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DNA Polymerase I Activity in *Escherichia coli* Is Influenced by Spot 42 RNA

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We have shown that the level of DNA polymerase I (Pol I) activity in *Escherichia coli* is influenced by the level of a 109-nucleotide RNA, spot 42 RNA. Deletion of the gene for spot 42 RNA results in a 20 to 25% decrease in Pol I activity, as assayed by nucleotide incorporation in cell extracts and a decrease in the ability of cells to grow in the presence of the DNA-alkylating agent methyl methanesulfonate. Also, a physiological reduction of the level of spot 42 RNA, by growth in media containing poor carbon sources, results in a corresponding decrease in Pol I activity. Conversely, overproduction of spot 42 RNA results in a 10 to 15% increase in Pol I activity in vitro. Thus, changes in the amount of spot 42 RNA result in relatively small but significant changes in Pol I activity.

DNA polymerase I (Pol I) of *Escherichia coli* functions both in the synthesis of DNA and in the repair of damage caused by a variety of chemical agents such as methyl methanesulfonate (MMS) and physical agents such as UV light (5, 13). Pol I synthesis does not appear to be autogenously regulated, nor is the rate of transcription of *polA*, the gene encoding Pol I, affected by DNA damage (28).

Located just 150 nucleotides downstream of the translation stop codon of the *polA* gene in the *E. coli* chromosome is a gene called *spf*, which encodes a 109-nucleotide RNA, spot 42 RNA (9). Spot 42 RNA is moderately abundant (100 to 200 molecules per cell) and has a half-life of 12 to 13 min (21, 22); accumulation of this RNA in vivo is negatively regulated by cyclic AMP and cyclic AMP receptor protein (D. A. Polayes, P. W. Rice, and J. E. Dahlberg, submitted for publication). To date, the biological role of spot 42 RNA has been unclear. Deletion of the *spf* gene is not lethal, but results in a slight growth impairment of one strain under particular conditions (7). In contrast, overproduction of spot 42 RNA has adverse effects on the growth of most strains of *E. coli* (19, 20).

Because of the proximity of the *spf* and *polA* genes, we questioned whether they or their products might influence each other. In particular, we studied the influence of spot 42 RNA on the level of Pol I activity in cells, as assayed in extracts or by the ability of cells to survive treatment with MMS. In the experiments presented in this report, we showed that deletion of the *spf* gene results in a decrease in Pol I activity, as assayed both in vivo and in vitro, and that overproduction of spot 42 RNA results in an increase in Pol I activity.

MATERIALS AND METHODS

Materials. Restriction endonucleases were obtained from Promega Biotec. The Klenow fragment of DNA polymerase I used for dideoxy sequencing was from Boehringer Mannheim Biochemicals. The deoxynucleoside triphosphates and S1 nuclease were from Pharmacia, Inc.; [α-^{32}P]dATP (410 Ci/mmol) was from Amersham Corp. The *Xho*II-*Eco*RI converter linker was from Worthington Diagnostics, and the *Eco*RI and *Bgl*II linkers were from New England BioLabs, Inc. MMS, calf thymus DNA, kanamycin, tetracycline, and ampicillin were from Sigma Chemical Co. The oligonucleotide used in M13 site-directed mutagenesis was synthesized by the Protein/DNA Synthesis/Sequencing Laboratory, University of Wisconsin Biotechnology Center, Madison. LB contains (per liter) 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 5 g of NaCl (18). When present, ampicillin was used at 200 μg/ml, tetracycline was used at 15 μg/ml, and kanamycin was used at 12.5 μg/ml, unless otherwise stated in the text.

Strains and plasmids. The *E. coli* K-12 strains used (1) are described in Table 1, and the plasmids used for linear transformations into JC7623 are outlined in Fig. 1. The point mutation in JED2010 was constructed by site-directed mutagenesis (29). To promote integration by homologous recombination, the DNA to be inserted (the *spf* gene and the 1.2-kilobase-pair [kbp] kanamycin resistance gene) was flanked on the 5′ side by 3 kbp of *polA* sequences and on the 3′ side by the 1 kbp of *E. coli* sequences normally found on the 3′ side of the *spf* gene (Fig. 1). All fusions were verified by sequencing (17, 23). The last three numbers in the JED strain designations correspond to the numbers of the plasmid used in the linear transformation procedure, and the first number refers to the parental strain background: JED2 cells were derived from JC7623 cells by transformation, and JED3 cells were derived from M7042 cells by P1-mediated transduction. Strain JED1 cells were used in construction of other strains and are described in Table 1.

pJD14 (formerly called pRD14) was made from pRD1, a pBR322 derivative (19) which had been linearized at the unique *Bgl*II site in the *polA* gene and digested with *Bal*31 (16) to remove the *polA* promoter; the *Bam*HI and *Sal*I sites in pBR322 sequences were destroyed and *Eco*RI and *Bam*HI sites were created at position +130 of the *spf* gene by partial digestion with *Xho*II and addition of a *Xho*II-*Eco*RI converter linker; a *Bam*HI fragment from pUC-4K (27) containing the kanamycin resistance gene from Tn*903* was inserted into the newly generated *Bam*HI site at position +130. When the resulting plasmid, pJD14, was linearized at the *Eco*RI

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TABLE 1. Strains used in this study

Strain[a]	Relevant genotype and phenotype	Source or reference
JC7623	F⁻ *thi his-4 proA2 argE3 thr-1 leuB6 ara-14 lacY1 galK2 xyl-5 mtl-1 supE44 tsx-33 rpsL31 recB21 recC22 sbcB15*	B. Bachmann (14)
JC9311	F⁻ *galK2 lacY1 mtl-1 xyl-5 argE3 proA2 thi-1 aroD6 tsx-29 supE37*(Am) *recB21 recC22 sbcB37*	S. R. Kushner
MG1655	Prototroph	B. Bachmann
CM5409	F⁺ Δ(*gal-bio*) *thi-1 relA1 spoT1 polA1* Tn*10*	S. Linn
M7042	*lacZu118, rpsL* (Sm^r)	J. Beckwith
JED2014	JC7623 *spf*$^+$ Km^r	This work
JED2001	JC7623 Δ*spf* Km^r	This work
JED2013	JC7623 Φ(*spf-13-lacZYA*) Km^r	This work
JED2004	JC7623 Δ*spf4* Km^r	This work
JED2005	JC7623 *spf*$^+$ Km^r	This work
JED2010	JC7623 *spf*(G27) Km^r	This work
JW1071	*zbf-507*::Tn*10 trp-49 lacZ125 relA1 spoT1*	B. Bachmann
JED1033[b]	JC7623 *zbf-507*::Tn*10 sup*^{0}	P1 · JW1071 × JC7623 to Tet^r
JED2110	JED1033 *spf*(G27) Km^r	P1 · JED2010 × JED1033 to Km^r
JED3014	M7042 *spf*$^+$ Km^r	P1 · JED2014 × M7042 to Km^r
JED3001	M7042 Δ*spf* Km^r	P1 · JED2001 × M7042 to Km^r

[a] The numbers in the JED strain names refer to the number of the plasmid used to transform the parent strain, with the prefixes 2− and 3− for strains derived from JC7623 and M7042, respectively. JED2005 differs from JED2014 by the insertion of the kanamycin resistance gene between *polA* and *spf* in the former strain (see Fig. 1).

[b] Strain JC7623 is *supE44* and was made *sup*^{0} by transduction with a P1 *vir* lysate grown on JW1071 (18). The transductants were selected on LB-tetracycline plates and screened for *sup*^{0} with λcb2P$_3$ (from F. Blattner, University of Wisconsin, Madison). Cells resistant to λcb2P$_3$ and sensitive to λ vir were scored as *sup*^{0}.

site at position +130 and digested with *Bal* 31 (15), several plasmids with deletions extending into the 5′ flanking region of *spf* were obtained. After addition of *Bgl*II linkers, the *Bal* 31-damaged kanamycin resistance gene was replaced with a *Bam*HI fragment containing a complete kanamycin resistance gene. pJD2001 lacked the entire *spf* coding region and 58 nucleotides in the 5′ flanking region. pJD2013 was generated from a less extensive deletion into which a (*Eco*RI-*Sal*I) fragment from pMC1403 containing the *lacZYA* genes (3) was inserted at position +60. The truncated *spf* gene in pJD2004 containing the coding sequence of *spf* from +8 to +130 was isolated as an *Rsa*I-*Eco*RI fragment from pJD14 DNA and inserted into a *Bal* 31 derivative that lacked sequences downstream of position −18. pJD2005 was derived by introduction of a *Sau*3A redigestion product of an isolated (6) 747-bp *Eco*RV-*Sal*I fragment from pRD6A (18) that contains the *spf* gene inserted into the *Sal*I site downstream of the kanamycin resistance gene. The sequence 3′ of the *spf* gene in pJD005 is the gene for the 22-kilodalton protein normally downstream of *spf*.

Linear transformation and P1 transductions. Insertion of variant *spf* genes into the chromosome was by transformation (8) of JC7623 cells by plasmid DNAs (20 μg) linearized with *Pvu*II. Transformants were selected on LB-kanamycin plates and screened for Ap^s. DNA isolated from Km^r Ap^s colonies (4) was digested with *Xho*I and analyzed by Southern blotting after electrophoresis in 0.8% agarose gels. Southern blots (26) were probed with a SP6-generated *spf* probe which contains the *spf* gene from +8 to +130 or with a 5′-flanking probe corresponding to the 2.2-kbp *Xho*I-*Bam*HI fragment of pJD14.

P1 lysates were prepared by the method of Miller (18). Recipient cells were made either Δ*spf* Km^r with a P1 *vir* lysate grown on JED2001 or *spf*$^+$ Km^r with a P1 *vir* lysate grown on JED2014 (25). Insertion of the new *spf* gene (and the associated kanamycin resistance gene) into the chromosome was determined by Southern blot analysis of DNA of the recipient cells (26).

Pol I assays. Cells were diluted 1:200 from an overnight culture and grown in LB to an optical density at 600 nm (OD_{600}) of 0.5. Cells were pelleted, washed, and sonicated as described by Kingsbury and Helinski (12). The extracts were assayed (24) with nicked calf thymus DNA as the template and normalized for protein concentrations (2).

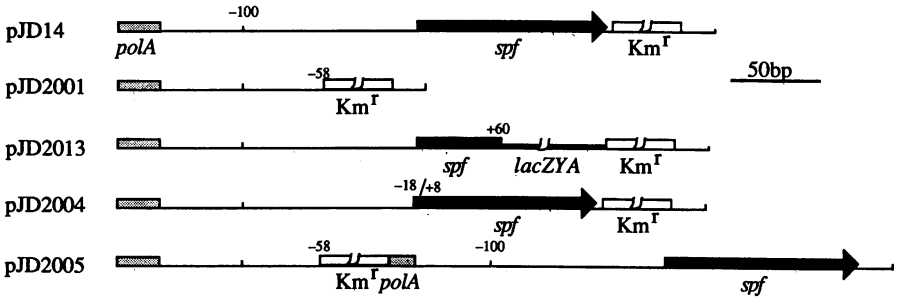

FIG. 1. Plasmids used in chromosome insertions. Only the portion of the plasmid in which insertions and deletions were made is indicated. The *spf* gene (▬▶), kanamycin resistance gene (▭), and *polA* gene (▭) are indicated. The thin line to the right of the kanamycin resistance gene contains sequences normally present 3′ of the *spf* gene, an open reading frame for a 22-kilodalton protein transcribed toward *spf* (9). The *polA*, *spf*, and kanamycin resistance genes are all transcribed from left to right. The 6.2-kbp DNA fragment carrying the *lacZYA* genes is shown (▬).

TABLE 2. Effect of *spf* on Pol I activity

Relevant allele	Pol I activity (nmol/mg)[a] in strain[b]:	
	M7042	JC7623
spf$^+$ (±pBR322)[c]	11.1 ± 0.6 (1)	11.1 ± 0.8 (1)
Δ*spf* (±pBR322)[c]	8.6 ± 0.6 (0.78)	8.3 ± 0.6 (0.75)
spf$^+$ (pRD6A)	12.5 ± 0.5 (1.13)	ND[d]
Δ*spf* (pRD6A)	12.8 ± 0.3 (1.15)	ND

[a] Pol I activity is presented as specific activity in nanomoles of dATP incorporated per milligram of protein ± the standard deviation of the mean (n = 5). The relative activities of the extracts were normalized to the value obtained for *spf*$^+$ cells and are shown in the parentheses.
[b] Cells were grown in LB for six generations, until the culture reached an OD_{600} of 0.5.
[c] *spf*$^+$ and Δ*spf* derivatives of M7042 were transformed with pBR322; nontransformed cells had equal activity. JC7623 cells were not transformed with plasmids.
[d] ND, Not determined.

RESULTS

Effect of *spf* gene on the level of Pol I activity in vitro. To test whether the level of spot 42 RNA affected the levels of Pol I we changed the level of this RNA by altering the number of *spf* genes per cell. The results show that regardless of the strain background, extracts from cells lacking a *spf* gene had 20 to 25% lower levels of Pol I activity (in each of five independent experiments) (Table 2). When cells of a *polA1* strain were assayed under the same conditions, less than 0.6% activity was measured (data not shown), demonstrating that the activity studied here is that of Pol I. Cells with the multicopy plasmid pRD6A, which contains the *spf* gene, overproduce spot 42 RNA; when M7042 cells were transformed with pRD6A they had higher than normal levels of Pol I activity.

The amount of spot 42 RNA in cells is reduced in the presence of 3′,5′-cyclic AMP (22). Growth of *spf*$^+$ cells in carbon sources that elevated cyclic AMP levels (and lowered spot 42 RNA levels [22]) resulted in reduced levels of Pol I activity (Table 3). A comparable reduction in Pol I activity was also obtained when *spf*$^+$ cells were treated with exogenously added cyclic AMP (data not shown). Significantly, these effects are dependent on the presence of the *spf* gene, showing that the changes in Pol I activity are due to events mediated by spot 42 RNA rather than by other effects of these sugars on cell metabolism.

Effect of *spf* gene on sensitivity of cells to MMS. We also tested whether the *spf* gene influenced the level of activity of Pol I in vivo. To do this, we used the resistance of cells to treatment with MMS as an indirect measure of the Pol I activity. Deletion of the *spf* gene resulted in increased sensitivity to MMS when assayed either by colony formation after MMS exposure (Fig. 2) or by growth in the presence of MMS (Fig. 3a); the increased sensitivity is particularly striking in cells that lack *recBC* functions (compare open and solid symbols in Fig. 2). Conversely, the ability of cells to grow in otherwise inhibitory concentrations of MMS correlates with overproduction of spot 42 RNA (as a result of the cells having multiple *spf* genes) (Fig. 3b). Thus, *spf* appears to alter Pol I levels in vivo as well as in vitro. The results in Fig. 3a also demonstrate that the effects are observed in several unrelated strains.

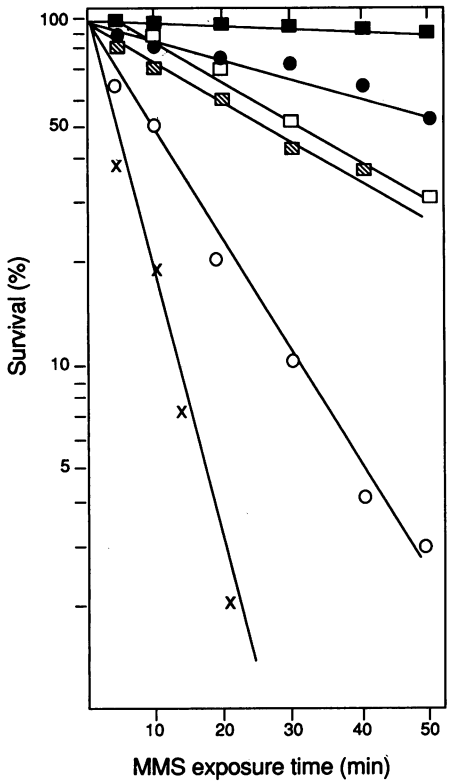

FIG. 2. Effect of the *spf* gene on the ability of cells to withstand treatment with MMS. Solid symbols represent cells derived from M7042; ■, *spf*$^+$ (JED3014); ●, Δ*spf* (JED3001). Open and shaded symbols represent cells derived from the *recBC sbcB* strain JC7623: □, *spf*$^+$ (JED2014); ○, Δ*spf* (JED2001); ▨, *spf*$^+$ (JED2005). This last strain resembles JED2014, except that the positions of the kanamycin resistance and *spf* genes were exchanged (Fig. 1). ×, *polA1* strain, CM5409. Cells were grown in M9 medium with 0.5% Casamino Acids (Difco), 0.5% glucose, and 10 μg of thiamine per ml to an OD_{600} of 0.5, treated with 20 mM MMS as described by Karran et al. (11), and plated on the above medium and 1.5% agar. Each point represents the average of results of three experiments.

TABLE 3. Effect of carbon sources on Pol I activity

Strain	Relevant allele	Pol I activity[a] with following carbon source[b]:		
		Glucose	Fructose	Succinate
JED2014	*spf*$^+$	11.3 ± 0.6	9.5 ± 0.9	8.7 ± 0.6
JED2001	Δ*spf*	8.3 ± 0.8	8.3 ± 0.8	8.3 ± 0.8

[a] Pol I activities are expressed as in Table 2, except that n = 3.
[b] Cells were grown in MOPS minimal medium containing either 0.2% glucose, 1% fructose, or 1% succinate; spot 42 RNA levels are highest when cells are grown in glucose and lowest when they are grown in succinate (7).

Mediation of effects of *spf* on Pol I activity by spot 42 RNA. To determine whether the effect of the *spf* gene on MMS sensitivity was due to the structure of the gene itself or to a transcription product (spot 42 RNA), we introduced partially deleted or mutated *spf* genes (Fig. 1) into cells. Isogenic derivatives of JC7623 that lacked either a functional *spf* promoter (JED2004) or the *rho*-independent *spf* terminator (JED2013) exhibited the phenotype of the complete deletion (Table 4), indicating that full MMS resistance required a functional *spf* gene and not simply sequences from one or the other end of that gene. Mutation of codon 3 in a putative short open reading frame in spot 42 RNA from tyrosine to chain termination (U-A-G) had no effect on MMS sensitivity.

The *spf* gene influences Pol I even when the *spf* and *polA* genes are not contiguous. Cells lacking the endogenous *spf*

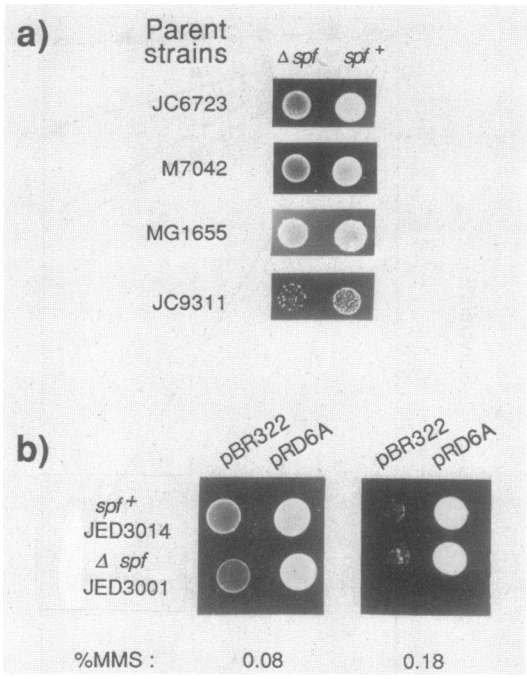

FIG. 3. Influence of the *spf* gene on the ability of cells to grow on plates containing MMS. *spf*$^+$ and Δ*spf* cells grown overnight in LB at 37°C were diluted to an OD_{600} of 0.01, and 5 μl was plated on LB plates containing 0.08% MMS (JC7623 and M7042 cells) or 0.10% MMS (MG1655 and JC9311 cells). (a) Spot tests of cells containing zero or one *spf* gene in the chromosome. Plates were incubated at 37°C for 18 h. (b) Spot tests of cells containing multiple copies of the *spf* gene. *spf*$^+$ (JED3014) and Δ*spf* (JED3001) derivatives of M7042 were transformed with pBR322 or pRD6A. Transformed cells were plated on LB-ampicillin-MMS plates and incubated at 37°C for 18 h (0.08% MMS) or 42 h (0.18% MMS).

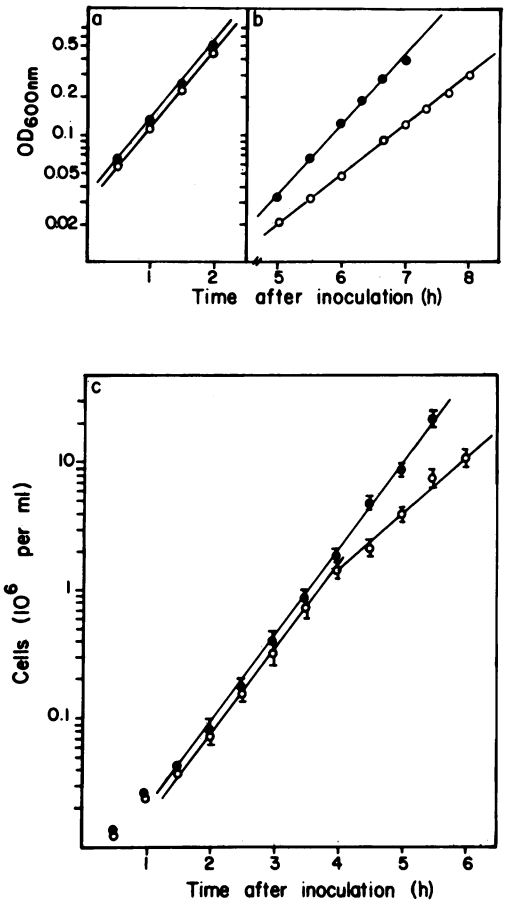

FIG. 4. Effect of deletion of *spf* on growth of *recBC sbcB* cells in LB liquid culture. Cultures of *spf*$^+$ cells (JED2014) (●) and Δ*spf* cells (JED2001) (○) were inoculated into LB and grown overnight at 37°C. Cells from overnight cultures were diluted in LB, and cell growth was monitored by measuring the OD_{600} (panels a and b) or by cell plating on LB-kanamycin plates (panel c). Cells were diluted to a calculated starting OD_{600} of 4×10^{-2} (panel a) or 4×10^{-5} (panels b and c). Each point in panel c represents the average of results for three different plates; error bars denote one standard deviation.

gene exhibited wild-type MMS resistance after introduction of an exogenous *spf* gene, even when that gene was separated from *polA* by the kanamycin resistance gene (JED2005 in Table 4 and Fig. 2). Therefore, the MMS sensitivity associated with the Δ*spf* deletion resulted from the loss of production of the *spf* gene product per se, rather than from disruption of the 3′ end of the *polA* gene.

Slow growth of JC7623 cells owing to deletion of the *spf* gene. Deletion of the *spf* gene from *recBC sbcB* cells (strain JC7623) resulted in an unusual alteration of the generation time that depended on the age of the culture. After five to seven generations of logarithmic growth in LB, Δ*spf* cells grew slower than *spf*$^+$ cells (Fig. 4b and c). The onset of slower growth correlated with the number of generations since inoculation, rather than with cell density (Fig. 4; data not shown), and was unaffected by preconditioning of the medium. This difference in growth rate was observed only in rich medium under high aeration (data not shown). The *recBC sbcB* background is necessary for expression of the slow-growth phenotype of Δ*spf*, since neither the *recBC*$^+$ *sbcB*$^+$ parent of JC7623 (AB1157) nor a *sbcB15* derivative of AB1157 (JC7689) exhibited the phenotype.

TABLE 4. Effect of deletions in *spf* on sensitivity to MMS and growth

Strain	Relevant allele	Growth in 0.08% MMS[a]	Generation time (min)[b]
JED2014	*spf*$^+$	+	30
JED2001	Δ*spf*(−57 to +130)	−	50
JED2013	Δ*spf*(+50 to +130)	−	50
JED2004	Δ*spf*(−17 to +7)	−	50
JED2005[c]	*spf*$^+$	+	30
JED2110	*spf*(G27) *sup*^{0}	+	30

[a] Growth MMS was measured at 0.08% MMS as shown in Fig. 3. Symbols: +, no obvious impairment of growth by 0.08% MMS; −, inhibition of growth by 0.08% MMS.

[b] Generation times were determined after growth for at least seven generations, as described for Fig. 4b.

[c] JED2005 has the kanamycin resistance gene transposed between *polA* and *spf* (cf. Fig. 1).

DISCUSSION

The data presented here demonstrate that spot 42 RNA, the product of the *spf* gene of *E. coli*, influences the level of activity of Pol I. Deletion of the *spf* gene results in a decrease in the Pol I activity in cell extracts and an increase in the sensitivity of cells to the DNA-alkylating agent MMS. The in

vitro incorporation experiments measured the activity of Pol I rather than that of another DNA polymerase, as evidenced by the reduction to <0.6% when the extract was prepared from a *polA* mutant.

For several reasons, it is likely that spot 42 RNA itself is responsible for modulation of the level of Pol I activity. First, the decrease is elicited by deletion of either the 5′ or the 3′ end of the *spf* gene; thus, deletion of the *spf* sequence per se does not disrupt transcription of *polA* unless *polA* uses signals from both ends of the *spf* gene. Second, the effects of deleting the chromosomal copy of *spf* are suppressed when functional *spf* sequences are separated from *polA* by the kanamycin resistance gene or when they are on a plasmid; thus, the *spf* gene functions in *trans*. Third, the level of Pol I activity in extracts is responsive to the level of transcription of *spf* in the cells prior to harvest.

The only product of the *spf* gene that has been identified is spot 42 RNA. We have shown elsewhere (20) that this RNA does not function as an mRNA, and so the possibility that an *spf*-encoded polypeptide exists is remote. Moreover, mutation of the third triplet of the open reading frame to a chain-terminating (U-A-G) codon has no effect on the Pol I activity in sup^0 cells. We conclude that spot 42 RNA itself must be the agent that is responsible for the effect on Pol I activity.

Under normal conditions, *E. coli* cells contain an excess of Pol I (10, 12), and so it is not surprising that the relatively small change in Pol I activity resulting from deletion of the *spf* gene results in no obvious phenotype under normal growth conditions (7). However, by monitoring cells under conditions when Pol I is required, such as when MMS is present, we were able to observe an effect of *spf*.

Deletion of part or all of the *spf* gene results in a lengthening of the generation time of *recBC sbcB* cells growing in rich medium. This may be attributable to the reduced level of Pol I activity in these cells, since *recBC* cells require Pol I (14) and the level may be insufficient when cells are growing rapidly (10).

The *spf*-mediated changes in the level of Pol I activity in vitro agree qualitatively with the in vivo changes as assayed by MMS sensitivity, but we cannot be certain that the two are causally related. Because the in vitro experiments used only net nucleotide polymerization as an assay of Pol I function, activities of this enzyme other than the polymerization activity per se might be altered. For example, if spot 42 RNA reduced Pol I-associated exonuclease activities, it would appear to elevate incorporation levels; however, that would not explain the effect of the *spf* gene on resistance to MMS, and so we believe that this alternative explanation is unlikely.

Because the effect of spot 42 RNA on Pol I activity is observed in vitro as well as in vivo, spot 42 RNA must alter either the number of Pol I molecules or the activity of each molecule. We are currently investigating whether the RNA alters the synthesis of Pol I or whether it binds directly to the enzyme and thereby alters one or more of its activities.

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