), are located adjacent to the RNAP-basic motif in this conserved region (Versalovic & Lupski, 1993). Mutations in these alleles probably affect the catalytic site of primase and preclude pRNA formation.

The carboxy terminus of DnaG is divergent among bacteria (Lupski & Godson, 1989; Ilyina *et al.*, 1992; Versalovic & Lupski, 1993) and probably does not comprise part of the catalytic site. This region may, instead, be important for protein–protein interactions of DnaG within the replisome complex. The *parB* and *dnaG2903* strains, unlike clones with the *dnaG3*, *dnaG308* and *dnaG399* alleles, can be suppressed by regulatory mutations that cause overexpression of the mutant primase (Katayama *et al.*, 1989; Grompe *et al.*, 1991; Britton & Lupski, 1995). If the changes in ParB and DnaG2903 reduce the affinity of primase for other replication proteins, overexpression may be necessary to overcome the defective interaction. Interestingly, trypsin cleavage of the carboxy terminus of T4 helicase preserves helicase activity but perturbs the interaction of T4 helicase with accessory replication proteins (Richardson & Nossal, 1989). As previously reported for other *E. coli* DNA replication proteins such as DNA polymerase I (Setlow & Kornberg, 1972) and DnaB helicase (Nakayama *et al.*, 1984), different domains within DnaG may be responsible for different functions, such as primase activity and intra-replisome protein–protein interactions.

A direct physical interaction of primase with other DNA

replication proteins has not been established. However, functional interactions between primase and either helicase or DNA polymerase holoenzyme have been demonstrated *in vitro* (Hiasa & Marians, 1994; Marians, 1992; Shrimankar *et al.*, 1992; Wu *et al.*, 1992). Recent evidence (Tougu *et al.*, 1994) strongly suggests that the carboxy terminus of DnaG is required for the interaction between *E. coli* primase and the DnaB helicase. A 16 kDa carboxy-terminal trypsin fragment of primase presumably competes with wild-type primase for binding DnaB in *in vitro* replication assays. *E. coli* DnaG requires DnaB helicase for primer synthesis on single-stranded template DNA (Shrimankar *et al.*, 1992). Helicase may be required to introduce particular secondary structures in the template DNA, or an actual physical contact between DnaB and DnaG may be required for primase activity (Tougu *et al.*, 1994).

Effective coordination of leading and lagging strand synthesis in an *ori_c*-dependent replication system was found to be dependent on *E. coli* primase concentrations (Hiasa & Marians, 1994). Low amounts of primase resulted in asymmetric *ori_c*-independent replication patterns, and sufficient amounts of primase were required to ensure formation of coordinated bidirectional replication forks. The necessary physical interactions between primase and helicase, DNA polymerase, or other cell cycle regulatory proteins might only be possible when sufficient amounts of wild-type primase are present (Hiasa & Marians, 1994). Indeed, if the missense substitutions in ParB and DnaG2903 reduce the binding affinity between DnaG and DnaB, the effective concentration of primase at the replication fork may be decreased. ParB and DnaG2903 may be less proficient at forming productive contacts between primase and helicase (or other replication proteins) and, hence, lack the ability to coordinate leading and lagging strand synthesis effectively.

The *parB* and *dnaG2903* strains support DNA replication at the restrictive temperature, but may have defects in the progression of the DNA replication fork which result in SOS induction. Although total DNA synthesis resembles that of the wild-type, SOS induction can be inferred by suppression of cell filamentation in SOS-defective *parB* and *dnaG2903* strains. Similar experiments with *priA* null mutants demonstrated induction of the SOS response and cell filamentation (Nurse *et al.*, 1991). A loss-of-function *sfiA* mutation also suppressed cell filamentation of strains containing mutations in the primosomal helicase gene, *priA* (Nurse *et al.*, 1991). While primase may be functional within the unsegregated nucleoids, the existence of gaps containing patches of ssDNA within anomalous replication forks may be sufficient to induce the SOS regulon. If uncoordinated DNA synthesis yields fewer and larger Okazaki fragments in the *parB* and *dnaG2903* strains, increased amounts of ssDNA may be sufficient for SOS induction (Higashitani *et al.*, 1992). DNA synthesis that occurs in the *parB* and *dnaG2903* strains at 42 °C may represent inducible stable DNA replication (iSDR) at

alternative origins (*oriM*), which is part of the SOS response (Magee & Kogoma, 1990; Magee *et al.*, 1992).

Since DNA synthesis and primer RNA formation appear to be intact in the *parB* and *dnaG2903* mutant strains, the possibility remains that primase may possess an additional function in the *E. coli* cell cycle. The *par* phenotype present in *parB* and *dnaG2903* mutant strains is not only due to SOS induction, suggesting that defective DNA replication and chromosome segregation must account, at least partly, for this phenotype. Primase, either alone or by its interaction with DnaB, may somehow link the DNA replication and chromosome segregation processes. Since the DNA replication and chromosome partitioning cycles are probably functioning in parallel (Nordstrom *et al.*, 1991), the cell must somehow activate its chromosome segregation mechanism while replicating its chromosomes. An understanding of the physical interactions between chromosome replication and segregation complexes inside the cell may reveal the key regulatory factors linking the two processes.

ACKNOWLEDGEMENTS

The authors thank Antonios Aliprantis for technical assistance. J. V. acknowledges the support of the NIH Medical Scientist Training Program at Baylor College of Medicine.

REFERENCES

- Adams, D. E., Shekhtman, E. M., Zechiedrich, E. L., Schmid, M. B. & Cozzarelli, N. R. (1992). The role of topoisomerase IV in partitioning bacterial replicons and the structure of catenated intermediates in DNA replication. *Cell* **71**, 277–288.
- Bianco, P. R. & Weinstock, G. M. (1994). Automated determination of β -galactosidase specific activity. *Biotechniques* **17**, 974–980.
- Bouche, J. P., Rowen, L. & Kornberg, A. (1978). The RNA primer synthesized by primase to initiate phage G4 DNA replication. *J Biol Chem* **253**, 765–769.
- Britton, R. A. & Lupski, J. R. (1995). Functional analysis of mutations in the transcription terminator T1 that suppress two *dnaG* alleles in *Escherichia coli*. *Mol Gen Genet* **246**, 729–733.
- Britton, R. A. & Lupski, J. R. (1997). Segregation of the bacterial chromosome. In *Bacterial Genomes: Physical Structure and Analysis*. Edited by F. J. de Bruijn, J. R. Lupski & G. M. Weinstock. New York, NY: Chapman & Hall (in press).
- de Bruijn, F. J. & Rossbach, S. (1994). Transposon mutagenesis. In *Methods for General and Molecular Bacteriology*, pp. 387–405. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.
- Calos, M. P. (1978). DNA sequence for a low-level promoter of the *lac* repressor gene and an 'up' promoter mutation. *Nature* **274**, 762–765.
- Carl, P. L. (1970). *Escherichia coli* mutants with temperature-sensitive synthesis of DNA. *Mol Gen Genet* **109**, 107–122.
- Churchward, G., Belin, D. & Nagamine, Y. (1984). A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. *Gene* **31**, 165–171.
- Elliott, T. & Roth, J. R. (1988). Characterization of Tn10d-Cam: a

- transposition-defective Tn10 specifying chloramphenicol resistance. *Mol Gen Genet* 213, 332–338.
- van der Ende, A., Baker, T. A., Ogawa, T. & Kornberg, A. (1985). Initiation of enzymatic replication at the origin of the *Escherichia coli* chromosome: primase as the sole priming enzyme. *Proc Natl Acad Sci USA* 82, 3954–3958.
- Grompe, M., Versalovic, J., Koeuth, T. & Lupski, J. R. (1991). Mutations in the *Escherichia coli dnaG* gene suggest coupling between DNA replication and chromosome partitioning. *J Bacteriol* 173, 1268–1278.
- Gross, J. D. (1972). DNA replication in bacteria. *Curr Top Microbiol Immunol* 57, 39–74.
- Heath, J. D., Perkins, J. D., Sharma, B. & Weinstock, G. (1992). NotI genomic cleavage map of *Escherichia coli* K-12 strain MG1655. *J Bacteriol* 174, 558–567.
- Hiasa, H. & Marians, K. J. (1994). Primase couples leading and lagging strand DNA synthesis from *ori_c*. *J Biol Chem* 269, 6058–6063.
- Hiasa, H., Tanaka, K., Sakai, H., Yoshida, K., Honda, Y., Komano, T. & Godson, G. N. (1989). Distinct functional contributions of three potential secondary structures in the phage G4 origin of complementary DNA strand synthesis. *Gene* 84, 17–22.
- Higashitani, N., Higashitani, A., Roth, A. & Horiuchi, K. (1992). SOS induction in *Escherichia coli* by infection with mutant filamentous phage that are defective in initiation of complementary-strand DNA synthesis. *J Bacteriol* 174, 1612–1618.
- Hiraga, S., Niki, H., Ogura, T., Ichinose, C., Mori, H., Ezaki, B. & Jaffe, E. (1989). Chromosome partitioning in *Escherichia coli*: novel mutants producing anucleate cells. *J Bacteriol* 171, 1496–1505.
- Hirota, Y., Mordoh, J. & Jacob, F. (1970). On the process of cellular division in *Escherichia coli*. III. Thermosensitive mutants of *E. coli* altered in the process of DNA initiation. *J Mol Biol* 53, 369–387.
- Ilyina, T. V., Gorbalenya, A. E. & Koonin, E. V. (1992). Organization and evolution of bacterial and bacteriophage primase–helicase systems. *J Mol Evol* 34, 351–357.
- Katayama, T., Murakami, Y., Wada, C., Ohmori, H., Yura, T. & Nagata, T. (1989). Genetic suppression of a *dnaG* mutation in *Escherichia coli*. *J Bacteriol* 171, 1465–1491.
- Kogan, S., Doherty, M. & Gitschier, J. (1987). An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. *N Engl J Med* 317, 985–990.
- Kornberg, A., Baker, T. A. (1992). *DNA Replication*, 2nd edn. New York: W. H. Freeman.
- Lark, K. (1972). Genetic control over the initiation of the synthesis of the short deoxynucleotide chains in *E. coli*. *Nature New Biol* 240, 237–240.
- Lupski, J. R. & Godson, G. N. (1989). DNA–DNA, and DNA–RNA–protein: orchestration by a single complex operon. *BioEssays* 10, 152–157.
- Magee, T. R. & Kogoma, T. (1990). Requirement of RecBC enzyme and an elevated level of activated RecA for induced stable DNA replication in *Escherichia coli*. *J Bacteriol* 172, 1834–1839.
- Magee, T. R., Asai, T., Malka, D. & Kogoma, T. (1992). DNA damage-inducible origins of DNA replication in *Escherichia coli*. *EMBO J* 11, 4219–4225.
- Marians, K. J. (1992). Prokaryotic DNA replication. *Annu Rev Biochem* 61, 673–719.
- Marinus, M. G. & Adelberg, E. A. (1970). Vegetative replication and transfer replication of deoxyribonucleic acid in temperature-sensitive mutants of *Escherichia coli* K-12. *J Bacteriol* 104, 1266–1272.
- Mendelman, L. V., Notarnicola, S. M. & Richardson, C. C. (1993). Evidence for distinct primase and helicase domains in the 63-kDa gene 4 protein of bacteriophage T7. *J Biol Chem* 268, 27208–27213.
- Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Mount, D. W., Low, K. B. & Edmiston, S. J. (1972). Dominant mutations (*lex*) in *Escherichia coli* which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. *J Bacteriol* 112, 886–893.
- Murakami, Y., Nagata, T., Schwarz, W., Wada, C. & Yura, T. (1985). Novel *dnaG* mutation in a *dnaP* mutant of *Escherichia coli*. *J Bacteriol* 162, 830–832.
- Mustaev, A. A. & Godson, G. N. (1995). Studies of the functional topography of the catalytic center of *Escherichia coli* primase. *J Biol Chem* 270, 15711–15718.
- Nakamura, Y. (1984). Amber *dnaG* mutation exerting a polar effect on the synthesis of RNA polymerase sigma factor in *Escherichia coli*. *Mol Gen Genet* 196, 179–182.
- Nakayama, N., Arai, N., Kaziro, Y. & Arai, K.-I. (1984). Structural and functional studies of the *dnaB* protein using limited proteolysis. *J Biol Chem* 259, 88–96.
- Nordstrom, K., Bernander, R. & Dasgupta, S. (1991). The *Escherichia coli* cell cycle: one cycle or multiple independent processes that are co-ordinated? *Mol Microbiol* 5, 769–774.
- Norris, V., Alliotte, T., Jaffe, A. & D'Ari, R. (1986). DNA replication termination in *Escherichia coli parB* (a *dnaG* allele), *parA*, and *gyrB* mutants affected in DNA distribution. *J Bacteriol* 168, 494–504.
- Nurse, P., Zavitz, K. H. & Marians, K. J. (1991). Inactivation of the *Escherichia coli* PriA DNA replication protein induces the SOS response. *J Bacteriol* 173, 6686–6693.
- Piatk, M., Jr, Luk, K.-C., Williams, B. & Lifson, J. D. (1993). Quantitative competitive polymerase chain reaction for accurate quantitation of HIV DNA and RNA species. *Biotechniques* 14, 70–81.
- Ray, D. S. & Schekmann, R. W. (1969). Replication of bacteriophage M13. I. Sedimentation analysis of crude lysates of M13-infected bacteria. *Biochim Biophys Acta* 179, 398–407.
- Richardson, R. W. & Nossal, N. G. (1989). Trypsin cleavage in the COOH terminus of the bacteriophage T4 gene 41 DNA helicase alters the primase–helicase activities of the T4 replication complex *in vitro*. *J Biol Chem* 264, 4732–4739.
- Roa, B. B., Garcia, C. A., Suter, U., Kulpa, D., Wise, C. A., Mueller, J., Welcher, A. A., Snipes, G. J., Shooter, E. M., Patel, P. I. & Lupski, J. R. (1993). Charcot-Marie-Tooth disease type IA: association with a spontaneous point mutation in the PMP22 gene. *N Engl J Med* 329, 96–101.
- Rowen, L. & Kornberg, A. (1978). A ribo-deoxynucleotide primer synthesized by primase. *J Biol Chem* 253, 770–774.
- Sakai, H. & Godson, G. N. (1985). Isolation and construction of mutants of the G4 minus strand origin: analysis of their *in vivo* activity. *Biochim Biophys Acta* 826, 30–37.
- Schmid, M. B. (1990). A locus affecting nucleoid segregation in *Salmonella typhimurium*. *J Bacteriol* 172, 5416–5424.
- Setlow, P. & Kornberg, A. (1972). Deoxyribonucleic acid polymerase: two distinct enzymes in one polypeptide. *J Biol Chem* 247, 232–240.

- Shrimankar, P., Stordal, L. & Maurer, R. (1992).** Purification and characterization of a mutant DnaB protein specifically defective in ATP hydrolysis. *J Bacteriol* **174**, 7689–7696.
- Silver, S. & Wendt, L. (1967).** Mechanism of action of phenethyl alcohol: breakdown of the cellular permeability barrier. *J Bacteriol* **93**, 560–566.
- Sun, W., Tormo, J., Steitz, T. A. & Godson, G. N. (1994).** Domains of *Escherichia coli* primase: functional activity of a 47-kDa N-terminal proteolytic fragment. *Proc Natl Acad Sci USA* **91**, 11462–11466.
- Tougu, K., Peng, H. & Marians, K. J. (1994).** Identification of a domain of *Escherichia coli* primase required for functional interaction with the DnaB helicase at the replication fork. *J Biol Chem* **269**, 4675–4682.
- Versalovic, J. & Lupski, J. R. (1993).** The *Haemophilus influenzae* *dnaG* sequence and conserved bacterial primase motifs. *Gene* **136**, 281–286.
- Wada, C. & Yura, T. (1974).** Phenethyl alcohol resistance in *Escherichia coli*. III. A temperature-sensitive mutation (*dnaP*) affecting DNA replication. *Genetics* **77**, 199–220.
- Wechsler, J. A. & Gross, J. D. (1971).** *Escherichia coli* mutants temperature-sensitive for DNA synthesis. *Mol Gen Genet* **113**, 273–284.
- Weinstock, G. M. (1991).** Transduction in Gram-negative bacteria. In *Modern Microbial Genetics*, pp. 253–270. Edited by U. N. Streips & R. E. Yasbin. New York: Wiley-Liss.
- Wu, C. A., Zechner, E. L., Reems, J. A., McHenry, C. S. & Marians, K. J. (1992).** Coordinated leading- and lagging-strand synthesis at the *Escherichia coli* DNA replication fork. *J Biol Chem* **267**, 4074–4083.
- Zinder, N. D. & Boeke, J. D. (1982).** The filamentous phage (Ff) as vectors for recombinant DNA – a review. *Gene* **19**, 1–10.

.....
Received 31 May 1996; revised 1 October 1996; accepted 8 October 1996.