JOURNAL OF BACTERIOLOGY, Oct. 1973, p. 54-58
Vol. 116, No. 1
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Isolation and Characterization of a Mutant of *Salmonella typhimurium* Deficient in a Major Deoxyribonucleic Acid Polymerase Activity

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Received for publication 21 June 1973

A mutant of *Salmonella typhimurium* strain LT2 that is deficient in a major deoxyribonucleic acid (DNA) polymerase activity has been isolated and characterized. This mutant resembles the *pol* mutants of *E. coli* in that it has low DNA polymerase activity and it is sensitive to methyl methane sulfonate as well as ultraviolet irradiation. Revertants selected for methyl methane sulfonate resistance are no longer sensitive to ultraviolet irradiation and contain normal DNA polymerase levels. No direct role in replication can be ascribed to this polymerase activity since cells grow well in its absence. In addition, the LT2 plasmid has been shown to exist in the mutant strain.

De Lucia and Cairns (4) have isolated a mutant of *Escherichia coli*, *polA1*, which appears to lack deoxyribonucleic acid (DNA) polymerase I (9). Additionally, this mutant shows increased sensitivity to methyl methane sulfonate (MMS), ultraviolet (UV) irradiation, and X rays. Recombination is not significantly altered (6). The *polA1* mutation is a single, recessive amber mutation located between the *metE* and *rha* genes on the *E. coli* chromosome (6). The *polA1* mutation defines a gene, *polA*, which is the structural gene for DNA polymerase I (8).

In spite of the fact that the *polA1* mutant lacks measurable DNA polymerase I, the mutant multiplies at the same rate as the parent strain (4). This observation suggests that this enzyme is not an obligatory part of the DNA replication apparatus of *E. coli* (7). However, since the *polA1* mutant is more sensitive to MMS and to UV irradiation, DNA polymerase I may be important in the repair of damaged DNA (7).

Analogous mutants have been isolated in *Bacillus subtilis* (7, 11). In this paper, we report the isolation and characterization of mutants of *Salmonella typhimurium* that have greatly reduced levels of a major DNA polymerase activity. Yamamoto (personal communication) has also isolated similar mutants in *S. typhimurium*. We also show that a plasmid that is present in LT2 strains of *S. typhimurium* (5, 18) is maintained in the polymerase-deficient strain.

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MATERIALS AND METHODS

Media. Media for growth of bacterial and bacteriophage strains were prepared and supplemented when appropriate as described by Roth (15). MMS plates contained MMS at a final concentration of 300 μliters per liter of nutrient agar (13).

Bacteriophage. The following mutants of bacteriophage P22 were provided by John Roth: 7 UGA, 8 UGA, 200 UAG, and 202 UAG, which are unable to grow on a host not containing the appropriate nonsense suppressor. Myron Levine supplied the *erf* (essential recombination function) mutant of bacteriophage P22. A non-lysogenizing mutant of P22, phage L-11 (17), was used for preparing transducing lysates. The preparation of transducing lysates, transductions, and phage sensitivity tests have been described (15).

Bacterial strains. Strains of *S. typhimurium* LT2 and *E. coli* K-12 employed in this study are listed in Table 1.

Mutants of *S. typhimurium* LT2 strain AA2997 (*metE338 ara-9*), sensitive to MMS, were isolated by mutagenesis with diethyl sulfate (Aldrich Chemical Co.) as outlined by Roth (15). Two MMS-sensitive mutants (AA2998, *pol-1 metE338 ara-9*, and AA2999, *pol-2 metE338 ara-9*) were identified by replica plating survivors to MMS plates and nutrient agar plates.

To place the *pol*$^-$ mutation in a non-mutagenized genetic background, we prepared isogenic strains containing the *pol*$^-$ (AA3007, *pol-2 ara-9*) and *pol*$^+$ (AA3008, *pol*$^+$ *ara-9*) alleles. Bacteriophage grown on a spontaneous *metE*$^+$ revertant of AA2999 (*pol-2 metE338 ara-9*), AA3004 (*pol-2 metE*$^+$ *ara-9*), were used to transduce strain AA2997 (*metE338 ara-9*) to

TABLE 1. *Salmonella typhimurium and Escherichia coli strains*

Strain no.	Genotype[a]	Source
S. typhimurium strains		
LT2	Wild-type	B. N. Ames
SA534[b]	*serA13 rfa-3058*	K. E. Sanderson
TR35	*F'80his/ΔhisDCBHAFIE712 arg-501 ser-821*[c]	J. R. Roth
TR149[d]	*aroD5 hisT1529 hisG200*	J. R. Roth
TR226[d]	*aroD5 hisT1529 hisG200 sup-584*	J. R. Roth
TR248[e]	*cysA1348 hisC527*	J. R. Roth
TR251[e]	*cysA1348 hisC527 sup-501*	J. R. Roth
AA2997	*metE338 ara-9*	J. R. Roth
AA2998	*pol-1 metE338 ara-9*	See Materials and Methods
AA2999	*pol-2 metE338 ara-9*	See Materials and Methods
AA3004	*pol-2 ara-9*	See Materials and Methods
AA3007	*pol-2 ara-9*	See Materials and Methods
AA3008	*ara-9*	See Materials and Methods
AA3014	*metE338 ΔhisCBHAFIE,gnd2607 ara-9*	See Materials and Methods
AA3018	*pol-2 ΔhisCBHAFIE,gnd2607 ara-9*	See Materials and Methods
AA3019	*ΔhisCBHAFIE,gnd2607 ara-9*	See Materials and Methods
E. coli strains		
JG112	*polA1 thyA rha lacY14 str*^{r}	J. D. Gross
JG113	*thyA rha lacY14 str*^{r}	J. D. Gross

[a] Genetic symbols used for *S. typhimurium* strains are those used by Sanderson (16); genetic symbols used for *E. coli* strains are those used by Taylor and Trotter (20).
[b] Strain SA534 contains HfrK4; the order of gene transfer is *O-pryB-thr-trp---purA*.
[c] The symbol Δ indicates a multisite deletion mutation.
[d] *hisG200* is a UGA nonsense mutation.
[e] *hisC527* is a UAG nonsense mutation.

methionine prototrophy. One MMS-sensitive clone (AA3007, *pol-2 ara-9*) and one MMS-resistant clone (AA3008, *pol*$^+$ *ara-9*) were saved for further study. Similarly, isogenic strains containing the *pol*$^-$ (AA3018, *pol-2 ΔhisCBHAFIE,gnd2607 ara-9*) or *pol*$^+$ (AA3019, *pol*$^+$ *ΔhisCBHAFIE,gnd2607 ara-9*) alleles and a *his-gnd* deletion mutation were prepared by transducing strain AA3014 (*metE338 ΔhisCBHAFIE,gnd2607 ara-9*) to methionine prototrophy.

Spontaneous MMS-resistant revertants of strain AA3007 were purified by successively streaking for single colonies on MMS plates, then nutrient agar plates, and finally on MMS plates.

UV irradiation. Cells were irradiated as described by Campbell et al. (2). The dose rate at 50 cm was 2 ergs per mm^2 per s.

Assay of DNA polymerase activity. Cells growing in nutrient broth were harvested in the exponential phase of growth and sonically disrupted by a previously outlined procedure (21). The extract was centrifuged for 15 min at 15,900 $\times$ *g* and 4 C. DNA polymerase assays were performed as previously described (21) except the reaction mixture contained 12.8 mM $MgCl_2$ and thymidine 5'-triphosphate-*methyl*-^3H (^{3}H-dTTP, 17.5 Ci/mmol; Schwarz/Mann) at 1.45 $\times$ 10^7 counts per min per µmol. Protein was measured by the method of Lowry et al. (12) using bovine serum albumin as a standard.

Ultracentrifugation studies. CsCl (technical grade, Kawecki Berylco Industries, Inc.)-ethidium bromide density equilibrium centrifugation studies were performed with cleared lysates as detailed by Clewell (3).

RESULTS

Isolation and preliminary characterization of MMS-sensitive mutants. Two MMS-sensitive mutants were isolated from a mutagenized stock of strain AA2997. Both strains, AA2998 and AA2999, have very low levels of DNA polymerase activity (Table 2). For comparison, the DNA polymerase activity found in isogenic *E. coli polA1* and *pol*$^+$ strains are also given in Table 2. Preliminary tests showed that strains AA2998 and AA2999 were more sensitive to UV irradiation than was the parent strain, AA2997. This combination of MMS sensitivity, UV sensitivity, and reduced DNA polymerase activity exhibited by both strains AA2998 and AA2999 is also exhibited by the *pol* mutants of *E. coli* (4, 6). These results strongly suggested that these strains were of the *pol*$^-$ type; hence the mutations in strains AA2998 and AA2999 were designated *pol-1* and *pol-2*, respectively.

Further characterization of the pol-2 mutation. For further study, the *pol-2* mutation

TABLE 2. *DNA polymerase assays of extracts of pol⁺ and pol⁻ strains*

Strain	Pertinent genotype	Net incorporation of ³H-dTTP into acid-precipitable material[a] (counts/min)	³H-dTTP incorporated per ml of extract (nmol)	Spec act[b] (U/mg)
S. typhimurium				
AA2997	*pol⁺*	1,685	29.0	2.44
AA2998	*pol-1*	0	0	0
AA2999	*pol-2*	35	0.6	0.04
AA3007	*pol-2*	36	0.6	0.05
AA3008	*pol⁺*	1,675	28.8	2.48
E. coli				
JG113	*pol⁺*	2,524	43.4	3.12
JG112	*polA1*	2	<0.1	<0.01

[a] A blank consisting of dilution buffer in place of cell extract generally incorporated less than 60 counts/min.

[b] Expressed as units per milligram of protein. One unit of DNA polymerase activity is defined as the incorporation of 1 nmol of ³H-dTTP into acid-precipitable material in 5 min at 37 C.

was transduced out of its original background, and the isogenic strains, AA3007 and AA3008, were prepared as outlined in Materials and Methods. The *pol-2* mutation was approximately 0.1% (1/1,000; 1/500) jointly transduced with *metE*. The *pol-2* mutation has no obvious effect on cell growth since the generation times of the isogenic *pol⁺* and *pol⁻* strains were identical in nutrient broth (34 min) and minimal medium (46 min). The data presented in Table 2 show that the *pol-2* mutation in the unmutagenized background (strain AA3007) retains its low DNA polymerase activity, while the polymerase activity of the isogenic *pol⁺* strain (AA3008) is comparable to that of the original parent (AA2997). When crude extracts of strains AA3007 and AA2997 were mixed, the DNA polymerase activity in the mixed extract was approximately the sum of the DNA polymerase activity in the separate extracts, suggesting that the low DNA polymerase activity in strain AA3007 extracts is not due to a diffusible inhibitor.

UV sensitivity. Figure 1 presents the UV irradiation survival curves of strains AA3007 and AA3008. The AA3007 strain is more sensitive to UV irradiation than is strain AA3008. The survival curve also indicates that strain AA3007 is more sensitive to low UV doses than to higher doses. This difference is not due to a small population of UV-resistant cells in cultures of strain AA3007. When three colonies of strain AA3007 surviving the highest dose of irradiation were picked and retested for UV sensitivity, they gave survival curves that were identical to that shown for strain AA3007.

MMS-resistant revertants of strain AA3007. To demonstrate that the MMS sensitivity, UV sensitivity, and low DNA polymerase levels of strain AA3007 are caused by a single mutation, a total of 11 spontaneous MMS-resistant revertants of strain AA3007 were selected at 25 or 37 C. All of the revertants were sensitive to phage P22, and all except strain AA3035 retained the *ara-9* marker. These revertants selected for resistance to MMS si-

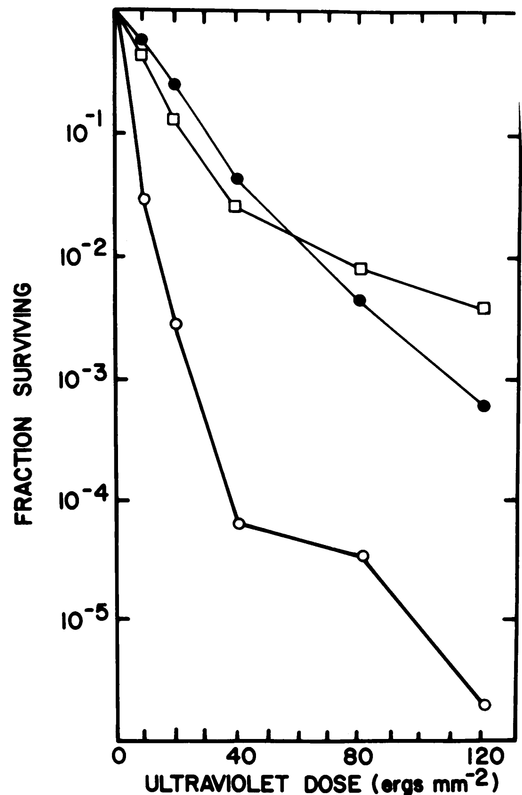

FIG. 1. *UV irradiation survival curves. Bacteria were harvested in the logarithmic phase of growth and resuspended at approximately 10⁸ cells/ml in 0.85% NaCl. Samples of 6.5 ml were placed in a glass petri dish on a gently rotating platform and irradiated with a 15-W germicidal lamp at a distance of 50 cm. Samples were irradiated, diluted, and plated onto nutrient agar plates in subdued light. The plates were incubated at 37 C in the dark and counted after 18 h. Symbols:* ○, *AA3007 (pol-2 ara-9);* ●, *AA3008 (pol⁺ ara-9);* □, *AA3039 (pol⁺ ara-9), a spontaneous MMS-resistant revertant of strain AA3007.*

multaneously regained resistance to UV irradiation and regained the ability to synthesize approximately normal levels of DNA polymerase activity. A typical UV-irradiation survival curve for one of the revertants is presented in Fig. 1. These results strongly suggest that the multiple phenotypes of strain AA3007, MMS sensitivity, UV sensitivity, and low DNA polymerase activity, result from a single mutation. Furthermore, the MMS-resistant revertants are not due to the appearance of nonsense suppressor mutations since all of the revertants were insensitive to two UGA and two UAG mutants of bacteriophage P22. Control experiments showed that bacterial strains carrying the appropriate suppressor mutation were sensitive to the UGA or UAG bacteriophage mutants. Therefore, by this criterion, the revertants do not contain nonsense suppressor mutations.

A temperature-sensitive revertant of strain AA3007. One of the MMS-resistant revertants, strain AA3040, exhibits temperature sensitivity for several in vivo properties of the pol^- mutation. In addition to being temperature sensitive for MMS-resistance (Table 3), AA3040 cells are more sensitive to UV irradiation when irradiated cells are plated at 37 C than when they are plated at 25 C.

Yamagami and Yamamoto (22) and Botstein and Matz (1) have isolated mutants of bacteriophage P22 that fail to grow on recombination deficient (*recA*) hosts. These mutants, designated *erf* mutants, are deficient in a phage-specified general recombination system. Yamamoto (personal communication) has shown that *erf* mutants are unable to grow on *S. typhimurium* pol^- strains. The data presented in Table 3 indicate that at 25 and 37 C the *pol-2* mutant yields plaques with *erf* phage at a low efficiency, while the pol^+ strain and a MMS-resistant revertant (AA3039) yield plaques with *erf* phage more efficiently. However, strain AA3040 is temperature sensitive in its ability to yield plaques with *erf* phage.

When strain AA3040 was grown and assayed at 25 C, very little DNA polymerase activity was detected by our in vitro assay (Table 3), and no activity was detected at 42 C. Therefore, this strain appears to contain a temperature-sensitive *pol* mutation. Monk and Kinross (13) have isolated a similar mutant in *E. coli.*

Recombinational properties of the pol-2 mutant. Isogenic strains containing a *his-gnd* deletion and the *pol-2* mutation (AA3018) or the pol^+ allele (AA3019) were prepared as outlined in Materials and Methods. The DNA polymerase activities in these strains were similar to those of strains AA3007 and AA3008, respectively. To determine whether the *pol-2* mutation alters the ability of the cell to effect genetic recombination, we introduced a wild-type *his* region into strains AA3018 and AA3019 by conjugation and transduction. There was no significant difference between the recombinational abilities of strains AA3018 and AA3019. Both strains were also equally efficient in accepting and maintaining an F′*his* episome.

CsCl-ethidium bromide density equilibrium centrifugation. Recent reports of the existence of a plasmid in LT2 strains (5, 18) prompted us to see if the *pol* gene is required for maintenance of this plasmid. Cleared lysates (3) of strains AA3007 and AA3008 were centrifuged to equilibrium in CsCl-ethidium bromide density gradients. The CsCl-ethidium bromide density gradients of each strain con-

TABLE 3. *In vivo and in vitro characteristics of a temperature-sensitive revertant (AA3040) of Strain AA3007 (pol-2 ara-9)*

Strain	Pertinent genotype or characteristic	Efficiency of plating[a]				Sensitivity to MMS[b]		DNA polymerase spec act[c] at 25 C (U/mg)
		25 C		37 C		25 C	37 C	
		P22	P22 *erf*	P22	P22 *erf*			
AA3008	pol^+	1.0	1.0	1.0	1.0	Resistant	Resistant	0.21
AA3007	*pol-2*	0.7	1×10^{-5}	0.5	$2 \times 1)^{-5}$	Sensitive	Sensitive	0.01
AA3039[d]	pol^+	0.2	0.5	0.2	0.9	Resistant	Resistant	0.14
AA3040[d]	Temperature-sensitive *pol* revertant	1.0	0.3	0.6	$<10^{-6}$	Resistant	Sensitive	0.02

[a] The efficiencies of plating given are relative to the number of plaques formed on strain AA3008 (pol^+ *ara-9*).

[b] Sensitivity or resistance to MMS refers to the absence or presence of growth on MMS plates. All strains grew on nutrient agar plates at 25 and 37 C.

[c] Expressed as units per milligram of protein. One unit of DNA polymerase activity is defined as the incorporation of 1 nmol of ^{3}H-dTTP into acid-precipitable material in 5 min at 25 C.

[d] Strains AA3039 and AA3040 are MMS-resistant revertants of strain AA3007 (*pol-2 ara-9*) isolated at 25 C.

tained a denser satellite band of covalently closed circular DNA (14) in addition to a lighter DNA component corresponding to chromosomal DNA. Therefore, the plasmid in LT2 does not absolutely require the *pol* gene product for its maintenance.

DISCUSSION

The results reported above suggest that the MMS-sensitive mutants isolated from *S. typhimurium* are analogous to the *E. coli polA1* mutant. Recent evidence from complement fixation studies does suggest that *S. typhimurium* contains a DNA polymerase that is closely related to the *E. coli* DNA polymerase I (19).

Although the *polA1* mutation is 17% jointly transduced with *metE* by phage P1 in *E. coli* (6), the *pol-2* mutation described above is approximately 0.1% jointly transduced with *metE* by phage P22. This large discrepancy in frequency of joint transduction is probably based on the fact that the amount of DNA transduced by phage P22 is significantly less than that transduced by phage P1 (16).

Although little is known about the enzymology of DNA polymerase(s) in *S. typhimurium*, the isolation of these *pol*$^-$ mutants should facilitate such studies. We have not established rigorously that the mutants described in this paper are deficient in a DNA polymerase which is equivalent to DNA polymerase I (9) of *E. coli*. Our assay conditions for DNA polymerase do not allow us to identify additional DNA polymerase activities in strain AA3007 that might be due to the equivalent of DNA polymerase II (reviewed by Gross [7]) and III (10) in *E. coli*. The assays for these DNA polymerases differ considerably from the assay for DNA polymerase I used in this work.

ACKNOWLEDGMENTS

We are grateful to N. Yamamoto for communicating his results prior to publication.

This investigation was supported by Public Health Service grant GM-18309 from the National Institute of General Medical Sciences, an Institutional Research Grant (IN-40K) from the American Cancer Society to the University of Michigan, and a grant from the University of Michigan Cancer Research Institute. One of us (H.J.W.) is a recipient of a Public Health Service Research Career Development Award (GM-70565) from the National Institute of General Medical Sciences.

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