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Streptococcus pneumoniae polA Gene Is Expressed in *Escherichia coli* and Can Functionally Substitute for the *E. coli polA* Gene

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The *Streptococcus pneumoniae polA*$^+$ gene was introduced into *Escherichia coli* on the recombinant plasmid pSM31, which is based on the pSC101 replicon. Extracts of *E. coli polA5* mutants containing pSM31 showed DNA polymerase activity, indicating that the pneumococcal DNA polymerase I was expressed in the heterospecific host. Complete complementation of the *E. coli polA5* mutation by the pneumococcal *polA*$^+$ gene was detected in excision repair of DNA damage.

DNA polymerase I (PolI) of *Escherichia coli* has been considered a good model for analyzing the mechanism of DNA replication (5). The enzymatic functions of PolI include discontinuous DNA synthesis in the processing of Okazaki fragments and in the excision repair of damaged DNA. The *polA*$^+$ gene of *E. coli*, which encodes PolI, has been sequenced (4), and the base changes in various mutations have been determined (3). Cloning of the wild-type *polA*$^+$ gene of *E. coli* in multiple copies was possible only by placing its expression under the inducible control of the bacteriophage lambda p_L promoter (13) or by subcloning the entire *polA*$^+$ gene on plasmids with a moderate copy number (15). The pneumococcal *polA* gene has been cloned in *Streptococcus pneumoniae* (12) by using the multicopy plasmid vector pLS1 (9). In addition, this gene could be subcloned in an *E. coli* expression vector (18) in which the pneumococcal *polA*$^+$ gene was placed under the control of the phage T7 ϕ10 promoter (12). *S. pneumoniae* PolI has been shown to be synthesized in three bacterial hosts, viz., *Bacillus subtilis*, *E. coli*, and *S. pneumoniae* (12), and to complement *polA* mutants of *B. subtilis* (S. Martinez, P. López, M. Espinosa, and S. A. Lacks, submitted for publication). The pneumococcal enzyme is similar to *E. coli* PolI with regard to its polypeptide size and enzymatic activities (4, 5, 12). Furthermore, both enzymes are susceptible to specific proteolytic fragmentation by subtilisin and show homology in their amino acid sequences (López et al., unpublished data). This report describes the subcloning and expression of the pneumococcal *polA*$^+$ gene in *E. coli* under its natural control signals. In addition, functional complementation of *E. coli polA5* mutants by PolI from *S. pneumoniae* is reported.

Cloning of the pneumococcal *polA*$^+$ gene in *E. coli* was performed by ligation (at a ratio of 10:1) of the 4.4-kilobase-pair *Eco*RI fragment of the recombinant plasmid pSM22 (12), which contains the entire gene, with the *Eco*RI-linearized plasmid vector pLG339 (17). This vector carries the pSC101 origin of replication and was chosen because of its ability to replicate in an *E. coli polA5* background. The ligation mixture was used to transform strain C600 (*thr-1 thi-1 leu-6 lacY1 fhuA21 supE44*) by the method of Kushner (6). Transformants were selected in agar medium containing tetracycline (10 μg/ml) and kanamycin (25 μg/ml). The plasmid content was analyzed for 20 clones, and 12 of them contained a plasmid larger than the vector pLG339. The recombinant plasmid, called pSM31, was thus isolated (Fig. 1). Restriction mapping of pSM31 demonstrated the integrity of the pneumococcal insert. Plasmid pSM31 was then transferred to *E. coli* MB1034 (HfrH *thi-1 rel-1* λ$^-$ *polA5*). In this background, pSM31 was structurally and segregationally stable for at least 120 generations in the absence of selective pressure.

Since the pneumococcal *polA* gene is present in pSM31 together with its own regulatory signals, we wanted to determine whether this gene was expressed and whether its

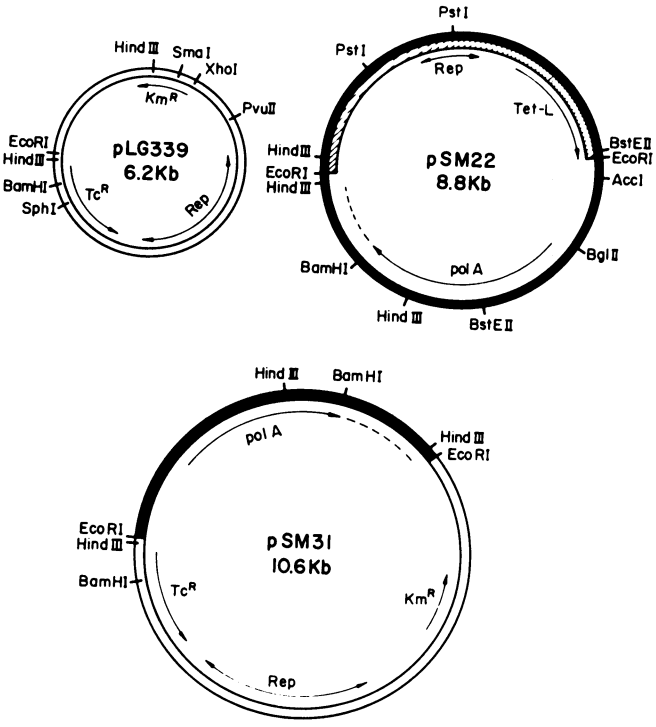

FIG. 1. Physical maps of pLG339, pSM22, and pSM31. →, Transcription of genes; ▭, pLG339 genes; ▬, *S. pneumoniae* chromosomal insert; ▬, pLS1 genes; Kb, kilobase pairs. Only relevant restriction sites are shown.

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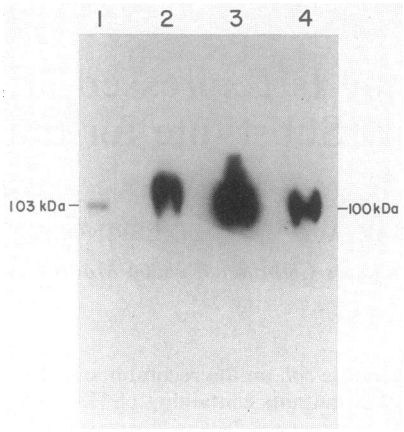

FIG. 2. Gel polymerase assay of extracts from *E. coli* MB1043 *polA5*(pLG339) (lane 1), *E. coli* C600 (lane 2), *E. coli* MB1043 *polA5*(pSM31) (lane 3), and *S. pneumoniae* 641(pSM22) (lane 4). In lane 4 only 5 μg of protein was loaded; 100 μg was loaded in lanes 1 to 3. kDa, Kilodaltons.

product was active in a heterospecific host. To this end, crude extracts of *E. coli* and *S. pneumoniae* were prepared by the passage of a cell slurry through a French pressure cell as described previously (12), and the exonuclease and polymerase activities of PolI in the extracts were determined. Enzymatic activities in the cell extracts were detected in situ after electrophoresis in DNA-containing polyacrylamide gels in the presence of sodium dodecyl sulfate and renaturation of the enzymes (14). Nuclease activities in extracts of *S. pneumoniae* 641 (*end-1 noz-19 exo-2*), a mutant strain that lacks both the major endonuclease and exonuclease (7, 8), harboring pSM22 showed an increase in the PolI exonuclease activity as compared with that of the strain without the plasmid (12; results not shown). The nuclease activity of the native pneumococcal PolI cloned in pSM31 was clearly observed in cell extracts of the *E. coli* MB1043 *polA5* (pSM31) mutant strain at the expected position of 100 kilodaltons; no detectable *S. pneumoniae* PolI nuclease activity could be seen for the *polA5* mutant harboring the vector pLG339 (data not shown). Detection of DNA polymerase in situ was performed as described previously (16). *E. coli* MB1043 *polA5*(pLG339) exhibited a reduced polymerase activity (Fig. 2, lane 1) as compared with that of the *polA*$^+$ strain (lane 2). This reduction resulted from the *polA5* mutation, which gives rise to a protein showing decreased processivity in polymerization (3). The position of the *E. coli* PolI band in the gel corresponds to its molecular mass of 103 kilodaltons (Fig. 2, lanes 1 and 2). *E. coli* MB1043 *polA5*(pSM31) produced active pneumococcal PolI as demonstrated by the high polymerizing activity observed (Fig. 2, lane 3). This activity banded at approximately the same position as the PolI of *S. pneumoniae* 641(pSM22) (Fig. 2, lane 4), which is slightly smaller than the *E. coli* PolI. This difference was more visible in less-exposed autoradiograms (data not shown). From these results, we conclude that the pneumococcal PolI is synthesized as an active product by *E. coli*.

To evaluate the DNA polymerase activities, the aforementioned cell extracts of *E. coli* and *S. pneumoniae* were assayed in vitro for their ability to catalyze the incorporation of labeled [*methyl*-^{3}H]dTTP into nicked salmon sperm DNA (12). The assays were performed in the presence of 250 mM KCl, which enhanced the pneumococcal enzymatic activity, although the conditions were not optimal for the *E. coli* PolI. An 11-fold increase in polymerase activity was obtained for *E. coli* MB1043 *polA5* when this strain harbored pSM31 (Table 1). This increase was due to expression of the pneumococcal PolI in *E. coli*. The level of PolI activity in *E. coli* MB1043 *polA5*(pSM31) was the same as that in *S. pneumoniae* 641 without a plasmid. However, inasmuch as pSM31 has a copy number of approximately 7 (17), the heterospecific expression of the pneumococcal *polA* gene seemed not to be as efficient as the homospecific expression. Furthermore, a single copy of the *E. coli polA*$^+$ gene gives the same level of polymerase activity as several copies of the pneumococcal *polA* gene cloned in pSM31. Since these results are difficult to reconcile with those shown in Fig. 2,

TABLE 1. DNA polymerase activity in cell extracts

Host strain	Plasmid	Polymerase sp act (U/mg of protein)
S. pneumoniae 641 *polA*$^+$	None	138
	pSM22	2,627
E. coli C600 *polA*$^+$	None	222.3
E. coli MB1034 *polA5*	pLG339	16.7
	pSM31	186.7

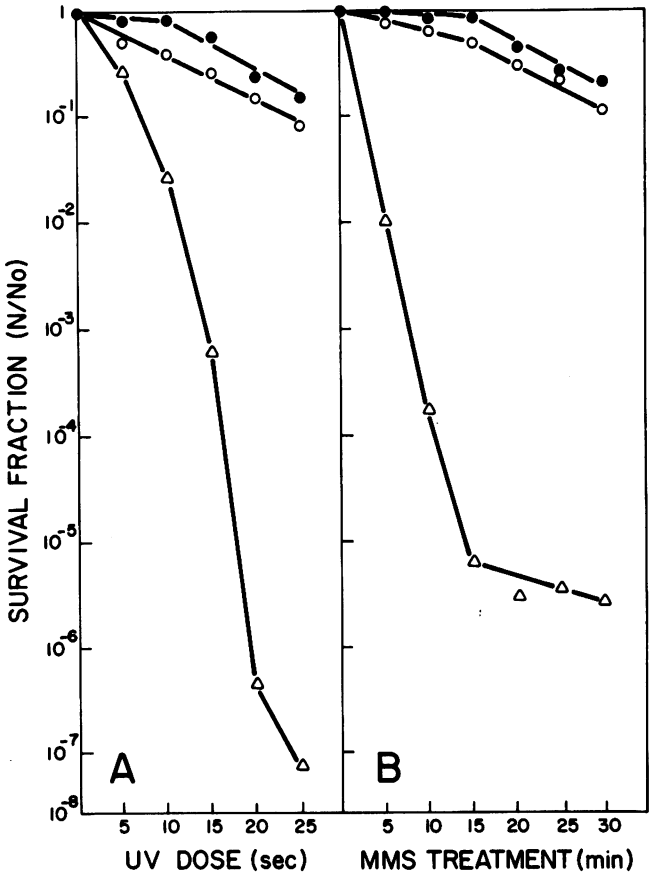

FIG. 3. Effect of pSM31 on resistance to UV light (A) and methyl methanesulfonate (B) in *E. coli* MB1043 *polA5*(pLG339) (△), MB1043 *polA5*(pSM31) (○), and C600 (●).

an alternative explanation could be that competition for substrate between pneumococcal PolI and the low-processing altered *polA5* product may occur in our assays with unfractionated extracts. As a consequence, we may be underestimating the pneumococcal PolI values in the *E. coli polA5* background. Also, it is difficult to quantitate two different enzymes under standard conditions. This may be true because in extracts prepared from *S. pneumoniae*, there was a correlation between gene dosage (1 copy in the chromosome or 24 copies in pSM22) and polymerase activity (Table 1 and reference 12). We conclude that in addition to being stable in *E. coli*, the *S. pneumoniae polA*$^+$ gene is expressed, although maybe not fully, under its own regulatory signals in a gram-negative host.

In vitro assays do not necessarily reflect the in vivo situation. To test the possibility of a functional complementation of the *E. coli* MB1043 *polA5* mutation by the pneumococcal PolI, experiments were performed to measure DNA excision repair. Cultures of *E. coli*, with or without plasmids, were grown to 10^8 CFU/ml in M9 medium (11) supplemented with the appropriate nutrients. The cultures were treated with UV light or with methyl methanesulfonate. For UV light treatment, a germicidal UV lamp emitting predominantly at 254 nm with a flux of 2.7 J/m^2 per s was employed. Cell viability was determined by plating appropriate dilutions. All manipulations were performed in dim light or in the dark to avoid photoreactivation. Treatment of *E. coli* cells with 25 mM methyl methanesulfonate was carried out as described previously (1). The results are shown in Fig. 3. Under our experimental conditions, *E. coli* MB1043 *polA5*(pLG339) was very sensitive to DNA-damaging agents. However, the presence of pSM31 in the *polA5* background enhanced the excision repair ability of strain MB1043 and fully restored the *polA*$^+$ phenotype. Evidence for a different in vivo function of the pneumococcal PolI enzyme in *E. coli* was recently reported (9). In that case, the pneumococcal *polA* gene contained in pSM22 allowed replication of that plasmid in both *polA*$^+$ and *polA* mutant strains of *E. coli*, whereas the pLS1 replicon present in pSM22 could only be established in *polA*$^+$ strains. Although we have not observed homology between the *E. coli* and *S. pneumoniae polA* genes at the level of DNA hybridization (data not shown), our results demonstrate that the pneumococcal PolI complements its *E. coli* counterpart in the processes of DNA excision repair. This complementation is remarkable in the case of a protein that appears to be a component of a multifunctional complex (2). It indicates a similarity at the structural level between the PolI proteins of gram-positive and gram-negative bacteria. It is noteworthy that preliminary results from our laboratory on the DNA sequence of the pneumococcal *polA* gene have shown homology at the amino acid level between PolI from *E. coli* and from *S. pneumoniae*. Complementation between proteins that play a fundamental role in the physiology of procaryotes, such as RecA and PolI, may be a general feature, although complementation for DNA repair is partial for RecA (10) and complete for PolI (this work).

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LITERATURE CITED

1. **Hase, T., and Y. Masamune.** 1981. Mutants of *Shigella* sonnei deficient in DNA polymerase I. J. Biochem. **90**:149–155.
2. **Husain, I., B. Van Houten, D. C. Thomas, M. Abdel-Monem, and A. Sancar.** 1985. Effect of DNA polymerase I and DNA helicase II on the turnover rate of UvrABC excision nuclease. Proc. Natl. Acad. Sci. USA **82**:6774–6778.
3. **Joyce, C. M., D. M. Fujii, H. S. Laks, C. M. Hughes, and N. D. F. Grindley.** 1985. Genetic mapping and DNA sequence analysis of mutations in the *polA* gene of *Escherichia coli*. J. Mol. Biol. **186**:283–293.
4. **Joyce, C. M., W. S. Kelley, and N. D. F. Grindley.** 1982. Nucleotide sequence of the *Escherichia coli polA* gene and primary structure of DNA polymerase I. J. Biol. Chem. **257**: 1958–1964.
5. **Kornberg, A.** 1980. DNA replication. W. H. Freeman and Co., San Francisco.
6. **Kushner, S. R.** 1978. An improved method for transformation of *Escherichia coli* with ColEI derived plasmid, p. 17–23. *In* H. W. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier, Amsterdam.
7. **Lacks, S. A.** 1970. Mutants of *Diplococcus pneumoniae* that lack deoxyribonucleases and other activities possibly pertinent to genetic transformation. J. Bacteriol. **101**:373–383.
8. **Lacks, S. A., B. Greenberg, and M. Neuberger.** 1975. Identification of a deoxyribonuclease implicated in genetic transformation of *Diplococcus pneumoniae*. J. Bacteriol. **123**:222–232.
9. **Lacks, S. A., P. López, B. Greenberg, and M. Espinosa.** 1986. Identification and analysis of genes for tetracycline resistance and replication functions in the broad-host-range plasmid pLS1. J. Mol. Biol. **192**:753–765.
10. **Love, P. E., and R. E. Yasbin.** 1986. Induction of the *Bacillus subtilis* SOS-like response by *Escherichia coli* RecA protein. Proc. Natl. Acad. Sci. USA **83**:5204–5208.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Martinez, S., P. López, M. Espinosa, and S. A. Lacks.** 1986. Cloning of a gene encoding a DNA polymerase-exonuclease of *Streptococcus pneumoniae*. Gene **44**:79–88.
13. **Minkley, E. G., Jr., A. T. Leney, J. B. Bodner, M. M. Panicker, and W. E. Brown.** 1984. *Escherichia coli* DNA polymerase I. Construction of a plasmid for amplification and an improved purification scheme. J. Biol. Chem. **259**:10386–10392.
14. **Rosenthal, A. L., and S. A. Lacks.** 1977. Nuclease detection in SDS-polyacrylamide gel electrophoresis. Anal. Biochem. **80**:76–90.
15. **Spanos, A., and S. G. Sedgwick.** 1984. Plasmid cloning and expression of the *E. coli polA*$^+$ gene in *S. cerevisiae*. Curr. Genet. **8**:333–340.
16. **Spanos, A., S. G. Sedgwick, G. I. Yarranton, U. Hübscher, and G. R. Banks.** 1981. Detection of the catalytic activities of DNA polymerases and their associated exonucleases following SDS-polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:1825–1839.
17. **Stoker, N. G., N. F. Fairweather, and B. G. Spratt.** 1982. Versatile low-copy-number plasmid vectors for cloning in *Escherichia coli*. Gene **18**:335–341.
18. **Studier, F. W., and B. A. Moffatt.** 1986. Use of T7 RNA polymerase to direct selective, high-level expression of cloned genes. J. Mol. Biol. **189**:113–130.