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Genetics

Isolation and characterization of a λ*polA* transducing phage

(DNA polymerase I/gene amplification/*in vitro* recombinants)

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ABSTRACT **A plaque-forming λ*polA* phage was isolated from a population of transducing phage made *in vitro* from *Escherichia coli* DNA and a phage vector digested with restriction endonuclease *Hin*dIII. *Amber* mutations, in genes whose products are necessary for late protein synthesis (*Q*) and cell lysis (*S*), were crossed into the λ*polA* phage. Infection of either *polA*$^+$ or *polA*$^-$ bacteria with this phage, under conditions permitting DNA replication but preventing phage production and lysis, elevated the levels of DNA polymerase I to between 75- and 100-fold that detected in a wild-type strain. The kinetics of enzyme production suggest that the *polA* gene is transcribed from its own promoter rather than from any of the well-characterized phage promoters. The fragment of *E. coli* DNA within the λ*polA* phage comprises approximately 5000 base pairs, sufficient to accommodate the *polA* gene and one, or two, coding sequences for smaller proteins.**

DNA polymerase I of *Escherichia coli* is a multifunctional enzyme having both polymerizing and nucleolytic activities. Compelling arguments based on the properties of several genetic variants indicate that polymerase I has an essential role in DNA replication (1). The enzyme comprises a single polypeptide of 109,000 daltons (2) and is present *in vivo* at approximately 400 molecules per cell (3). The means of achieving any regulation of the level of this enzyme are not understood. The structural gene for polymerase I, *polA*, lies midway between *metE* and *rha* (4), and its orientation with respect to these markers has been established (5).

To further the analysis of *polA* and its product we have isolated a plaque-forming lambda transducing phage carrying the *polA* gene. This phage is genetically stable, and we show here that the level of DNA polymerase I produced after infection of *E. coli* may be up to 100 times that detected in an uninfected Pol$^+$ *E. coli* strain. In addition to furthering our understanding of the *polA* gene and its control, this phage should simplify the production of large quantities of normal and variant species of DNA polymerase I.

MATERIALS AND METHODS

The λ/*E. coli* hybrid bacteriophage were isolated by using the Red$^-$ integration-deficient, replacement vector, NM742 (6) detailed in Fig. 3. The fragment of *E. coli* DNA was transferred to an integration-proficient vector, NM540 (7). A P1(EK1) containment level was used. This vector was also used as a helper to mediate integration of integration-deficient transducing phages. An h^{80} att^{80} c1857 *nin*5 *Qam*73 *Sam*7 phage was the donor of the c1857 *nin*5 *Qam*73 *Sam*7 markers. Other phages, λ$^+$, λ*cI*, λ*vir*, λ*b*2, and λ*imm*^{21}*c*I, were standard phages described elsewhere (8). Bacterial strains are listed in Table 1.

Media and general bacterial and bacteriophage plating techniques were as described (7). The *polA* phenotype was tested on L medium supplemented with 0.04% methyl methanesulfonate (MeMes). Preparations of phage lysates for DNA extraction, DNA preparations, restriction endonuclease digestion, ligation reactions, and transfection of *E. coli* cells with recombinant phage DNA were as described in a previous communication (7).

Electrophoresis of DNA in agarose gels (6) and electrophoresis of proteins in sodium dodecyl sulfate($NaDodSO_4$)/polyacrylamide gels (5) has been described. Preparations of restriction endonuclease *Hin*dIII were kindly supplied by E. Southern and K. Kaiser; *Eco*RI was prepared as described by Yoshimori (16); and *E. coli* DNA polymerase I was prepared as described by Kelley and Grindley (17). Other enzymes were from commercial sources as indicated (6).

Transducing phages were detected by their ability to complement mutations in appropriate bacterial hosts. Thus, λ*gln*$^+$ phages were isolated as "Gln$^+$" plaques on the *glnA*$^-$ host, M5000 (13), in the absence of glutamine and λ*chlB*$^+$ phages were sought as "*chlB*$^+$" plaques, or as lysogens, growing anaerobically in a prototrophic *chlB*$^-$ strain, C127 (12), on lactate nitrate medium.

RESULTS

Isolation of a λ*polA* Phage. Populations of recombinants were made by using DNA from *E. coli* strain ED8659, digested with *Hin*dIII, as donor DNA and the phage λ vector NM742. Lambda phage that are Red$^-$ form plaques with low efficiency on *polA*$^-$ *E. coli* strains (18), and we therefore chose to use a Red$^-$ vector phage hoping that the inclusion of the *E. coli* *polA*$^+$ gene would enhance the vector's ability to form plaques. However, this selective technique by itself is not an absolute one since *red*$^-$ phage do form small plaques on a *polA1* host at an appreciable frequency. Furthermore, the efficiency of this plaque formation is increased by the inclusion of various fragments of DNA (ref. 19; N. E. Murray, unpublished results). Pools of recombinants were therefore plated for plaque formation on a *polA1* host merely to enrich for putative λ*polA* recombinants. A lysate was made from these plaque-forming phage and was tested for its ability to transduce a *polA1 E. coli* strain to MeMes resistance in the presence of an integration proficient helper phage.

Many MeMes-resistant colonies were selected, and most were lysogenic. Induction of these apparently Pol$^+$ lysogens yielded lysates containing two types of phage. One comprised integration-proficient, Red$^+$ helper phages able to form plaques on either KB8 (*polA1*) or N3098 (*lig*ts7) bacteria. The other, recognized by a slightly clear plaque morphology, were Red$^-$,

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Abbreviations: MeMes, methyl methanesulfonate; $NaDodSO_4$, sodium dodecyl sulfate.

Table 1. Bacterial strains

Strain	Relevant features	Source	Ref.
C600	*supE, tonA*	F. W. Stahl	9
Ymel	*supF*	A. D. Kaiser	
W3350	*sup°*	J. R. Davison	10
KB8	*endA, polA1, sup°*	J. P. Brockes	8
N3098	*lig*ts7, *supF*	M. M. Gottesman	
W1485	prototroph, *supE*	N. C. Franklin	11
C127	*chlB* derivative of W1485	W. A. Venables	12
M5000	*glnA2, trpA9825*	B. J. Bachmann	13
ED8659	*trpAC9 supE supF hsdR⁻ M⁺S⁺ met⁻ trpR*	W. J. Brammar	14
JG108	*metE⁻ polA⁺ rha⁻ sup°*	J. D. Gross via J. A. Wechsler	15
CM3599	*metE⁻ polA1 rha⁺ sup°*	Phage P1CM transduction of JG108	15

Strains C600, Ymel, and W3350 were used for construction, maintenance, and testing of phage stocks carrying phage *amber* mutations. Other strains were utilized as described in the *text*.

as shown by their failure to form plaques on a *lig*ts7 strain at 37° (18). However, these phages not only formed plaques on a *polA1* host, but transduced such a host to MeMes resistance. These putative λ*polA* phages were chosen for further characterization.

Our original selection used bacteria in which the lesion in the *polA* gene was an *amber* mutation; our transducing phages failed to suppress a series of *amber* mutations in other genes, and were therefore not the carrier of a suppressor gene.

λ*polA* Phage Codes for DNA Polymerase I and Can Be Used to Increase Yields of This Enzyme. Infection of a suppressor-free host by a λ*cI857.trp Qam73 Sam7* phage allows replication of the phage and prolonged expression of an incorporated bacterial gene from its own promoter (20). We have therefore made a λ*polA* derivative including *amber* mutations in genes *Q* and *S* and have monitored the specific activities of DNA polymerase I after infection of both wild-type and *polA⁻* strains of *E. coli* (Fig. 1). In this experiment the maximal levels of polymerase achieved after 200–230 min are approximately 75 times the normal level when the host is wild type and 100 times the normal level when the host is a *polA1* strain. In three separate experiments infections of these two strains indicated a more efficient expression of the *polA* gene in the *polA1* bacteria than in the isogenic *polA⁺* cells. Similar infection experiments using isogenic strains carrying other *polA⁻* alleles resulted in polymerase amplification to intermediate levels while infections of *E. coli* with nontransducing phage produced no significant stimulation of polymerase synthesis.

Further evidence for the production of DNA polymerase I was obtained by analyzing extracts of the infected cells by electrophoresis on $NaDodSO_4$/polyacrylamide gels (Fig. 2). A band migrating at the same rate as an authentic sample of DNA polymerase I (109,000 daltons) was first detected 45 min after infection. Maximal levels of this polypeptide appear to be attained by 210 min. These results confirmed those obtained by assay for polymerizing activity (Fig. 1); DNA polymerase I is made specifically and in large quantity by the λ*polA* phage.

Evidence for Presence of *polA* Promoter. The continued production of DNA polymerase by this *cro⁺ Q⁻* phage is consistent with transcription from a bacterial rather than a phage promoter, since transcription from the early λ promoter would be turned off by the product of the *cro* gene within the first 10 min after infection (24) and transcription from the late promoter is dependent on the *Q* gene product (25). Further evidence for the presence of a bacterial promoter was obtained by examining the characteristics of lysogenic bacterial strains carrying this phage.

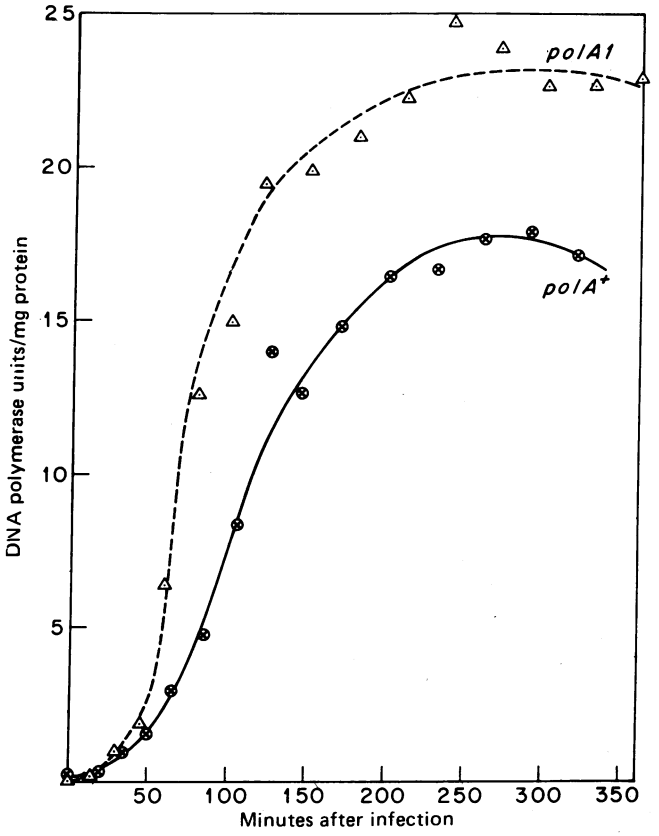

FIG. 1. DNA polymerase I synthesis after infection of wild-type and *polA1 E. coli* cells with phage lambda *polA⁺ c*I857 Q*am* S*am*. The *E. coli* strains JG108 (*polA⁺ sup°*) and CM3599 (*polA1 sup°*) were grown at 37° in L broth to a density of 5×10^8 cells per ml. Each logarithmic phase culture was collected by centrifugation, resuspended in 10 mM $MgCl_2$/0.10 M Tris·HCl, pH 7.6 at one-fiftieth its original volume, and mixed with an equal volume of phage to give a multiplicity of infection of two phage per bacterium. The phage were allowed to adsorb for 10 min at 37° and the infected culture was diluted to twice its original volume with warmed L broth. This culture was then aerated vigorously by shaking at 37°. Samples corresponding to 10^{10} infected cells were removed, pelleted by centrifugation, and frozen at the indicated intervals. After all samples had been collected, each was thawed, suspended in 3 ml of 0.1 M sodium glycinate buffer, pH 9.2 (at 37°), and lysed by ultrasonic disruption. Debris was removed by sedimentation and the cleared supernatant was assayed at various dilutions. Polymerase activity was determined essentially as described (21). Each assay contained 20 μmol of sodium glycinate (pH 9.2), 2 μmol of $MgCl_2$, 0.3 μmol of 2-mercaptoethanol, 10 nmol of each of the four deoxynucleoside triphosphates, dATP, dCTP, dGTP, and dTTP, and 40 nmol of nicked calf thymus DNA nucleotides plus 20 μl of diluted enzyme in a final volume of 0.30 ml. Incorporation of [^{3}H]dTTP was monitored, and activity was expressed in units as described by Setlow (21) based on a specific activity of [^{3}H]dTTP of 25 μCi/μmol. The nicked calf thymus DNA was prepared by the method of Fansler and Loeb (22) in which controlled nicking is carried out to optimize the primer/template capacity of the DNA for purified polymerase. Enzymatic specific activities measured in crude extracts using this DNA are approximately one-tenth lower than those reported by Setlow (21) for less extensively nicked DNA species even at saturating DNA concentrations. Protein concentrations were determined (23), and the data are presented as polymerase units per mg total protein.

MeMes-resistant transductants, isolated after infection of a *polA⁻* host with a λ*polA* phage together with an integration-

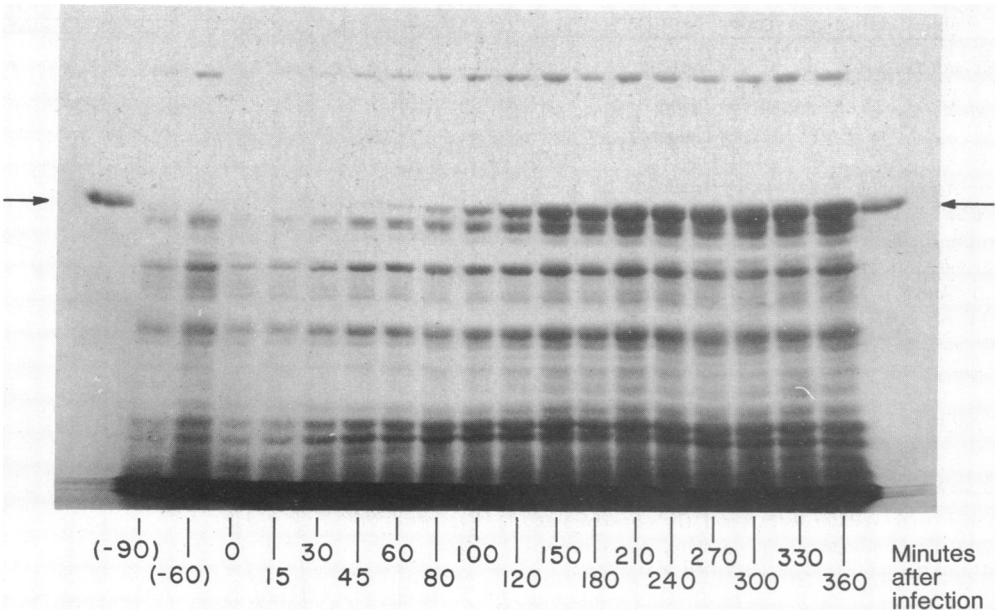

FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoresis of lysates of *polA1 E. coli* infected with phage lambda *polA*$^+$ cI857 Q*am* S*am*. The extracts of CM3599 (*polA1 sup*^{0}) that were assayed to obtain the data displayed in Fig. 1 were further analyzed by NaDodSO$_4$/acrylamide gel electrophoresis. A 7.5% gel was prepared as described (5) and 20-μl samples, corresponding to 20–60 μg of total crude extract protein, were mixed with sample buffer and loaded onto consecutive slots of the gel corresponding to the times of sampling after infection. (Two samples prepared from the logarithmic phase culture at 60 and 90 min before infection were included as controls.) DNA polymerase I standards (109,000 daltons), prepared as described (17), were included in the outer slots of the gel (arrows). After electrophoresis the gel was stained with Coomassie blue R250, destained, and photographed.

proficient helper, were expected to be dilysogens in which the helper and the λ*polA* phages were integrated in tandem within the attachment site of the bacterial chromosome. This interpretation was supported by the finding that superinfection with a heteroimmune phage (λ*b2imm*$^\lambda$) cured the bacteria of the resident prophages and restored the MeMes-sensitive phenotype. As expected, a *polA1* host lysogenized by an integration-proficient λ*polA* phage also became MeMes resistant. In either the tandem dilysogen or the monolysogen integrated at the phage attachment site, expression of the *polA* gene cannot be from a major λ promoter because these are repressed. Although it seems likely that the promoter for the *polA* gene has been included in the λ*polA* phage, the possibility remains that attachment of *polA* to a novel promoter was demanded by the original selection for an MeMes-resistant lysogen.

Bacterial genes within transducing phages may be expressed from phage promoters during lytic growth of the phage (24, 26, 27), and there is no need to invoke the presence of any other promoter. Putative λ*polA* phages, detected only by their ability to form plaques on a *polA1* host, were isolated and dilysogens were made in a *polA1* host by using a heteroimmune (λ$^+$) helper phage. The only selection imposed was for the immunity (*imm*^{21}) of the λ*polA* phage. As before, the lysogens were MeMes resistant. The *polA* gene within our λ*polA* phage is presumably expressed from its own promoter when the λ promoters are repressed. Constitutive transcription from a minor λ promoter is unlikely to evoke the MeMes-resistant phenotype (27).

Physical Analysis of DNA from λ*polA* Phage. DNA from a λ*polA* phage was made and digested with *Hin*dIII and the fragments were separated by electrophoresis on an agarose gel (Fig. 3). The λ*polA* phage (Fig. 3, track 2) includes a fragment of DNA smaller than that containing the *trpE* gene of *E. coli* (11.6% λ$^+$ DNA, ref. 27; Fig. 3, track 3) but slightly larger than the second smallest fragment resulting from digestion of λ$^+$ DNA with *Eco*RI (9.8% λ$^+$ DNA, ref. 28; Fig. 3, tracks 1 and 5). The size of the DNA fragment containing the *polA* gene is thus between 11.6 and 9.8% of the λ genome, or approximately 5000 bases. This fragment does not contain targets for either *Eco*RI or *Bam*HI (data not shown).

Genetic Evidence for Other Closely Linked Genes. The 5000-base fragment of DNA within the λ*polA* phage could code for the polymerase gene, requiring about 3000 bases, and at the most, two other genes. It is not, therefore, surprising that this phage includes neither *chlB* nor *glnA*, two genes that are relatively close to *polA* on the current genetic map (29).

In order to test further for linkage of the genes *chlB* and *glnA* to *polA*, we attempted to construct derivatives of the λ*polA* phage that had acquired these genes. To do this, we isolated a clone of wild type *E. coli* W1485 in which the integration deficient λ*polA* phage had formed a stable lysogen. Integration of the transducing phage into the bacterial chromosome at the *polA* locus was presumed to be via generalized recombination as the result of DNA sequence homology. Subsequent induction of the phage should result in the formation of further transducing phage derivatives that should contain DNA from the genes adjacent to *polA*. By this method λ*glnA* phages were selected, but no λ*chlB* transducing phage was detected and the λ*glnA polA* phages did not transduce C127, a *chlB*$^-$ strain to ChlB$^+$.

λ*glnA* phages were also selected directly from the pooled lysates of λ *E. coli* recombinants. A lysogen of such a λ*gln* phage was made; from an induced lysate a λ*glnA polA* phage was isolated, but again, no λ*chlB* phage was detected. These data cast doubt upon the present tentative map order *metE polA chlB glnA rha* (29). Either *chlB* is more remote from *polA* than suggested or transducing phages, including *chlB* or a closely linked marker, cannot readily be propagated.

DISCUSSION

We have isolated a stable, plaque-forming derivative of bacteriophage lambda carrying the *E. coli polA*$^+$ gene. As cur-

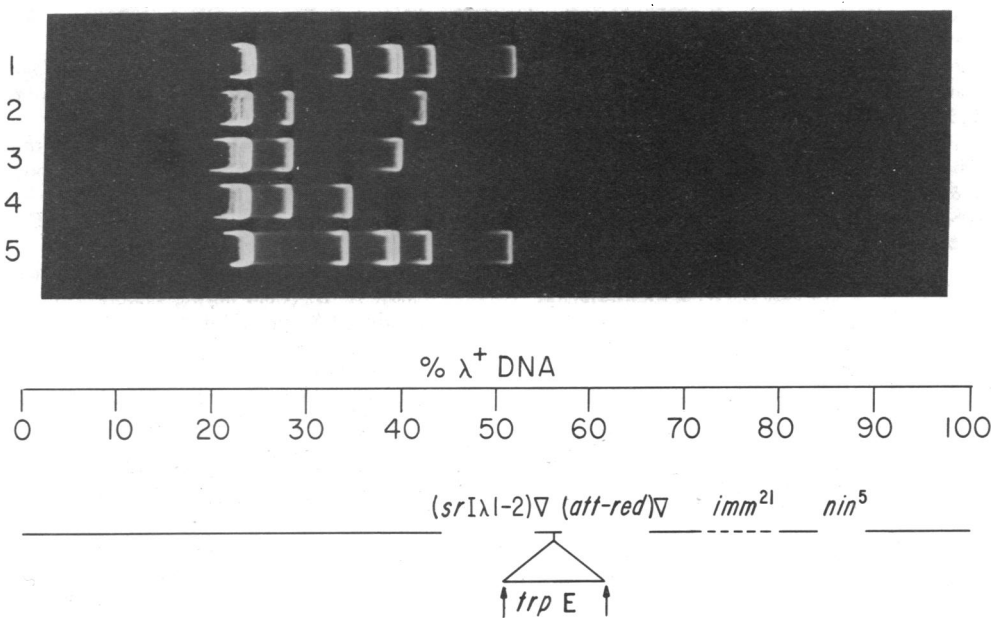

FIG. 3. Electrophoretic analysis of restriction enzyme digests of DNA from λ*polA* phage. Tracks 1 and 5 are λ^+ DNA digested by *Eco*RI. The fifth fragment is estimated to be 9.8% of the length of the λ genome (28). Track 2 is λ*polA* DNA digested with *Hin*dIII. The fragment migrating with the highest mobility contains the *polA* gene. Track 3 is DNA from the λ*trpE* vector (NM742) digested with *Hin*dIII. The fastest moving fragment (11.6% of the λ^+ genome, ref. 27) contains the *trpE* gene of *E. coli*. Track 4 is a derivative of the same vector in which the central fragment has been replaced by a fragment containing the *supF* gene. Below the gel analyses is a diagram of the λ*trpE* vector, NM742. The genotype of this phage is λ(*srI* 1-2) ∇ (*trpE*) (*att-red*) ∇ imm^{21} cI^+ *nin*5 *shin*d6°.

rently constituted, our phage allows expression of this gene under the control of its own promoter to yield high levels of DNA polymerase I during lytic infection of either wild-type or $polA^-$ *E. coli*. Amplifications of the $polA^+$ gene product by approximately two orders of magnitude over uninfected wild-type levels have been demonstrated. Based on an estimate of 400 molecules of DNA polymerase I per normal cell (3), this implies 40,000 molecules of polymerase per infected cell, representing about 4% of the total cellular protein by weight. Further experiments, not detailed here, indicate that integration-proficient derivatives of the λ*polA* phage may be induced by temperature shifts to give similar elevations of polymerase levels. From a λ*lig* phage a 500-fold overproduction of DNA ligase has been reported (30), but in these experiments advantage was also taken of a mutation in the promoter for the ligase gene. Phages of this type obviously will facilitate the preparation of large quantities of such enzymes for biochemical studies.

It seems unlikely that we shall better our yields by transferring the *polA* gene and its promoter to a relaxed plasmid. Attempts to maintain such a plasmid containing the $polA^+$ gene have been unsuccessful although we have been able to propagate plasmids in which the fragment includes the *polA1* allele (K. Chalmers and N. E. Murray, unpublished results). It is probable that overproduction of polymerase I resulting from multiple copies of the *polA* gene and its promoter is detrimental to the cell. This emphasizes the importance of being able to control the expression of genes present in many copies in a relaxed plasmid.

The mechanism by which DNA polymerase I is normally controlled remains to be elucidated. Assuming that the λ*polA* phage includes the normal bacterial promoter, we can conclude that the increased gene dosage due to replication of the phage DNA allows effective production of the polymerase.

The results of several infection experiments indicate that the λ*polA* phage induces polymerase production more efficiently in a *polA1* cell line than in its isogenic $polA^+$ parent strain. These data do not permit extensive speculation about the mechanism of regulation of polymerase synthesis at present.

λ*polA* transducing phages have also been isolated (J. A. Reehl, personal communication) after the integration of a λ derivative in close proximity to the *glnA* gene by the techniques described by Shrenk and Weisberg (31). Like the phage described here, these *in vivo*-derived phages form stable $PolA^+$ lysogens in $polA^-$ cells.

The λ*polA* transducing phage makes possible a large variety of genetic experiments designed to elucidate the structure of DNA polymerase I. Currently existing alleles of *polA* may be transferred to the λ*polA* phages, which can then be used to produce mutant enzyme for biochemical analysis and for fine-structure genetic mapping. In addition, it should now be possible to isolate a new spectrum of useful mutations in the *polA* gene via direct mutagenesis of the phage; *amber* and deletion mutations would be particularly useful. Since the phage described apparently includes the promoter for the *polA* gene, it should also be possible to isolate mutations affecting the expression of the *polA* gene.

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