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Temperature-Sensitive Mutants for the Replication of Plasmids in *Escherichia coli*: Requirement for Deoxyribonucleic Acid Polymerase I in the Replication of the Plasmid $ColE_1$

DAVID T. KINGSBURY[1] AND DONALD R. HELINSKI

Department of Biology, University of California, San Diego, La Jolla, California 92037

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An *Escherichia coli* mutant (*polA1*), defective in deoxyribonucleic acid (DNA) polymerase I, (EC 2.7.7.7) is unable to maintain colicinogenic factor E1 (ColE1), whereas several sex factor plasmids are maintained normally in this strain. *polA1* mutant strains containing these sex factor plasmids do not exhibit a readily detectable plasmid-induced polymerase activity. A series of *E. coli* mutants that are temperature sensitive for ColE1 maintenance, but able to maintain other plasmids, were isolated and shown to fall into two phenotypic groups. Mutants in one group are defective specifically in ColE1 maintenance at 43 C, but exhibit normal DNA polymerase I activity. Mutations in the second group map in the *polA* gene of *E. coli*, and bacteria carrying these mutations are sensitive to methylmethanesulfonate (MMS). Revertants that were selected either for MMS resistance or the ability to maintain ColE1 were normal for both properties. The DNA polymerase I enzyme of two of these mutants shows a pronounced temperature sensitivity when compared to the wild-type enzyme. An examination of the role of DNA polymerase I in ColE1 maintenance indicates that it is essential for normal replication of the plasmid. In addition, the presence of a functional DNA polymerase I in both the donor and recipient cell is required for the ColV-promoted conjugal transfer of ColE1 and establishment of the plasmid in the recipient cell.

The colicinogenic factor E1 (ColE1) is an extrachromosomal deoxyribonucleic acid (DNA) element normally found in certain strains of *Escherichia coli*. This molecule can be isolated from the cell as either a supercoiled DNA duplex (13) or as a relaxation complex of supercoiled DNA and protein (3). The ColE1 factor has a molecular weight of 4.2×10^6 and exists in multiple copies per cell (1).

It has been demonstrated that the ColE1 molecule, a plasmid that lacks sex factor activity, is not maintained in an *E. coli* mutant that is defective in DNA polymerase I (EC 2.7.7.7) (*polA1*) (5), whereas other *E. coli* plasmids, all of which exhibit sex factor activity, are maintained normally in this mutant (11). The nature of the requirement of DNA polymerase I for ColE1 maintenance was not clear from this previous study. The present work describes the properties of a class of temperature-sensitive mutants of *E. coli* that are unable to maintain specifically the ColE1 plasmid. One group of these mutants exhibits a temperature-sensitive DNA polymerase I. These mutants are defective in the replication and conjugal transfer of the ColE1 factor.

[1] Present address: Department of Medical Microbiology, College of Medicine, University of California, Irvine, Calif. 92664.

MATERIALS AND METHODS

Bacteria. The bacterial strains used throughout this study are described in Table 1. Unless otherwise noted, the source of the strains was our own collection.

Media. The defined media used throughout consisted of M9 salts supplemented with the particular requirements of each strain. M9 salts contained the following in grams per liter: 6.0 g of Na_2HPO_4, 3.0 g of

TABLE 1. *E. coli strains and their characteristics*

Strain	Properties[a]	Parent strain	Source[b]
W3110 *thy*⁻	Thy⁻		1
W3110 *polA1*	Thy⁻, *polA1*	W3110 *thy*⁻	1
JG108	*rha*, Thy⁻, *lacZ*, *metE*, Str-r	W3110 *thy*⁻	1
AB1206	Thi⁻, *his*, Gal⁻, Pro⁻, Lac⁻ Str-r, deletion Ilv-Arg/F'14 (*ilv-arg*)		1
K30	ColEl⁺, ColV⁺		
K94	ColV⁺		
J5-3R1	Pro⁻, Met⁻, R1⁺ (fi⁺, Km, Cm, Sm, Su)		2
J5-3 R64	Pro⁻, Met⁻, R64⁺ (fi⁻, Tc, Sm)		2
JC411	*his*, *argG*, *metB*, *leu*, *strA*, *malA*, *xyl*, *lacY*		
C600/*az*	*thr*, *leu*, Thi⁻, Lac⁻, *azi-r*		
YS40/*az*	*his*, Pro⁻, Str-r, *azi-r*		
YS40/*azE*	same as YS40/*az* except colicin E-r	YS40/*az*	
YS40/*azV*	same as YS40/*az* except colicin V-r	YS40/*az*	
C6000 F'*lac*	*thr*, *leu*, Thi, F'*lac*⁺		
DK100	*his*, *argG*, *metB*, *leu*, *strA*, Thy⁻ *xyl*, *lacY*, ColE1⁺	JC411	
DK404	Thy⁻, *lacZ*, *metE*, Str-r, ColE1⁺	JG108	

[a] Gene symbols are those suggested by Taylor and Trotter (14). Str-r, colicin E-r, colicin V-r, and *azi-r* refer to resistance to these agents.
[b] Sources: 1, Julian Gross; 2, E. Meynell via W. Belser.

KH_2PO_4, 5.0 g of NaCl, 1.0 g of NH_4Cl, 10^{-3} M $MgSO_4$, and 10^{-4} M $CaCl_2$. When amino acids were required the L-isomer of each amino acid was added at 50 μg/ml; thymine was added at a level of 20 μg/ml in agar and 2 μg/ml in liquid. Vitamin-free Casamino Acids (Difco) were used at a concentration of 4 g/liter. Glucose was added as a carbon source at a final concentration of 0.4%.

Enriched media were used for the detection of colicin production, the titration of colicin, the titration of bacteriophage, and the performance of genetic crosses. L medium (12) consisted of 10.0 g of tryptone (Difco) per liter, 5.0 g of yeast extract (Difco) per liter, 5.0 g of NaCl per liter, and 1.0 g of glucose per liter. AB3 medium consisted of 17.5 g of antibiotic medium 3 (Difco) per liter. When solid media were needed, 17.5 g of agar (Difco) per liter was added for hard agar and 6 g/liter for soft agar.

Detection of colicinogenic colonies. Colicin production by single colonies was assayed by the procedure of Fredericq (6). The quantitative determination of transfer frequency of the Col factors utilized the triple overlay technique of Fredericq (6).

Conjugal transfer of plasmids. Transfer of the ColE1 factor, which lacks sex factor activity, was promoted with a ColV factor. Log-phase cultures were prepared by growth of the donor and recipient in L broth to a cell density of approximately 3.5×10^8/ml. The donor and recipient cells were mixed in equal proportions and incubated in static culture for the appropriate time for the particular experiment. Wherever possible, streptomycin or azide counter selection was used to eliminate the donor cells.

The ColV, ColI, R1, R64, and F' factors were generally transferred by overnight growth of the donor and recipient cells together in static culture followed by plating on a selective agar medium.

Bacteriophage transductions. Bacteriophage P1kc transducing lysates were prepared, and the transductions were carried out as described by Lennox (12).

Preparation of cell lysate for DNA isolation and sucrose density centrifugation. Sucrose density gradient analysis was performed on Brij 58 cleared lysates of lysozyme spheroplasts prepared as described earlier (3). Sucrose gradient centrifugation was performed in a Spinco model L2 or L4 ultracentrifuge in either an SW65 or SW50.1 rotor at 50,000 rpm, or 45,000 rpm, respectively. Centrifugation was carried out at 15 C for 150 min through a 5-ml linear 5 to 20% sucrose density gradient containing 0.5 M NaCl and 0.005 M ethylenediaminetetraacetic acid (EDTA), pH 8.0. Fractions of 0.15 ml were collected from the bottom of each tube directly onto Whatman no. 1 filter paper squares or into Triton X100-toluene scintillation fluid in a counting vial as described previously (4).

DNA polymerase assays. Brij 58 lysates were prepared from washed cells that were suspended in 0.05 M tris(hydroxymethyl)aminomethane (pH 8.0) containing 10% sucrose. Lysozyme was added at 100 μg/ml, and the cell suspension was maintained for 5 min at 0 to 1 C. EDTA was then added to a concentration of 0.005 M followed by an additional 5 min of incubation in the cold. Brij-Mg^{2+} (20% Brij, 0.2 M $MgSO_4$) was added to a final concentration of 5% Brij and 0.05 M $MgSO_4$. This mixture was incubated for an additional 30 min at 0 to 1 C. The thick lysate was centrifuged at 19,000 rpm for 20 min in an SS34 rotor in a Sorvall RC2-B centrifuge at 4 C. The supernatant fraction was assayed for DNA polymerase activity.

The polymerase reaction mixture contained 20 μg of sonically treated salmon sperm DNA per ml and 15

nmol each of deoxycytosine, deoxyadenine, and deoxyguanine triphosphate per ml. ³H-thymidine triphosphate (5 Ci/mmol) was added without carrier to a final concentration of 3 to 7 nM/ml. The complete reaction mixture was incubated for a 3-min period. DNA synthesis was assayed by the procedure of Blatti et al. (2) with Whatman DE-81 filter paper circles.

RESULTS

DNA polymerase I activities of polA1 cells carrying bacterial plasmids. Previous work (11) demonstrated that *polA1* cells would support the replication of a wide variety of bacterial plasmids but that the ColE1 plasmid was not maintained in this mutant. Table 2 shows the DNA polymerase I activities of the *polA1* mutant of strain W3110 carrying different plasmid molecules. Clearly only F′14 induced the production of a new polymerase activity in these cells. F′14, however, is known to carry the *polA*⁺ allele and, therefore, would be expected to restore the normal or near normal polymerase I enzyme level. In addition, the presence of these plasmids, with the exception of F′14, did not promote the replication of the ColE1 factor. Either the other extrachromosomal elements do not code for a polymerase I-like activity or the DNA polymerase determined by these plasmids is not in a readily detectable state.

Specificity of the mutants temperature sensitive for the replication of ColE1 and DNA polymerase I. A large number of mutants temperature sensitive for ColE1 replication were isolated after nitrosoguanadine mutagenesis of strain DK100, a thymine-requiring derivative of the *E. coli* strain JC411 (ColE1), and subsequent selection with 5-bromouracil (D. T. Kingsbury and D. R. Helinski, Bacteriol. Proc., p. 55, 1970; Kingsbury and Helinski, manuscript in press). These mutants varied with respect to the effect of the mutation on the maintenance of other bacterial plasmids; however, one class was specific for only the ColE plasmids. The properties of this class of ColE-specific mutants are summarized in Table 3. All of these mutants showed normal or near normal growth rates and chromosomal DNA replication at the restrictive temperature (43 C) and were able to maintain all the extrachromosomal DNA elements tested with the exception of ColE1 and to a lesser extent colicinogenic factor E2 (ColE2).

DNA polymerase activities of ColE1 replication mutants. Since these mutants exhibited a phenotype which resembled that of the *polA1* mutant with regard to plasmid replication, they were examined for their sensitivity to methylmethanesulfonate (MMS) and their DNA polymerase I activities. The mutants fall into two classes with respect to their sensitivity to MMS at the restrictive temperature (Table 4). Those mutants (group A) which show MMS sensitivity also show a DNA polymerase activity that is temperature sensitive. The MMS-resistant isolates (group B) show a normal polymerase activity. Table 4 also shows that two of the mutants which demonstrate a temperature-sensitive polymerase have normal, or greater than normal, polymerase activity and become MMS resistant when they carry the F′14 plasmid. The group B mutants, which possess normal DNA polymerase I activity, have been described in more detail in another report (Kingsbury and Helinski, manuscript in press).

Map position of the mutation in the group A ColE1 replication mutants. The restoration of normal polymerase activity in strains TS214 and TS216 by F′14 (Table 4) indicated that the gene affected in these mutants was at least near

TABLE 2. *DNA polymerase I activity in polA1 cells carrying various bacterial plasmids*

Strain	Polymerase activity[a]	
	30 C	50 C
JG108	100	160
JG108 *polA1*	<2	<2
JG108 *polA1* ColV⁺	<2	<2
JG108 *polA1* F′*lac*⁺	<2	<2
JG108 *polA1* F′14⁺	96	155
JG108 *polA1* R64⁺	<2	<2
JG108 *polA1* R1⁺	<2	<2

[a] Percentage of control polymerase activity after normalizing for protein concentration. The incorporation at 30 C in the wild-type strain JG108 was considered 100%.

TABLE 3. *Properties of temperature-sensitive mutants specifically affected in ColE1 replication*

Property	Mutational group	
	A	B
Growth at the nonpermissive temperature	Normal	Normal
Replication of ColE1	ts[a]	ts
Replication of F′*lac*, ColI, ColV, R1, R64	Normal	Normal
Complementation by F′14	Yes	Yes
DNA polymerase activity and growth in the presence of 0.05% MMS	ts	Normal
Rate of shutoff of ColE1 replication	Immediate	Slow

[a] ts, Temperature sensitive.

TABLE 4. *DNA polymerase I activities of various temperature-sensitive ColE1 replication mutants*

Strain no.	Mutational group	Polymerase activity			MMS[a]	
		30 C	50 C	50 C for 15 min then 30 C	30 C	43 C
DK100		100	100	154[b]	R	R
TS201	B	94[c]	151	100	R	R
TS205	B	94	134	300	R	R
TS213	B	100	110	66	R	R
TS214	A	82	16	94	S	S
TS214 F'14+	A	105	105	150	R	R
TS216	A	100	50	44	R	S
TS216 F'14	A	150	120	160	R	R
TS225	A	105	55	58	R	S
TS232	A	100	85	94	R	S

[a] R denotes the ability to grow in the presence of 0.05% MMS, and S denotes the inability to grow under these conditions.

[b] Percentage of 30 C activity remaining after 15 min of incubation at 50 C followed by assay of enzyme activity at 30 C.

[c] Percentage of control (DK100) polymerase activity after normalizing for protein concentration. The controls at 30 and 50 C were each considered 100%. The difference between the activities at 30 and 50 C was approximately twofold.

the *polA* locus. The presence of F'14 in these strains not only restored normal polymerase activity and a normal level of resistance to MMS, but also restored the ability to replicate the ColE1 factor, consistent with the notion that the same gene affects all three properties. This was examined more carefully by the selection and examination of a number of revertant colonies selected either for the ability to replicate ColE1 at the restrictive temperature or for MMS resistance at 43 C. Seventy-five revertants of TS214 and sixty revertants of TS216 were selected either for MMS resistance at 43 C or for normal ColE1 replication at 43 C. In every one of these revertants, regardless of the phenotype selected, both properties had reverted together.

Every mutant shown in Table 4 to have an altered polymerase activity could be complemented by F'14 both for MMS sensitivity and the ability to replicate ColE1. The tentative mapping of the mutations in the *metB* region of the chromosome was supported also by crosses between various Hfr cells and the TS214 and TS216 mutants. Two types of fine mapping experiments more clearly defined the position of these mutational sites and located the TS214 and TS216 mutations at or very near the *polA1* site. First, bacteriophage transducing lysates were prepared from TS214, TS216, and *polA1* mutant cells. These transducing phage were then used to transduce the *metE* marker into a *polA*+, *metE* strain carrying the ColE1 plasmid (DK404). The rate of co-transduction of sensitivity to MMS with the *metE* marker was very similar for each of the donor strains (Table 5). Furthermore, the ColE1 replication mutation was co-transduced with MMS sensitivity in every case. This is consistent with the ColE1 replication mutation being in the DNA polymerase I gene.

A second transduction experiment involved the determination of the number of MMS-resistant transductants obtained from the infection of *polA1*, TS216, and TS214 cells with transducing lysates prepared from wild-type *polA1* and TS214 cells. The number of recombinant cells obtained from the transducing lysates from the mutant cells was less than 1% of that obtained from the phage prepared from the wild-type cells. The efficiency of transduction for the various crosses was normalized by using the recombination frequency of the unlinked *his* marker determined for each cross.

Growth characteristics and rate of ColE1 segregation in the temperature-sensitive mutants. De Leucia and Cairns (5) reported that their *polA1* mutant. which had only a barely detectable DNA polymerase I activity, grew normally in all media and showed only an increase lag period prior to exponential growth. The growth of strains TS214 and TS216 was examined to determine the effect on cell growth of a shift from the permissive to the nonpermissive temperature. The experiment (Fig. 1) not

TABLE 5. *Transduction of the polA1 and ColE1 replication mutations into pol+ cells*

Donor strain	Transductant colonies[a] with phenotypes:							
	Met+ MMSr ColE1+		Met+ MMSr ColE1-		Met+ MMSs ColE1+		Met+ MMSs ColE1-	
	No.	%	No.	%	No.	%	No.	%
polA1	74	81	0	0	0	0	17	19
TS214	71	78	0	0	0	0	20	22
TS216	101	81	0	0	0	0	24	19

[a] Strain DK404 (*pol+*, *metE*) was treated with a lysate of Plkc grown on W3110 *polA1*, TS214, or TS216, at a multiplicity of infection of 0.1 and plated on minimal media to select *met+* transductants. Each Met+ colony was tested for its ability to produce colicin E1 and its resistance to MMS. Initial selection was done at 34 C, with the subsequent MMS and colicin testing done at 43 C.

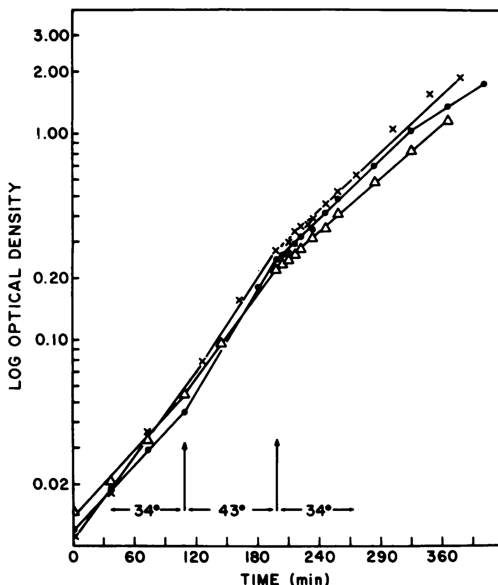

FIG. 1. *Growth of strains DK100, TS214, and TS216 at the permissive and nonpermissive temperatures. Exponentially growing cells were transferred to fresh L broth at 34 C. After two to three generations at 34 C, the cultures were shifted to 43 C for an additional three generations followed by a return to the permissive temperature. Symbols:* ×, *DK100;* ●, *TS214;* Δ, *TS216.*

only shows that there is no change in the growth of the mutants when shifted to the nonpermissive temperature, but that there is no effect of lowering the temperature after several generations at the high temperature. Since the procedure employed for the selection of these mutants demanded cell division at 43 C, these experiments do not test the role of the enzyme in chromosomal DNA replication.

A similar experiment was performed to determine the rate of segregation of the ColE1 factor from the mutant cells. Exponentially growing cells at 34 C were shifted to 43 C and grown for many generations. When the cell density reached that of late log phase, the cultures were diluted into fresh, prewarmed broth and growth continued. At various intervals, samples were plated at 34 C to determine the percentage of the cells which contained the ColE1 factor (Fig. 2). After a lag of about three generations, there is an exponential decrease in the number of ColE1-containing cells. The rate of loss is approximately what would be expected if the mutation had an immediate effect on ColE1 replication and the ColE1 molecules present were being distributed randomly to the daughter cells.

Rate of shut off of ColE1 DNA synthesis. To examine directly the shutoff of ColE1 DNA synthesis after the transfer to the nonpermissive temperature, cultures of TS214, TS216, and control cells were grown at 33 C to a density of approximately 3×10^8/ml in a medium containing M9 salts, Casamino Acids, thymine, and glucose. Each culture was shifted to a 43 C waterbath, and ^{3}H-thymidine was added to a portion for a 20-min period. After one generation of growth at 43 C, the cultures were diluted to 3×10^8/ml with prewarmed medium, and again a portion was removed for incubation in the presence of ^{3}H-thymidine for 20 min. This procedure was repeated after three generations at 43 C. The amount of ColE1 DNA synthesized during these time periods was then determined by analysis of Brij-cleared lysates of the cells on sucrose density gradients. Supercoiled ColE1

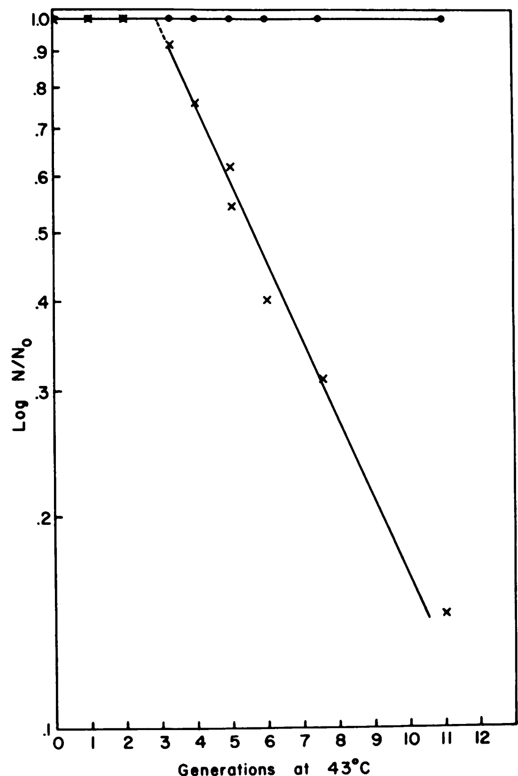

FIG. 2. *Rate of segregation of ColE1 from TS214 grown at 43 C. Broth cultures of strains DK100 and TS214 were grown in L broth in the presence of 100 μg of trypsin per ml for the indicated number of generations at 43 C. At various intervals, cells were plated on AB3 plates at 34 C and tested for the ability to produce colicin.* N/N_0 *refers to the number of Col*$^+$ *colonies divided by the total number of colonies. Symbols:* ●, *DK100;* ×, *TS214.*

DNA synthesis shut off almost immediately in the mutant cells after the transfer from the permissive to the nonpermissive temperature (Fig. 3). In addition, there was no significant accumulation of partially replicated or open circular material that was detectable by this procedure of extraction of the ColE1 DNA. The rate of ColE1 synthesis at 33 C, as determined by ^{3}H-thymidine incorporation, is identical in all three strains.

Properties of DNA polymerase I extracted from TS214, TS216, and control cells. To provide direct evidence that the DNA polymerase itself is temperature sensitive in these ColE1 DNA replication mutants, Brij lysates of strains TS214, TS216, and DK100 were prepared and examined. Portions of each lysate and 1:1 mixtures of the mutant and control lysates were measured into tubes and placed in a waterbath at 50 C. At 1-min intervals the tubes were removed from the 50 C bath and placed in a 30 C waterbath. After a 30-s equilibration, the triphosphate reaction mixture and primer DNA were added, and the incubation was allowed to proceed for 3 min. The reaction was stopped by placing the tubes in an ice waterbath.

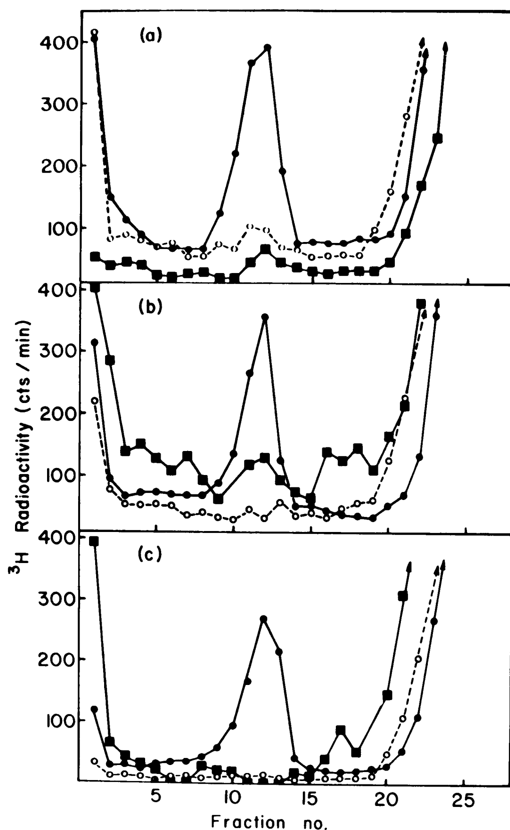

FIG. 3. Sucrose gradient analysis of ColE1 DNA pulse labeled at 43 C after various periods of preincubation at 43 C. In each case, a 10-ml sample of a culture (3 × 10^{8} cells per ml) was labeled for 20 min immediately after being shifted to 43 C (a), after one generation of growth at 43 C (b), and after three generations of growth at 43 C (c). Brij lysates were prepared and centrifuged in sucrose gradients as described in Materials and Methods. The number of generations was determined by the doubling of the optical density at 600 nm, and the cell concentrations for labeling were adjusted by dilution with medium prewarmed to 43 C. Symbols: ●, DK100; ○, TS214; ■, TS216.

The resulting temperature-inactivation profiles are shown in Fig. 4. Under the conditions

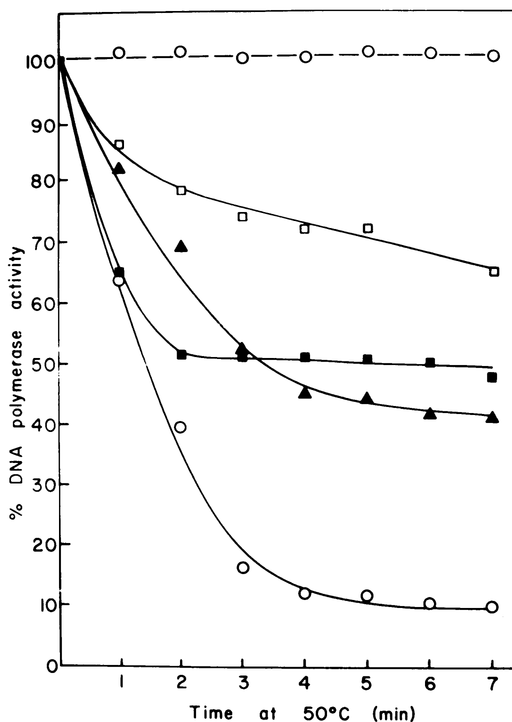

FIG. 4. Thermal stability of mutant DNA polymerases. Brij lysates of control (DK100) cells, TS214, and TS216 were assayed for the stability of the DNA polymerase I at 50 C. Samples (0.3 ml) were incubated at 50 C for various periods and placed in a 30 C water bath for 30 s followed by the addition of the four deoxytriphosphates and primer DNA (final volume 0.35 ml). After 3 min the reactions were spotted on diethylaminoethyl-cellulose disks, washed, and counted as described in Materials and Methods. Symbols: ○---○, strain DK100; ▲, TS214; ○——○, TS216; ■, TS214 mixed 1:1 with DK100; □, TS216 mixed 1:1 with DK100.

used here, the control enzyme preparation showed no significant inactivation over the 15-min period, whereas the polymerase activity in the extract of strain TS214 was 90% inactivated in 3 min. The activity in the strain TS216 extract was 60% inactivated after the 7-min incubation. The results with the mixtures of the temperature-sensitive enzymes and the control preparation supported the conclusion that these mutant cells possessed a thermal-labile DNA polymerase. Similar results were obtained with more highly purified preparations of DNA polymerase I from the TS214 mutant.

The role of DNA replication in the conjugal transfer of the ColE1 plasmid. The role of DNA replication in plasmid transfer was examined by using the TS214 mutant as either the donor or the recipient cell in the transfer of the ColE1 plasmid.

To examine the possible requirement for DNA synthesis in the donor, strains DK100 and TS214 were crossed with strain K94 to produce the doubly colicinogenic DK100 (ColV) and TS214 (ColV) strains. These strains were then examined for donor ability at 33 and 43 C. In addition, strain TS214 was cured by growing the colicinogenic culture at 43 C until non-colicinogenic segregants were produced, and the resulting non-colicinogenic strain, TS214C, was used as a recipient in the conjugal mating.

The examination of donor ability was carried out by growing the donor and recipient (C600 azi-r) strains at 33 C in L broth until the culture reached a cell density of 3×10^8/ml. At this point, portions were removed and donor and recipient cells were mixed (1:1) and incubated for 90 min at 43 C in L broth containing 0.5 mg of trypsin per ml. The remainder of the cultures were incubated at 43 C for one generation, diluted 1:1 with prewarmed broth, and mated under the same conditions. Control cultures held at 33 C were handled identically.

The ability of strain TS214C to act as a recipient in conjugal crosses was examined in a similar manner by utilizing K30 as the donor strain. The results of the two types of transfer experiment are shown in Table 6. In both cases the transfer of ColV, which replicates normally in strain TS214, was similar to the control. However, immediately after the temperature shift, the ability of strain TS214 to donate ColE1 was reduced by 80% while it was unable

TABLE 6. *The ability of strain TS214 to act as a recipient or a donor of ColE1 and ColV in conjugal transfer*

Strain[a]	Role in cross	Transfer temp.	Extra-chromosomal element	Col+/total[b]	Col+[b] (%)	Col+/total[c]	Col+[c] (%)
DK100 (ColV)	Donor	33	ColE1	262/500	52	238/350	68
		43	ColE1	29/140	21	80/189	42
		33	ColV	260/490	53	260/447	58
		43	ColV	38/156	24	104/202	51
TS214 (ColV)	Donor	33	ColE1	120/428	28	151/422	35
		43	ColE1	2/178	1	9/110	9
		33	ColV	132/420	31	221/563	39
		43	ColV	6/178	4	53/116	46
JC411	Recipient	33	ColE1	90/130	69	182/206	88
		43	ColE1	123/164	75	98/128	77
		33	ColV	48/98	49	42/119	36
		43	ColV	73/161	46	42/137	33
TS214C	Recipient	33	ColE1	120/482	28	78/223	35
		43	ColE1	0/82	0	0/92	0
		33	ColV	33/109	33	23/88	26
		43	ColV	46/94	49	40/89	45

[a] C600 *azi-r* was the recipient strain in the crosses with DK100 (ColV) and TS214 (colV) as donors. K30 was the donor strain in the crosses with JC411 and TS214C as recipients.

[b] Zero generations of growth at 43 C prior to mating at 43 C.

[c] One generation of growth at 43 C prior to mating at 43 C.

to receive ColE1 at the nonpermissive temperature. At 33 C the transfer of ColE1 was significantly reduced but still proceeded at a high rate.

DISCUSSION

The requirement for DNA polymerase I in the maintenance of the ColE1 factor has been established (11). The present experiments were designed to examine the role this enzyme plays in the maintenance of other plasmid molecules and to more clearly define the role this polymerase plays in ColE1 replication and conjugal transfer.

The ability of the other plasmids tested to replicate in *polA1* cells suggests that these molecules either do not require DNA polymerase I or code for a sex factor-specific polymerase. The failure to detect any new polymerase activity in *polA1* cells containing a wide variety of plasmids suggests that these molecules replicate via a mechanism more like that of the host chromosomal DNA replication system than like the ColE1 replication system.

The isolation of a series of temperature-sensitive mutants affected in ColE1 replication and DNA polymerase I activity is additional confirmation of the requirement for this enzyme. It was shown that the two characters, ColE1 replication and the temperature-sensitive polymerase, were determined by the same gene by both reversion studies and by mapping. The fine structure mapping of the ColE1 replication gene placed it at or near the *polA* locus. The reciprocal transduction experiments with *polA1* cells strongly suggests the identity of the genes carrying the *polA1*, TS214, and TS216 mutations. It is clear that the DNA polymerase I activities of Brij lysates of these temperature-sensitive mutants demonstrated an in vitro temperature sensitivity, providing additional evidence that the *polA* locus is the structural gene for DNA polymerase I.

The exact role of DNA polymerase I in ColE1 replication cannot be determined from the present experiments. The replication of the ColE1 factor stops immediately after the shift to the nonpermissive temperature. This was shown by both the rate of segregation of the ColE1 factor from the cells grown at 43 C and by pulse-labeling studies after the temperature shift. There are several obvious stages of replication in which polymerase I could play a role. It could be the actual replicating enzyme, serve a required repair function, or be involved in filling a terminal gap prior to the formation of the supercoiled structure of ColE1 DNA normally found in the bacterial cytoplasm. If this third possibility were the case, an accumulation of open circular intermediates might be expected at the nonpermissive temperature. Figure 3, however, demonstrates that there is no significant accumulation of material at the open circular (17*S*) position of the gradient. When very high-specific-activity ^{3}H-thymidine is used to label TS214, immediately upon shifting to the nonpermissive temperature a small peak of 17*S* material is seen after a 20-min period (D. Sherratt, personal communication). The amount of this material, however, is far less than that expected if simple terminal gap filling were the case. It is, of course, possible that this intermediate accumulates but remains at the replicating site and is not extracted by the procedure employed.

A required repair function for the enzyme cannot be ruled out if unrepaired breaks result in an inhibition of replication or if a mechanism of continuous nicking and repair (9) were used. This mechanism appears inconsistent with the finding of Inselburg and Fuke (10) that ColE1 molecules that apparently are in a partially replicated state can be isolated as covalently closed circles.

There is no evidence indicating that polymerase I is not the actual replicating enzyme for the ColE1 molecule. An examination of ColE1 replication in one of the conditional *dnaE* mutants of *E. coli* which is affected in DNA polymerase III and in chromosomal DNA chain elongation (7) has shown that ColE1 replicates semi-conservatively at the nonpermissive temperature for over a 90-min period (8).

The role of DNA replication during the conjugal transfer of the ColE1 factor was examined by using one of the temperature-sensitive polymerase I mutants as either the donor or recipient. These experiments support the view that plasmid replication is required in both the donor and recipient cell if proper conjugal transfer and establishment of the ColE1 plasmid are to occur. TS214, a mutant specific for ColE1 replication, allowed normal transfer of the ColV factor which was used to promote the ColE1 transfer. This suggests that plasmid-specific replication is required in both the donor and recipient at the time of transfer. It also suggests that, when ColE1 is transferred via the ColV factor, the sex factor replication system is not used at the time of transfer.

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