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# Mapping of a Mutation, *polB100*, Affecting Deoxyribonucleic Acid Polymerase II in *Escherichia coli* K-12

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Direct assay for deoxyribonucleic acid polymerase II in extracts has been used to screen recombinants for the *polB* allele in Hfr × F⁻ crosses, F-ductants in episome transfer, and transductants in generalized transduction by phage P1. The *polB* gene is located at 2 min on the *Escherichia coli* linkage map; it is 39 to 64% co-transducible with *leu*. A mutant, *E. coli polA1 polB100 polC* (ts), deficient in deoxyribonucleic acid polymerases I and II and having a thermolabile deoxyribonucleic acid polymerase III, has been prepared by the P1-mediated cross: P1 (HMS85 *polB100*) × *E. coli* BT1026 *polA1 polC* (ts) *leu*⁻.

Three deoxyribonucleic acid (DNA) polymerases, I, II, and III, have been identified and purified from extracts of *Escherichia coli* K-12 (18). Since the purified enzymes catalyze similar reactions, it has not been possible to define the cellular processes for which each enzyme is responsible on the basis of in vitro studies alone. One genetic approach has been to isolate mutants of *E. coli* lacking or having defective DNA polymerases and to study their physiology. *E. coli polA*⁻, *polB*⁻, and *polC*⁻ mutants have been isolated and shown to have defective DNA polymerases I, II, and III, respectively (6, 8, 10). *E. coli polA*⁻ and *polC*⁻ mutants have altered repair and replication phenotypes, respectively (18). However, no altered phenotype has been observed in *polB*⁻ mutants having a defective polymerase II (3, 10). Since the lack of a physiological defect could be due to undetectable residual enzyme activity, it was important to map the lesion to ultimately isolate a strain carrying a deletion of the *polB* locus. Furthermore, detailed mapping data should aid in the construction of strains carrying mutations affecting DNA metabolism in addition to the *polB100* mutation. (In keeping with the convention that every *pol* mutation has a different allele number, the *polB1* mutation described previously [3] has been changed to *polB100*.)

The *polB100* mutant was isolated by assaying for polymerase II activity in extracts prepared from a stock of heavily mutagenized *E. coli* K-12 (3). Since the *polB100* mutation does not lead to an obvious defect in bacterial cell function, it was also necessary to carry out mapping by assaying for polymerase II activity in recombinants obtained from bacterial matings or generalized transduction with phage P1. An independently arising *polB* mutant has been identified and mapped in a similar manner by Hirota et al. (10).

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## MATERIALS AND METHODS

**Strains.** All *E. coli* strains used (Table 1) are K-12 derivatives except strain BT1026, a K-12-C hybrid. Phage P1kc was used for generalized transductions.

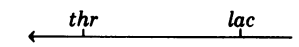
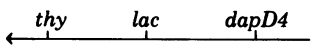
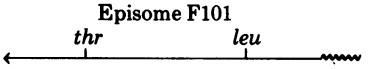
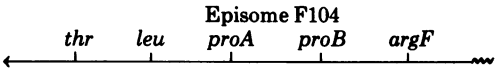
**Media.** Minimal medium was that of Vogel and Bonner (24), supplemented with 0.5% of the desired sugar, and 40 μg of any required amino acids per ml. Thymine was present at 10 μg/ml where necessary. L broth contained 1% tryptone (Difco), 1.0% NaCl, 0.5% yeast extract (Difco), and 0.1% glucose. MacConkey agar was prepared with MacConkey agar base (Difco) and contained 0.1% of the desired sugar. DL-α, ε-Diaminopimelic acid (Sigma Chemical Co.) was present at 40 μg/ml where necessary. Sodium azide was present at 1 mM. Streptomycin plates contained 200 μg of streptomycin per ml.

**Bacterial matings.** All bacterial matings were done by the method of Curtiss et al. (5). Matings were interrupted by dilution and 60 s of agitation on a Vortex mixer.

**Transduction procedures.** Transductions were carried out as described by Lennox (12). Phage P1kc, grown on an appropriate donor, was added to cells (5 × $10^8$ cells/ml) grown, at a multiplicity of infection of 1.0, on L broth containing 2.5 mM $CaCl_2$. After 20 min the cells were collected by centrifugation, suspended in a solution containing 10 mM $MgSO_4$, and plated onto appropriate plates.

**Assay for DNA polymerase II in extracts.** Bacteria were grown and extracts were prepared as previously described (3). Each reaction mixture contained 66 mM potassium phosphate buffer (pH 7.4); 6.6 mM $MgCl_2$; 0.033 mM (each) deoxycytosine, deoxyadeno-

TABLE 1. *Strains used in mapping*

| Strain | Characteristics | Source |
|---|---|---|
| Hfr males: | | |
| Hfr H (Hayes) |   | W. Hayes |
| AT982-Hfr KL16 |   | B. Low (13) |
| F′ strains: | | |
| KLF1/AB2463 |   | B. Low (13) |
| KLF4/AB2463 |   | |
| AB2463 | *ArgE3, his-4, leu-6, thr-1, proA2, recA13, mtl-1, xyl-5, ara-14, galK2 lacY1, tsx-33, str-31,* λ⁻, *sup-37, thi*⁻ | Coli Genetic Stock Center |
| F⁻ strains: | | |
| HMS49 | *E. coli* W3110 which is *thy*⁻ | J. Gross (6) |
| HMS83 | *polA1 polB100;* all females used in mapping were derivatives of this strain | Campbell et al. (3) |
| HMS83-1 | *leu*⁻ *polA1 polB100* | This paper |
| HMS83-2 | *thr*⁻ *polA1 polB100* | This paper |
| HMS83-3 | $(tonB\text{-}trp)_{del}$ his⁻ | This paper |
| HMS85 | *polA*⁺ *polB100* | Campbell et al. (3) |
| Others: | | |
| X178 | Colicins V and B | Gottesman and Beckwith (9) |
| A324 | *pro*⁻ *str azi* | E. P. Kennedy |
| CSH73 | Hfr H $(ara\text{-}leu)_{del}$ | B. Weiss (17) |
| BT1026 | *thy*⁻ *polA1 polC1026 endI*⁻ | F. Bonhoeffer (8) |

sine, deoxyguanine, and [$^3$H]deoxythymidine triphosphates ($1.2 \times 10^8$ counts per min per μmol); 0.25 mM "activated" salmon sperm DNA (1); and 0.05 ml of cell extract, in a final volume of 0.3 ml. The tubes were incubated at 37 C for 30 min, and the amount of acid-insoluble radioactivity was determined as described previously (3). The assay measures polymerases I and II; those specific for polymerase II can, therefore, only be performed with *polA* strains lacking most of normal DNA polymerase I. We have been unable to detect polymerase III by this assay; perhaps it is not active under these conditions or is inactivated by the lysis procedure.

**Chemicals.** [$^3$H]deoxythymidine, deoxyadenosine, deoxycytosine, and deoxyguanine triphosphates were obtained from Schwarz BioResearch. Salmon sperm DNA was purchased from the Sigma Chemical Co.

**Preparation of HMS83 tonB-trp deletions.** A spontaneous *trp*⁻ derivative of strain HMS83 was isolated by selection for a *tonB trp* deletion by the method of Spudich et al. (21). Colicins V and B were prepared from strain X178 (9). Phage φ80v was a gift of J. Beckwith.

**Preparation of strain HMS83 $(tonB\ trp)_{del}$ his⁻ by P2 eduction.** Strain HMS83 $(tonB\ trp)_{del}$ *his*⁻ was prepared by the method of Sunshine and Kelly (22). P2 lysogens of strain HMS83-3 were prepared and grown, and *his*⁻ colonies were identified among cured cells by replica plating.

**Penicillin selection.** Spontaneous auxotrophs were isolated by penicillin enrichment (17).

## RESULTS

Since the *polB100* mutation causes no known defect in bacterial cell functions, mapping was accomplished by assay of recombinants from bacterial matings or by generalized transduction with phage P1 for the DNA polymerase II activity.

**Approximate map position.** The approximate position of gene *polB* on the *E. coli* chromosome was determined by mating the donor strains AT982 (Hfr KL16) and Hfr H with *E. coli* HMS83 *polB100*. First, *E. coli* Hfr KL16 was mated with strain HMS83-3 *polB1 thy*⁻ *his*⁻ *trp*⁻ *lac*⁻. Several colonies of the Thy⁺, His⁺, and Trp⁺ recombinants appearing after various times of mating were purified, grown, and assayed for polymerase II. All remained PolB⁻. Three Lac⁺ recombinants, selected after 2 h of mating (same experiment), were also PolB⁻.

In a second cross between donor AT982 *dapD*⁻ and recipient HMS83, colonies which had gained the ability to ferment lactose were isolated and scored for the requirement of diaminopimelic acid. Extracts prepared from eight Dap⁻ recombinants were assayed for polymerase II activity, and six were found to have regained wild-type levels of the enzyme.

Hfr H was mated for 30 min with strain HMS83 to determine whether *polB* was between the points of origin of Hfr H and Hfr KL16. Lac⁺ recombinants were selected, and 15 out of 44 were *polB*⁺. Since this was the first time point at which Lac⁺ appeared in the mating, we concluded that *polB* was probably between the point of origin of Hfr H and *lac*.

**PolB activity in merodiploids.** To map the *polB* locus more precisely, we constructed partial diploids. A leucine-requiring derivative of strain HMS83 was prepared by penicillin selection. F'101 and F'104 were then introduced into *E. coli* HMS83-1 *polB100 leu*⁻ by bacterial conjugation. The donors were strains AB2463 carrying either F'101 or F'104. F' derivatives of strain HMS83 were selected as Leu⁺. Extracts of 10 Leu⁺ colonies for each F' derivative contained normal polymerase II activity, indicating that the *polB*⁺ allele was present on the episome. Since these derivatives (i) had the ability to act as donors of the *leu*⁺ allele and (ii) were sensitive to the male-specific phage fd, it is probable that they are *polB*⁺/*polB1* merodiploids. Since the cells were not cured of the episome, we cannot be sure that the strain was heterozygous for the *polB* gene.

**Mapping by P1 transduction.** Since F'101 carried the *polB* gene, it was clear that it was located near the threonine-leucine region of the chromosome, around 2 min on the Taylor-Trotter map (23). We therefore determined co-transduction frequencies with markers in this region (Fig. 1). Phage P1kc was grown on the donor HMS49 λ⁻*thy*⁻ *polA*⁺ *leu*⁺ *polB*⁺. The recipients were spontaneously arising threonine- or leucine-requiring derivatives of *E. coli* HMS83 *polB100* prepared by penicillin selection. The results (Table 2) indicate that *polB* is 39% co-transducible with *leu*. Since the co-transduction frequency of *polB* and *thr* is only 6%, the *polB* locus is closer to *leu* and, in view of results presented below, probably is to the right of *leu*.

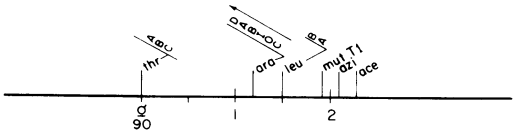



FIG. 1. *Linkage map of the thr-leu region of E. coli K-12. Data are those of Taylor and Trotter (23).*

TABLE 2. *Co-transduction frequencies with phage P1*[a]

| Donor | Recipient | No. with selected marker | | No. with unselected marker *polB*⁺ |
|---|---|---|---|---|
| | | *leu*⁺ | *thr*⁺ | |
| HMS49 *leu*⁺ *polB*⁺ | HMS83-1 *leu*⁻ *polB100* | 100 | | 39 |
| HMS49 *thr*⁺ *polB*⁺ | HMS83-2 *thr*⁻ *polB100* | | 48 | 3 |

[a] Phage P1kc was grown on strain HMS49 (*E. coli* W3110 *thy*⁻), and transductions were carried out as described. Selection plates contained minimal medium supplemented with glucose, 0.5%; lysine, 20 μg/ml; and thymine, 10 μg/ml. Transductants were scored for the *polB* marker by assay of extracts.

**Approximate gene order in the leu region.** A triple cross was performed by P1 transduction. The *leuA* and *leuB* loci are located at about 1.5 min on the *E. coli* map. A convenient nearby marker is *azi*, 0.5 min to the right of *leu*. P1 transduction was used to cross *E. coli* A324 *azi leu*⁺ with strain HMS83-1 *leu*⁻ *azi*⁺. In the first cross, selection was for Azi$^R$. The co-transduction frequency of *azi* and *polB* was 69% as determined by assay of the transductants for polymerase II (Table 3, cross A). In a second cross with the same strains, Leu⁺ colonies were selected and scored as Leu⁺Azi$^S$ or Leu⁺Azi$^R$; these transductants were then assayed separately for polymerase II activity (Table 3B). The *polB* locus co-transducers 69% with *azi* alone. The co-transduction frequency with *leu* is between 40 and 60%, based on results of Tables 2 and 3. If Leu⁺ and Azi$^R$ are inherited together, however, the fraction of Leu⁺Azi$^R$ transductants which have also become PolB⁺ rises to 82%. The transduction frequencies reported in Table 3 indicate that *polB* lies to the right of *leu* close to *azi*. Since a majority of the Leu⁺Azi$^R$ transductants have also inherited the *polB*⁺ allele, it is likely that *polB* lies between *leu* and *azi*. Whether *polB* lies to the right or the left of *azi*, however, cannot be determined unambiguously from the data.

**ara-leu deletions are PolB⁺.** There are a number of strains containing deletions extending from the *ara* operon through *leu*. *ara* is about 0.3 map units to the left of *leu*, between *thr* and *leu*. If the *polB* locus were between *thr* and *leu*, it would have to be very close to *leu*,

TABLE 3. *Ordering of genes in the leu region by transduction*[a]

| Selected marker | Unselected markers | No. of transductants | Transductants with unselected markers (%) |
|---|---|---|---|
| (A) *azi* | *polB*$^+$ | 9 | 69 |
| | *polB*$^-$ | 4 | 31 |
| (B) *leu*$^+$ | *azi polB*$^-$ | 9 | 11 |
| | *azi polB*$^+$ | 41 | 50 |
| | *azi*$^+$ *polB*$^-$ | 21 | 25 |
| | *azi*$^+$ *polB*$^+$ | 11 | 14 |

[a] In these P1 transductions, the donor was A324 *azi leu*$^+$ *polB*$^+$. The recipient was HMS83-1 *polB100 leu*$^-$ *azi*$^+$. (A) Selection was for azide resistance on minimal medium agar plates containing 1 mM $NaN_3$ and 40 μg of leucine per ml. (B) Selection was for *leu*$^+$. The Azi$^R$ and Azi$^S$ colonies were then scored and assayed for the presence or absence of polymerase II.

probably between *ara* and *leu*, in view of the transduction frequencies reported above (29). Thus, extracts of *ara-leu* deletions should be lacking polymerase II activity if *polB* is to the left of *leu*. Polymerase II, therefore, was purified from CSH73 $(ara\text{-}leu)_{del}$. Polymerase II activity was present in normal amounts in extracts of this deletion mutant. Therefore, *polB* is not between *ara* and *leu*. This evidence, though not conclusive, again suggests that *polB* is to the right of *leu*, close to *azi*.

**Preparation of a polA1 polB1 polC (ts) triple mutant.** Since studies on DNA metabolism in *E. coli* would be facilitated by the availability of a mutant having alterations in all three DNA polymerases, we prepared a *polA*$^-$ *polB*$^-$ *polC* (ts) strain We chose the *polC* 1026 mutation in strain *E. coli* BT1026 to combine with *polA1* and *polB100*, since Gefter et al. (8) and Livingston and Richardson (unpublished data) had shown that extracts of this strain contained a temperature-labile DNA polymerase III protein. Plkc was grown on strain HMS85 *polB100 leu*$^+$. Strain BT1026 *leu*$^-$ *polA1 polC* (ts) was then transduced to *leu* by the P1 lysate. Ten colonies were picked and assayed for polymerase II as in the screening assay. Three of the ten colonies contained no detectable polymerase II activity in the extracts. One was purified and is *E. coli* HMS126 *polA1 polB1 polC* (ts). Polymerase III was purified from this strain and shown to be thermolabile. The mutant is viable at 30 C and shows no obvious phenotype, distinguishing it from the parental double mutant *polA1 polC* (ts).

## DISCUSSION

Mutants defective in enzymes of DNA metabolism have been isolated, by assay for enzyme activity, in extracts of mutagenized cells. These mutants include ones defective in DNA polymerase I (6), DNA polymerase II (3), exonuclease I (C. Milcarek and B. Weiss, Fed. Proc. **30**:1156, 1971), and exonuclease III (15). When physiological defects can be attributed to the specific mutation, as in the case of the *polA* mutants (18), the mutants are extremely useful in assigning in vivo roles to the enzyme. However, when no physiological defect can be identified, as in the case of the *polB* mutants (3, 10), one cannot draw any conclusions with regard to the role of the enzyme in vivo.

However, the *polB100* mutant has been very useful for developing a purification procedure for polymerase III, since all other known DNA polymerases are absent (D. Livingston and C. C. Richardson, unpublished data). In addition, the *polA1 polB100* double mutant has been a useful donor strain in complementation assays with in vitro replication systems. Since fractions containing polymerases will always nonspecifically stimulate DNA synthesis, removal of polymerases has simplified interpretation of results. Hurwitz and his co-workers have used this strain to isolate the *dnaB*, *dnaC-D*, and *dnaG* proteins (25) in such assays (26–28). In addition, the same investigators have identified two factors in extracts of HMS83 *polA1 polB100* which alter the specificity of, and stimulate, DNA polymerase III (11).

Finally, we have shown, as have others (10, 16), that if a mutant can be isolated by assay in extracts the mutation can also be mapped. The marker can be scored by the same assay used for initial identification of the mutant even if the mutant shows no physiological defect.

The map position of *polB100* reported here is similar to that reported by Hirota et al. (10). Their *polB* mutation results in a residual polymerase II protein that is thermolabile, and therefore *polB* is probably the structural gene for polymerase II.

The *polB100* mutation lies at 2 min on the *E. coli* map, clearly distinct from *polA1* at 75 min (23). In addition, since *polB* is carried by F'101 and *polC* is not, *polB* must be distinct from *polC*. This indicates that polymerase II is a genetically distinct polymerase and not an altered or defective form of polymerases I or III.

Knowledge of the map position of *polB100* has facilitated the construction of strains carrying *polB100* in addition to other mutations

affecting DNA metabolism. Such multiple mutants are important since polymerase II may be involved in a function in vivo for which an alternative pathway exists. Thus, a defect in polymerase II would become apparent only in the presence of an additional mutation in the alternative pathway. The triple mutant, deficient in polymerases I and II and with thermolabile polymerase III, has been useful to some workers trying to elucidate the specific roles of the various polymerases in DNA repair (2, 14). Studies by Masker et al. (14) indicate that, in the absence of polymerases I and III, polymerase II can carry out repair synthesis in toluene-treated *E. coli* previously treated with ultraviolet irradiation.

When a mutation causing a deficiency in an enzyme extract, but which causes no physiological defect, is mapped in this way, the position may coincide with that of a previously described mutant having a phenotype possibly due to a defect in the enzyme in question. The map position of *polB100* is close to that reported by Cox and Yanofsky for *mutT1* (4). It is known that an altered polymerase can give rise to a mutator phenotype, since mutants in T4 polymerase show various degrees of mutator, antimutator, and neutral phenotype (7, 20). Muzyczka et al. have shown that it is the ratio of polymerase to nuclease activity in the mutants which determines their mutator phenotype (19).

Knowing the map position of the *polB* gene facilitates isolation of additional independent *polB*$^-$ mutations. For example, one could use mutagenesis with nitrosoguanidine and screen *leu*$^-$ auxotrophs which arise, since nitrosoguanidine causes multiple, linked mutations. If polymerase II is not essential, it may be possible to obtain a deletion of the *polB* gene during abnormal excision of phage lambda or Mu from lysogens.

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