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Conditional Lethality of *recA* and *recB* Derivatives of a Strain of *Escherichia coli* K-12 with a Temperature-Sensitive Deoxyribonucleic Acid Polymerase I

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We have isolated a strain of *Escherichia coli* K-12 carrying a mutation, *polA12*, that results in the synthesis of a temperature-sensitive deoxyribonucleic acid (DNA) polymerase I. The double mutants *polA12 recA56* and *polA12 recB21*, constructed at 30 C, are inviable at 42 C. About 90% of the cells of both double mutants die after 2 hr of incubation at 42 C. Both double mutants filament at 42 C and show a dependence on high cell density for growth at 30 C. In *polA12 recB21* cells at 42 C, DNA and protein synthesis gradually stop in parallel. In *polA12 recA56* cells, DNA synthesis continues for at least 1 hr at 42 C, and there is extensive DNA degradation. The results suggest that the primary lesion in these double mutants is not in DNA replication per se.

Mutants of *Escherichia coli* K-12 defective in their ability to form recombinants fall into three main groups *recA*, *recB*, and *recC* (5, 14, 25, 26). Cells lacking *recA* function show no genetic recombination (19). In addition they are highly sensitive to irradiation (7) and are characterized by spontaneous deoxyribonucleic acid (DNA) degradation which is accentuated by exposure to ultraviolet light (UV) (6). They are not UV-inducible for phage λ (3) and are altered in cell division processes (10, 15). The nature of the *recA* gene product is unknown. The *recB* and *recC* mutant strains show reduced recombination, intermediate UV sensitivity, and little DNA breakdown after irradiation. They are inducible for phage λ (24). Extracts of *recB* or *recC* mutant cells lack an adenosine triphosphate-dependent nuclease (2, 4, 21). The *recB* and *recC* genes may code for subunits of this enzyme.

The *recA* gene product and the *recB recC* nuclease appear to act together in repair of UV damage since the *recA recB* or *recA recC* double mutants show the same UV-sensitivity as a *recA* single mutant (24). However they do not show the DNA breakdown characteristic of a *recA* single mutant. *recA*, *recB*, or *recC* strains show a reduced growth rate and divide off a high proportion of dead cells during growth (5, 13).

De Lucia and Cairns (8) isolated a mutant of *E. coli*, *polA1*, which shows greatly reduced DNA polymerase I activity as well as increased sensitivity to UV, X-rays, and methyl methane sulfonate. Recombination in *polA1* mutants is only slightly reduced (11). In UV repair, DNA polymerase I functions as part of the excision repair pathway which depends also on *uvr* gene products and is independent of the *rec* repair pathway. It has been suggested that DNA polymerase I may "fill in" single-strand gaps in cell DNA (20).

Recently, Gross et al. (12) have shown that a double mutant lacking both the *recA* function and DNA polymerase I is inviable. Attempts to construct a *polA recB* double mutant have also been unsuccessful (J. D. Gross, Current Topics in Microbiology and Immunology, vol. 57; Springer Verlag, *in press*). It appears that there is a function or functions necessary for growth, that can only be carried out if either the *polA* or *rec* gene products are present.

In this paper we report the isolation of a strain with a temperature-sensitive *polA* mutation (*polA12*). The double mutants, *polA12 recA56* and *polA12 recB21*, were constructed

at 30 C. We have shown that both double mutants were unable to grow at 42 C and have examined their behavior at 30 C and upon transfer to 42 C.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1.

Media. Cultures were grown in M9 (glucose-salts) medium prepared as described by Anderson (1). The medium contained 10^{-3} M $MgSO_4$ and was supplemented with 0.5% Difco Casamino Acids (min-cas) or with all 20 amino acids at a final concentration of 20 μg/ml (min-aa). Viable counts were performed on nutrient agar plates (Oxoid no. 2 nutrient broth, 25 g; Davis New Zealand agar, 12.5 g; distilled water to 1 liter).

Nitrosoguanidine mutagenesis. Exponential-phase cells at 5×10^8/ml were incubated with 10 μg of nitrosoguanidine per ml for 30 min at 37 C. The cells were centrifuged, washed twice in buffer, resuspended in nutrient broth, incubated at 37 C with aeration to 2×10^9 cells/ml, and plated for single colonies on nutrient agar.

MMS plates. Methyl methane sulfonate (MMS), at a final concentration of 3 parts in 10^4, was added to molten nutrient agar cooled to 50 C. Plates were dried briefly (15 min, 60 C) and used the same day. To measure relative MMS sensitivities, exponential phase cultures were streaked onto a series of plates containing varied concentrations of MMS.

UV irradiation. A Hanovia bactericidal ultraviolet unit was used for UV irradiation. Dose rates were measured with a Latarjet dosimeter (18). To measure relative UV sensitivities, a series of 0.01-ml volumes of exponential-phase cells (10^8/ml) were spotted on a nutrient agar plate, and the UV dose was progressively increased by moving a shielding cardboard square across the plate.

P1 transduction. Phage P1 was added to cells grown in L broth (Difco tryptone, 10 g; Difco yeast extract, 5 g; NaCl, 10 g; distilled water to 1 liter) supplemented with 0.2% glucose and 0.005 M $CaCl_2$ (multiplicity of infection of about 1). After adsorption (15 min at 37 C), the cells were centrifuged and resuspended in 0.1 M sodium citrate, and 0.1-ml volumes were spread on selective minimal plates containing 0.001 M sodium citrate. P1 phage stocks were prepared by the confluent lysis method of Yanofsky and Lennox (27).

Hfr matings. Equal volumes of exponential-phase Hfr donor (*str-s*) and F^- recipient were mixed and incubated at 30 C for 1 hr. Diluted samples were spread onto selective minimal plates containing streptomycin (100 μg/ml) and incubated at 30 C. For the purposes of testing recombination proficiency and of approximate mapping, recombination tests were performed by cross-streaking exponential-phase donor Hfr and F^- recipient cells directly onto the selective medium.

Labeling experiments. Thymine-*methyl*-^3H (TRK127) and ^{14}C-leucine (CFA273) were obtained from Radiochemical Centre, Amersham, England.

Fully ^{3}H-thymine-labeled cells were prepared by overnight growth in min-cas supplemented with 1 μg of cold thymine per ml, 100 μg of deoxyguanosine per ml, and ^{3}H-thymine at a specific activity of 3 μCi/μg.

DNA synthesis was followed by the incorporation of ^{3}H-thymine in fully labeled cells diluted into fresh labeled medium.

Double labeling. Fully labeled cells were prepared by overnight growth in min-aa supplemented with 1 μg of cold thymine per ml, 100 μg of deoxyguanosine per ml, ^{3}H-thymine at 3 μCi/μg, and ^{14}C-leucine at 0.1 μCi/ml. DNA synthesis and protein synthesis were followed by incorporation of ^{3}H-thymine and ^{14}C-leucine in fully labeled cells diluted into fresh labeled medium.

Sampling procedure. Samples (50 μliter) were removed at various times to Whatman filter papers (3 mm by 2.5 cm) and immediately transferred to chilled 5% trichloroacetic acid. At 30 min after the

TABLE 1. *Escherichia coli strains*

No.	Characters[a]	Derived from	Source or reference
MM300	*ilv⁻ thy⁻ rha⁻ lac⁻ str-r*	W3110	M. Monk
MM736	HfrH prototroph (λ^-) *str-s*		M. Monk
HfrKL14	HfrKL14 prototroph *str-s*		W. Maas
JC5029	HfrKL16 *thr⁻ ilv⁻ spec^r str-s*	KL16	25
JC5088	HfrKL16 *thr⁻ ilv⁻ spec^r str-s recA56*	KL16	25
JC5412	HfrKL16 *thr⁻ ilv⁻ spec^r str-s recB21*	KL16	25
JG196	*F′ metE⁺ pol⁺ rha⁺/metE⁻ rha⁻ recA56*	W3110	J. D. Gross
JG197	*F′ metE⁺ polA1 rha⁺/metE⁻ rha⁻ recA56*	W3110	J. D. Gross
JG108	*metE⁻ rha⁻ lac⁻ thy⁻ str-r*	W3110	J. D. Gross
JG138	*thy⁻ rha⁻ lac⁻ polA1 str-r*	W3110	20
MM450	*recA56 thy⁻ rha⁻ lac⁻ str-r*	W3110	M. Monk
MM383	*polA12 thy⁻ rha⁻ lac⁻ str-r*	W3110	See text
MM384	*thy⁻ rha⁻ lac⁻str-r*	W3110	See text
MM385	*polA12 recA56 rha⁻ lac⁻ str-r*	W3110	See text
MM386	*polA12 rha⁻ lac⁻ str-r*	W3110	See text
MM387	*polA12 recB21 rha⁻ lac⁻ str-r*	W3110	See text

[a] Symbols for genetic markers are as listed by Taylor (23).

last sample was taken, the filters were washed three times with chilled 5% trichloroacetic acid, twice with ethanol, and twice with ether and allowed to dry. The filters were transferred to 5 ml of scintillation fluid (0.3% 2,5-diphenyloxazole, 0.03% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene) and counted in a Packard scintillation counter.

RESULTS

Isolation and genetic characterization of polA12. Cells of strain MM300 (Table 1) were mutagenized with nitrosoguanidine as described above. Colonies formed on nutrient agar at 37 C were replicated onto two nutrient plates containing 3 parts MMS in 10^4 and incubated at 30 and 42 C. Those colonies showing temperature-dependent MMS sensitivity (sensitive at 42 C and not at 30 C, or sensitive at 30 C and not at 42 C) were further tested for recombination ability at the two temperatures. None of the isolates tested was severely affected in recombination in cross streaks with HfrH (MM736) at 42 or 30 C, and presumably therefore none was heat- or cold-sensitive for *recA* function. The isolates were screened for map location of the relevant mutation by selection for MMS-resistant recombinants at 42 or at 30 C in cross streaks on streptomycin nutrient agar containing MMS, with each of two Hfr strains, Hfr KL14 *str-s* (transferring the *pol*$^+$ region early) and HfrKL16 *str-s* (JC5029) (transferring the *rec*$^+$ region early). One of the isolates, *tm6*, yielded a high frequency of MMS-resistant recombinants when mated with Hfr KL14 in this test and was characterized as a *polA* mutation as follows.

The isolate *tm6* (MMS-sensitive at 42 C) was mated with two F′ donors, JG196 and JG197 (Table 1), one carrying F′ *metE*$^+$ *pol*$^+$ *rha*$^+$, the other carrying F′ *metE*$^+$ *polA1 rha*$^+$ (Gross and Peacey, *personal communication*). Both donors transferred *rha*$^+$ to *tm6* with the same efficiency. All the *rha*$^+$ derivatives from mating with JG196 were MMS-resistant at 42 C, whereas only a minority of *rha*$^+$ derivatives from mating with JG197 were MMS-resistant at 42 C. We conclude that the mutation *tm6* fails to complement with *polA1* and is therefore a *polA* mutation. We have designated it *polA12*.

A P1 lysate was grown on the isolate *tm6*, and the *polA12* mutation transduced out of its original background into strain JG108 *metE*$^-$; *metE*$^+$ transductants were selected and scored for MMS sensitivity at 42 C. Fifteen per cent of the *metE*$^+$ transductants inherited *polA12*. This linkage agrees with that previously reported for *polA1* (11). A *metE*$^+$ *polA12* (MM383) and a *metE*$^+$ *pol*$^+$ (MM384) transductant were purified and used in all subsequent experiments.

The *polA12* strain was tested for sensitivity to a range of MMS concentrations and UV doses at 30 and 42 C as described above. It shows the same sensitivity as *polA1* (8) to MMS and UV at 42 C, and it is more resistant than *polA1* to MMS and UV at 30 C. It is, however, more sensitive to both of these treatments than wild-type cells and so is partially Pol$^-$ at 30 C.

Biochemical characterization of polA12. DNA polymerase I activity was assayed at 30 and 42 C in sonic cell extracts of isogenic *polA12*, *polA1*, and *pol*$^+$ strains with the results shown in Fig. 1. The *polA12* strain, like *polA1*, showed no in vitro polymerase I activity at 42 C and very little activity at 30 C. Assays of in vitro activity of *polA12* cells at 25 C showed no increased polymerase activity. It appears that although the mutant enzyme has substantial activity in vivo at low temperatures it is virtually inactive under our in vitro conditions.

Temperature sensitivity of the double mutants polA12 recA and polA12 recB. The *recA56* mutation and the *recB21* mutation were introduced into *thy*$^-$ *polA12 str-r* (MM383) by mating with donors Hfr KL16 *recA56 str-s* (JC5088), and Hfr KL16 *recB21 str-s* (JC5412), respectively. In both crosses, *thy*$^+$ recombinants were selected at 30 C on minimal glucose plates containing streptomycin. After 72 hr of incubation at 30 C, two types of *thy*$^+$ recombinants were visible, a large-colony type and a small-colony type. The small colonies in each cross were shown by replica plating to be temperature-sensitive for growth and were presumed to be *polA12 recA56* and *polA12 recB21* double mutants. Large and small colonies from each cross were picked and purified at 30 C.

The double mutants *polA12 recA56* (MM385) and *polA12 recB21* (MM387) failed to grow at 42 C. In both cases the double mutants were made temperature-resistant by the introduction of F′ *pol*$^+$ (from donor JG196), but not by the introduction of F′ *polA1* (from donor JG197). This demonstrates that the conditional lethality in each case is relieved by the introduction of a gene specifying an active DNA polymerase I. The strains *polA12 recA56* and *polA12 recB21* were more sensitive to UV at 30 C than the corresponding *recA* and *recB* single mutants, respectively.

Growth of the double mutants. The double

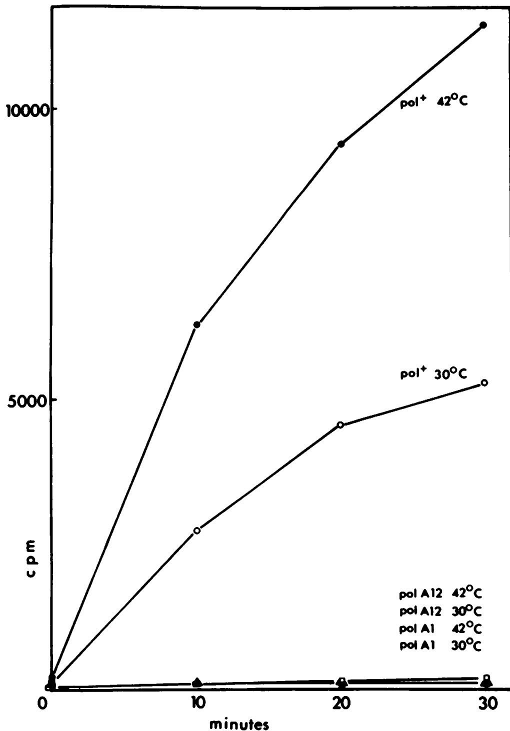

FIG. 1. *DNA polymerase I activity in extracts of polA1 (JG138), polA12 (MM383), and pol+ (MM384) cells. Cells were grown with aeration at 30 C in nutrient broth to about 5 × 10^8/ml. Each culture was then chilled, centrifuged, and suspended in 0.05 M Tris (pH 8) at a concentration of 10^{10} cells/ml. The three suspensions were disrupted by sonic vibration and centrifuged (3,000 × g for 15 min). Reaction mixtures were set up at 4 C as follows: 0.2 ml of supernatant fluid, 0.02 ml of calf thymus DNA (final concentration 20 μg/ml), 0.05 ml of tri-phosphate solution (final concentrations: deoxyguanosine triphosphate, deoxyadenosine triphosphate, and deoxycytosine triphosphate, 2.5 × 10^{-5} M; ^{3}H-thymidine triphosphate, specific activity 125 Ci/mole, 10^{-5} M; and $MgCl_2$, 6 × 10^{-3} M). Two reaction mixtures were set up for each supernatant fraction. One was incubated at 30 C (open symbols), one at 42 C (solid symbols). At intervals, 0.05-ml samples were taken onto paper discs (Whatman, 3 mm by 2.5 cm) and immersed immediately in 5% trichloroacetic acid containing 0.01 M sodium pyrophosphate. The discs were washed five times with acid and twice with ethanol, dried, and counted. (▲, △) polA1; (■, □) polA12; (●, ○) pol+.*

mutants *polA12 recA56* and *polA12 recB21* grow poorly at 30 C. They exhibit heavy growth in the confluent region of the streak at 30 C, but take 3 days to form readily visible single colonies. It appears that isolated single cells of the double mutants have difficulty in forming a colony at 30 C. Colony formation at 30 C is enhanced by plating with 0.1 ml of supernatant fluid of sonically disrupted cells, or with 0.1 ml of chloroform-killed cells. This could be due to the presence in cell extracts of a division-promoting substance (9) as these double mutants show filamentation at 30 C.

Figure 2 shows growth curves (viable counts and optical density measurements) of the two double mutants and a *polA12* single mutant at 30 C and after shift to 42 C. At 30 C both double mutants show an optical density to viable count ratio about 10 times higher than that for wild-type cells. On shift to 42 C, about 90% of the cells of both double mutants die

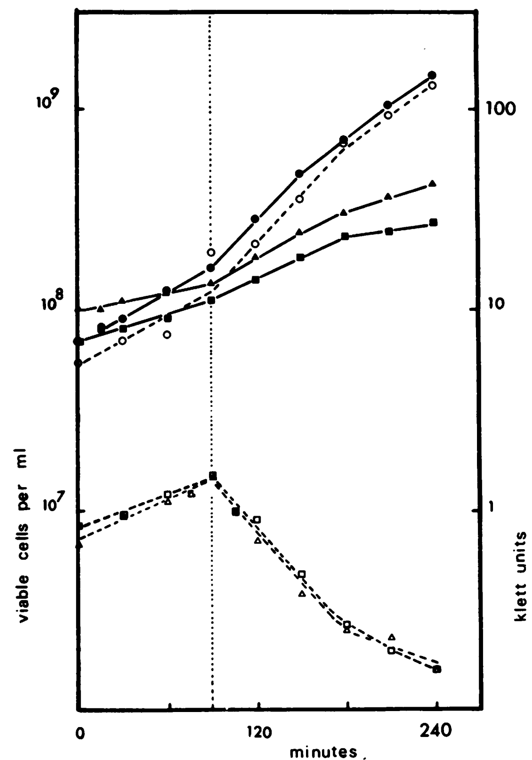

FIG. 2. *Growth curves of polA12 (MM386), polA12 recA56 (MM385), and polA12 recB21 (MM387). Overnight cultures grown at 30 C in mincas were diluted 1 in 2 (polA12 recA56 and polA12 recB21) or 1 in 10 (polA12) in the same medium and incubated with aeration at 30 C. After 90 min the cultures were transferred to 42 C, and incubation with aeration was continued. Optical density (solid curves) was followed in a Klett-Summerson colorimeter. Viable counts (dashed curves) were measured by plating diluted samples in soft agar along with 0.1 ml of chloroformed-killed, 10× concentrated, stationary-phase wild-type (W3110) cells and incubating 2 to 4 days at 30 C. (●, ○) polA12; (■, □) polA12 recA56; (▲, △) polA12 recB21.*

within 2 hr. The optical density of each of the double mutants continues to increase for several hours after the shift to 42 C and then ceases before saturation of the medium.

Microscopic examination of cells of the double mutants grown in liquid culture shows variable morphology at 30 C, and after about 3 hr of incubation at 42 C many of the cells are filaments.

DNA and protein synthesis in the double mutants at 30 and at 42 C. To determine the type of macromolecular synthesis affected at 42 C in the double mutants we examined incorporation of ^{3}H-thymine and ^{14}C-leucine at 30 and 42 C into cells of *polA12*, *polA12 recA56*, and *polA12 recB21* (Fig. 3). In the *polA12 recA56* cells, net DNA synthesis continues for at least 1 hr after the shift to 42 C; this is followed by a loss of counts incorporated into the DNA while protein synthesis continues. The double mutant *polA12 recB21* shows no breakdown and continues to synthesize DNA and protein in a normal ratio; net DNA and protein increase about threefold before synthesis ceases.

DNA breakdown at 30 and 42 C. All experiments of the type shown in Fig. 3 demonstrated a substantial loss of net counts in the DNA of *polA12 recA56* cells after they were incubated for 1 hr at high temperature. To determine whether this breakdown commences at this time or at some earlier time we labeled cells at 30 C, washed the cells free of label, and determined the stability of the incorporated counts upon transfer to 42 C. The results for cells of *polA12*, *polA12 recA56*, *polA12 recB21*, and of an isogenic *recA56* strain (MM450) are shown in Fig. 4. The dashed lines show ^{3}H-thymine incorporation at 30 C and in a portion of the culture transferred to 42 C in continued presence of label. The solid lines show stability of the acid-insoluble counts incorporated at 30 C in samples washed free of radioactivity and incubated at 30 and at 42 C. In cells of *polA12*, and *polA12 recB21* there is little loss of label at either temperature. The *recA56* control cells show 17% DNA breakdown at 30 C and 43% DNA breakdown at 42 C. The reason for the greater DNA degradation at 42 C in *recA* cells is not known, but it has also been observed by D. Korn (*personal communication*). In the double mutant *polA12 recA56*, DNA degradation at 42 C is markedly and consistently increased. There is breakdown of 20% of the DNA at 30 C, and of 75% at 42 C. This DNA degradation in *polA12 recA56* cells starts immediately after the shift to 42 C. We conclude that the net incorporation of counts in the *polA12 recA56* double mutant on transfer to high temperature is the resultant of continued DNA synthesis and of an abnormal rate of DNA breakdown. In the case of the *polA12 recB21* cells, net incorporation at 42 C (dashed curve) appears to be a direct measure of DNA synthesis. It seems that substantial DNA synthesis occurs at 42 C in cells of both double mutants in the apparent absence of both the *pol* and *rec* functions.

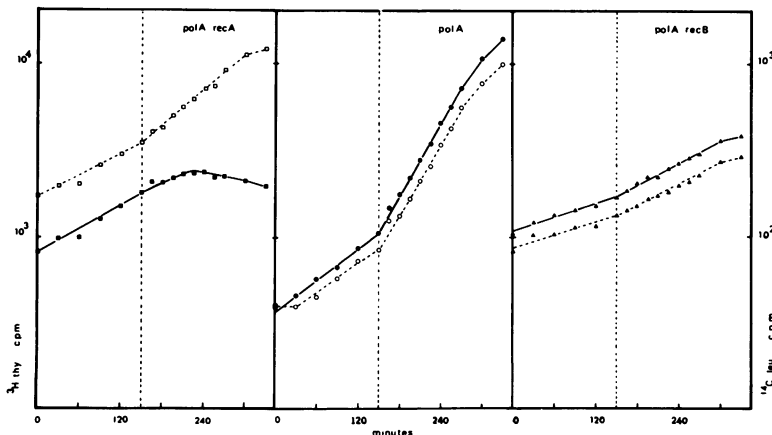

FIG. 3. *Incorporation of ^{3}H-thymine and ^{14}C-leucine during growth at 30 and 42 C. Cell DNA and protein were fully labeled by overnight growth at 30 C in min-aa containing ^{3}H-thymine (specific activity 3 μCi/μg), and ^{14}C-leucine (final concentration 0.1 μCi/ml). Cells were diluted 1 in 2 (polA12 recA56 and polA12 recB21), or 1 in 10 (polA12) in the same labeled medium and incubated with aeration at 30 C. After 150 min the cultures were transferred to 42 C and incubation with aeration continued. Incorporation of ^{3}H-thymine (solid curves) and ^{14}C-leucine (dashed curves) labels were measured as described in text. (●, ○) polA12; (■, □) polA12 recA56; (▲, △) polA12 recB21.*

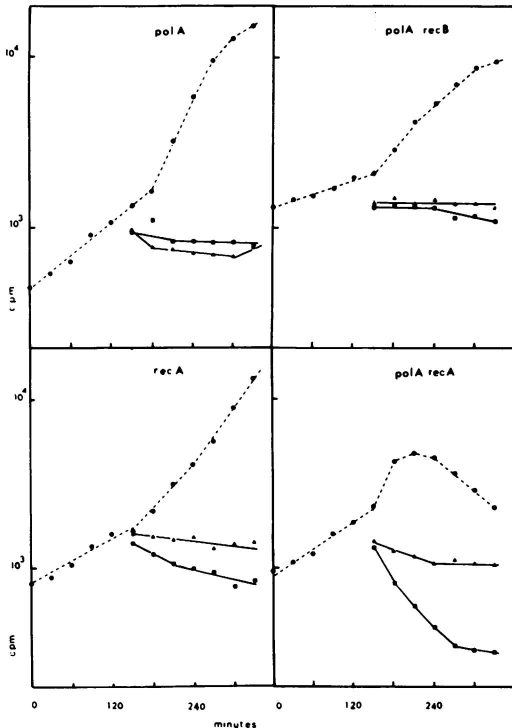

FIG. 4. *DNA breakdown at 30 and at 42 C in cells of recA56, polA12, polA12 recA56, and polA12 recB21. Cell DNA was fully labeled by overnight growth of the cultures at 30 C in min-cas containing ^{3}H-thymine (specific activity 3 μCi/μg). Cells were diluted 1 in 2 (polA12 recA56 and polA12 recB21) or 1 in 10 (polA12 and recA56) in the same labeled medium and incubated with aeration at 30 C. After 120 min a portion of each culture was filtered, washed, resuspended in nonlabeled medium containing 100 μg of cold thymine per ml and reincubated at 30 C. After 30 min, half of each washed culture was shifted to 42 C, and the other half was maintained at 30 C. [The remaining portion of the original cells incubated in the continued presence of label (dashed curves) was also shifted to 42 C at this time.] At intervals, acid-insoluble ^{3}H-thymine counts were measured as described in the text. (●) Incorporation of ^{3}H-thymine at 30 and 42 C; (▲) loss of acid-insoluble counts at 30 C; (■) loss of acid-insoluble counts at 42 C.*

DISCUSSION

Kelley and Whitfield (16) have demonstrated that *polA* is the structural gene for DNA polymerase I. We have isolated a mutation in the *polA* gene, *polA12*, which results in the synthesis of a temperature-sensitive DNA polymerase I. *polA12* cells show the same sensitivity to UV and MMS at 42 C as the classical *polA1* strain but are substantially more resistant than *polA1* at 30 C. *polA12* cells have no in vitro polymerase I activity at 42 C, and very little, if any, activity at 30 C.

recA56 or *recB21* derivatives of the *polA12* strain grow poorly at 30 C, and are inviable at 42 C. There is a 90% decrease in numbers of viable cells of both double mutants after 2 hr of incubation at 42 C. The conditional lethality of these double mutants confirms the findings of Gross et al. (12) that there is a function or functions necessary for growth that can only be carried out if either the *polA* or *rec* gene products are present. The relevant functions of the *polA* and *rec* gene products are not known. They could represent alternative enzyme pathways in the performance of some function vital for (i) the progression of the replication fork, i.e., for DNA synthesis per se; (ii) initiation or termination of rounds of DNA replication, or with DNA segregation; or (iii) repair of discontinuities or gaps in the DNA. Such gaps may occur ahead of the replication fork to allow rotation of the DNA; repair processes would then be required to repair the breaks before replication fragmented the DNA (see J. D. Gross, Current Topics in Microbiology and Immunology, vol. 57; Springer Verlag, *in press*). Alternatively, *rec* and *pol* products could function to join Okazaki fragments behind the replication fork. They could also be required to repair single-strand gaps arising fortuitously during growth.

Our studies with conditional lethal combinations of *polA12* with *recA56* or *recB21* mutations show that in each case substantial DNA synthesis occurs at 42 C in the absence of *pol* and *rec* functions. Therefore, although the product of this synthesis has not been characterized, it is unlikely that these functions play an essential role in the progression of the replication fork itself.

A major finding in this work is that there is no preferential inhibition of DNA synthesis in *polA12 recB21* cells at 42 C. In fact, total DNA and protein increase in parallel before both syntheses cease. We are forced to consider the possibility that *polA* and *recB* gene products are involved in some function equally necessary for DNA and protein synthesis. It is conceivable that an event in the initiation or termination of rounds of DNA replication is necessary to permit sequential transcription of the *E. coli* genome similar to that observed in *Bacillus subtilis* (17). Due to the slow growth rate of *polA12 recB21* cells at the low temperature, and the possible existence of multiple growing forks, we are unable to say whether or not synthesis ceases at 42 C at the ends of rounds of replication. It may be noted that

Sironi et al. (22) have recently shown that infection of *recB*$^-$ cells (but not *recB*$^+$ cells) with phage P2 *old*$^+$ results eventually in the approximately parallel arrest of DNA, ribonucleic acid, and protein synthesis.

In *polA12 recA56* cells the most striking result is the extensive DNA degradation after transfer to 42 C. The absence of this degradation in *polA12 recB21* cells appears to suggest that the primary lesion in the two strains is different. However, it might be argued that the difference is only superficial and results from the fact that DNA breakdown requires the *recB,C*-coded nuclease. It is noteworthy in this connection that the extensive DNA breakdown observed after UV irradiation of *recA* cells is not the cause of their high UV sensitivity since *recA recB* double mutants show the same high UV sensitivity in the absence of DNA degradation. Whether or not the primary lesion in the two strains is of the same nature, it is clear that the *recB* function is, if anything, more important to the normal growth of *E. coli* than the *recA* function, since *recB* single mutants grow less well than *recA* single mutants (Willetts, *personal communication*), and the same is true at 30 C for the corresponding derivatives of *polA12* (Fig. 2, 3, and 4). It is hoped that examination of the single-strand molecular weights of the DNA of the two double mutants after transfer to high temperature will throw some light on the nature of the lesion in the two strains.

The situation in which one gene product can substitute for another in performing an essential function may occur quite generally. This may greatly complicate analysis of the role of specific enzymes in cellular processes.

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