The A and B proteins encoded by the temperate bacteriophage Mu are involved in the high efficiency DNA transposition reaction which is the distinguishing feature of this phage. The genes encoding these early proteins were cloned in an expression vector under the control of the bacteriophage λ leftward promoter. Under optimal conditions gpB was overproduced to account for 15% of the total cellular protein. The protein was purified to near homogeneity as determined by silver staining. Sequence analysis of the N terminus confirmed the identity of the purified protein as gpB. Proteolytic processing of the B protein does not occur at the amino terminus; the terminal methionine residue is quantitatively deamidylated. The protein, which was found to be basic and a general DNA binding protein, was insoluble at low ionic strength in the absence, but not in the presence, of DNA. The B protein also displayed a tendency to aggregate at high ionic strength where it was soluble in the absence of DNA. In addition, the protein was characterized as to its amino acid composition and extinction coefficient at 280 nm. The purified protein is active in a soluble in vitro transposition-replication system.

The temperate bacteriophage Mu is a giant transposon which utilizes DNA transposition as a means of propagating its genetic material (Toussaint and Réisibois, 1983; Klukner, 1981). The integrative replication of Mu DNA is a process which closely resembles the transposition of other prokaryotic transposable elements except that in the case of Mu the in vivo reaction occurs at a much higher frequency. Bacteriophage Mu is also unique among transposons in that the type of transposition end products generated appears to be developmentally regulated (Chaconas et al., 1981b; 1983). Infecting DNA integrates to give primarily simple insertions while the reactive DNA at the ends of the Mu genome remains to be defined. The proteins involved in Mu DNA transposition are specified by both the host (Toussaint and Faelen, 1974) and phage genomes. The products of the Mu early genes A and B appear to be directly involved in the integrative replication process. Mu A- mutants show no integration and B- mutants show a 10-100-fold reduction in transposition (O'Day et al., 1978; Faelen et al., 1978; Harshey, 1983). Physical association of mini-Mu plasmids with the host chromosome also does not occur in the absence of the A or B protein (Chaconas et al., 1980, 1981c) and significant levels of Mu DNA replication do not occur in A- or B- mutants (Wijffelman and Lotterman, 1977; Waggoner et al., 1981). The presence of other early Mu genes has also been reported to stimulate Mu DNA replication; however, these genes are not essential and may have only an indirect involvement (Waggoner et al., 1981; Goosen et al., 1982).

The mechanism by which Mu DNA transposition and replication occurs remains obscure. A contributing factor to this lack of knowledge is the difficulty involved in studying the Mu A and B proteins because of their extremely low intracellular concentrations; during Mu development these low proteins are difficult to detect by two-dimensional polyacrylamide gel electrophoresis of total extracts from radioactively labeled Escherichia coli cells. Furthermore, establishment of in vitro systems for Mu DNA transposition has only recently been accomplished. Higgins et al. (1983a, 1983b) have reported Mu DNA replication in vitro on cellophane discs, and during the preparation of this manuscript Mizuuchi (1983) published the first soluble in vitro transposition system. We now report the expression of the Mu A and B proteins under the control of the bacteriophage λ leftward promoter carried on a multicopy plasmid. The Mu B protein is amplified to represent 15% of the total cellular protein and we present the first report of the purification of this protein.

**EXPERIMENTAL PROCEDURES**

3. Portions of this paper (including "Experimental Procedures," Figs. 4-6, and Footnote 4) are presented in miniprint at the end of this paper. The abbreviations used are: SDS, sodium dodecyl sulfate; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PEI, polyethyleneimine; MSH, β-mercaptoethanol; PTH, phenylthiohydantoin; HPLC, high performance liquid chromatography. Miniprint is easily read with the aid of a magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-691, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverley Press.

**Amplification and Purification of the Bacteriophage Mu Encoded B Transposition Protein**

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RESULTS AND DISCUSSION

Construction of Recombinant Plasmids—Recombinant plasmids were constructed using the plasmid vector pKC30 which carries the bacteriophage \( \lambda \) leftward promoter. Expression in this system can be regulated by a temperature-sensitive repressor, and the great promoter strength coupled with high plasmid copy makes this system useful for amplification of many proteins in \( E. coli \) (Rosenberg et al., 1983). The Mu DNA used in the plasmid constructions was derived from the mutant phages (Fig. 1) Mucts62-13/4 (Goosen et al., 1982) and Mucts62Xcam5 (Chow and Bukhari, 1978). The former strain carries an IS1 insertion about 225 base pairs after the end of the Mu B gene. Cleavage of this DNA with \( B_{al}1 \) results in the liberation of a fragment carrying the 3’ end of the \( \text{ner} \) gene followed by the \( A \) and \( B \) genes in their entirety. Several plasmids were derived from insertion of \( B_{al}1 \) fragments of the phage DNA into pKC30 and are shown in Fig. 2. The most important of these is pGC511 which has the Mu A and \( B \) genes inserted in the proper orientation downstream from \( \text{PL} \). The 3’ end of the \( \text{ner} \) gene is present on the inserted DNA and provides translational stops in all three reading frames to eliminate the possibility of a fusion between the \( \lambda N \) protein and the Mu A protein. Analogous plasmids carrying only the Mu A gene were generated by the same rationale using the mutant phage Mucts62Xcam5 which carries a Tn9 insertion about 250 base pairs after the start of the \( B \) gene. These plasmids are also shown in Fig. 2.

Functional \( A \) or \( A \) and \( B \) genes are carried on the plasmids with inserts in the proper orientation as demonstrated by the following criteria:

1) Complementation of Mu Aam and Bam mutant phages in spot tests.
2) A high level of \( \text{in vivo} \) transposition of a mini-Mukan catalyzed by the \( A \) and \( B \) proteins provided in \( \text{trans} \) from pGC511 and a low but significant level of mini-Mukan transposition with gpA alone provided from pGG05 (Chaconas et al., 1984).
3) Amplification of the Mu A and \( B \) proteins and the ability of extracts from the overproducing strains to stimulate Mu DNA transposition \( \text{in vitro} \) (see Fig. 8).

Control plasmids also carrying the \( A \) or \( A \) and \( B \) genes but in the wrong orientation were also recovered. It was found, however, that low levels of transposition could be promoted by these plasmids \( \text{in vivo} \), probably because of readthrough into \( A \) and \( B \) from the \( \beta \)-lactamase promoter of pBR322.

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**Fig. 1.** Restriction map of Mucts62-13/4 and Mucts62Xcam5 phage DNA. The upper restriction maps are of the entire genomes, drawn to the scale at the top of the figure. The lower restriction maps show the left ends blown up to the scale at the bottom of the figure. The thin lines denote Mu DNA and the thick lines the internal sequences of Tn9. The striped boxes indicate IS1 sequences and the wavy lines denote host DNA found at the ends of the Mu phage DNA. Beneath the enlarged restriction map is a diagram of the position of the Mu \( P_i \) and \( P_o \) promoters, and the position of the Mu genes transcribed. The arrows indicate the direction of transcription. The position of the IS1 insertion in Mucts62-13/4 is taken from Goosen et al. (1982) and the Tn9 insertion in Mucts62Xcam5 from Chow and Bukhari (1978). The orientation of the insertions is based upon our unpublished data. The positions of Mu promoters, genes, and restriction enzyme sites are taken from Priess et al. (1982), Schumann et al. (1980), and Kahmann et al. (1977). The positions of the restriction sites in IS1 are taken from Ohtsubo and Ohtsubo (1978). The scales are calibrated in kilobases.
Purification of the Mu B Protein

(Chaconas et al., 1984). For this reason another control plasmid (pGC333) was recovered which contained no insertion but had a small deletion which knocked out the λ N gene. A by-product of the cloning work on the A and B genes was the plasmid pGC180 which carries the Mu c gene (immunity repressor) under the control of P_L.

**FIG. 2.** Restriction maps of recombinant plasmids derived from pKC30. Plasmids pGC511, pGC240, and pGC180 contain BglII fragments of Mucts62-13/4 phage DNA cloned into the HpaI site of the plasmid vector pKC30. Plasmid pGC333 is a derivative of pKC30 with a deletion of approximately 165 base pairs (indicated by Δ) at the HpaI site within the λ N gene. Plasmids pGG04, pGG05, and pGG06 contain BglII fragments of Mucts62Xcam5 phage DNA in the HpaI site of pKC30. The solid dot in pGC180 indicates the junction of λ DNA with host sequences found at the left end of Mu. Beneath each restriction map is a diagram of the position of the λ P_L promoter in each plasmid and the position of the Mu genes transcribed. The arrows indicate the direction of transcription. Plasmid pGC180 contains the Mu repressor (c) gene. The positions of restriction sites in the pBR322 sequences are taken from Sutcliffe (1978) and the position of P_L and the restriction sites in the λ DNA are from Sanger et al. (1982). Other information was taken from the sources indicated in the legend to Fig. 1. The scale at the bottom is calibrated in kilobases.
Purification of the Mu B Protein

Amplification of the Mu B Protein—Thermal induction of the leftward promoter on pGC511 (Fig. 3) resulted in the dramatic appearance of a protein the size of gpB (33,000 daltons, Giphart-Gassler et al., 1981) on sodium dodecyl sulfate-polyacrylamide gels. The 33,000-dalton polypeptide was not observed with the control plasmids pGC333 or pGC240 and was therefore assumed to be the B protein. The amplification leveled off with gpB comprising almost 15% of the total cellular protein. Under these conditions we have not detected the A protein. This is of interest since in vivo experiments demonstrated the presence of a functional A gene which was transcribed from PL. Furthermore, the A gene is transcribed before the B gene in the same operon, so the message for gpA must be present. It is possible that the A protein is not amplified to the same extent as B because it is rapidly degraded; Pato and Reich (1982) have reported a half-life of approximately 3 min for the A protein. It is also possible that translational inefficiency and/or translational control mechanisms are responsible for the low yields of gpA in this system. Using lower percentage gels and with pGG04 and pGG05 which carry A but not B we have detected amplified gpA by Coomassie blue staining, but at levels much lower than for gpB (Chaconas et al., 1984).

Purification of the Mu B Protein—Starting from strain GC115 which harbors the overproducing plasmid pGC511 we purified the Mu B protein to near homogeneity. Since there was no available assay for gpB, the purification was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. During preliminary work to devise a purification protocol we found that gpB was very soluble at high concentrations in low ionic strength buffer (50 mM NaCl) as long as there was DNA present in the extract. As soon as the DNA was removed from the extract, however, the protein became insoluble with some precipitation occurring even in 0.5 M NaCl. The precipitation was found to be irreversible unless urea or guanidine HCl was used for solubilization, suggesting that the protein had denatured. Variation in pH or the presence of detergents, divalent metal ions, dimethyl sulfoxide, glycerol, ATP, deoxynucleoside triphosphates, or monophosphates did not change the solubility properties of gpB. The insolubility of the protein at low ionic strength created an obvious problem with respect to...

FIG. 3. Kinetics of synthesis of the Mu B protein directed by the λ leftward promoter in pGC511. Strain GC115 which carries this plasmid was grown in LB broth containing 100 μg/ml of ampicillin at 32 °C to a density of about 6×10^8 cells/ml. The culture was shifted to 43 °C, and aliquots were removed for analysis at the indicated times. A, Coomassie blue stain of the acrylamide gel used to analyze the protein samples. Slot M contains molecular mass markers. The molecular masses are given in kilodaltons. B, quantification of the percentage of the total protein comprising gpB by densitometric analysis of the gel shown in panel A.

FIG. 7. Sodium dodecyl sulfate-polyacrylamide gel analysis of gpB purification stages. The 12% gel with a 6% stack contained about 8 μg of B protein per slot. Lane 1, total cells; lane 2, supernatant of cell lysate; lane 3, resuspended streptomycin sulfate pellet; lane 4, pooled fractions from hydroxylapatite chromatography; lane 5, pooled fractions from DEAE-Sepharose chromatography; lane 6, pooled fractions from CM-Sepharose chromatography; lane M, standards whose molecular masses are indicated in kilodaltons. The gel was first stained with Coomassie blue and subsequently stained with silver to accentuate any trace contaminants. The apparent molecular mass of the purified protein is 33,000 daltons as determined from this gel.
the use of ion exchange chromatography in the purification procedure. We therefore unsuccessfully attempted to obtain a cleare preparation of B protein using DNA binding at low salt and a variety of steps at 0.5 M NaCl or above (hydroxylapatite chromatography, hydrophobic chromatography, dye matrix chromatography, and gel filtration). We finally resorted to denaturation of the protein in 6.2 M urea to eliminate the solubility problems in the final two steps of the purification. The final purification procedure described under "Experimental Procedures" results in the recovery of 5-7 mg of protein from 1 liter of cells. The entire purification can be completed in 8 h and the final preparation is nearly homogeneous as can be seen in Fig. 7. Only a few trace contaminants can be visualized by an overdeveloped silver strain.

Because denaturation in urea was used during the purification procedure we were concerned that the renatured protein might be inactive or have altered properties. It is noteworthy, however, that the total time during which the protein was in contact with highly purified urea was less than 4 h. Carbamylation of amino groups in proteins is a slow reaction requiring much longer times, and carbamylation of thiol groups, although rapid, is readily reversible above neutral pH (Cohen and Oppenheimer, 1977). Furthermore, several proteins involved in DNA metabolism with solubility problems similar to that of gpB have been recently purified using solubilization in urea. Both the bacteriophage lambda O and P proteins (Tsuruoka and Matsubara, 1982) and the Tn3 resolvase (Krasnow and Cozzarelli, 1983) retained their ability to stimulate lambda DNA replication or site specific recombination, in vitro after removal of the urea. In the case of the Mu B protein the solubility properties of the native protein are restored upon renaturation out of urea. In addition, similar chromatographic behavior on gel filtration and heaxyl-Sepharose was observed. These findings demonstrate a structural similarity between gpB before and after purification in urea. We also assayed the purified B protein by in vitro complementation in the soluble transposition-replication system of Mizuuchi (1983). Fig. 8 shows that the purified protein renatured from urea is as active in the complementation assay as the streptomycin sulfate fraction which had not been denatured and renatured. The maximal level of Mu DNA synthesis under the assay conditions used was about 22 pmol which corresponds to 10.5% of the Mu DNA template and is in good agreement with the 5-10% efficiency observed by Mizuuchi (1983).

Besides assaying the ability of purified gpB to stimulate Mu DNA replication, we also analyzed the transposition end products generated in the in vitro system. Agarose gel electrophoresis of the reaction products (data not shown) revealed no detectable difference in the products formed with the renatured protein or the streptomycin sulfate fraction; in both cases replicon fusions were the predominant end products. These experiments demonstrate that the Mu B protein purified in urea by the protocol we have developed is functionally indistinguishable from an early purification fraction which has not been subjected to denaturation and renaturation.

**Physical Characterization of the Mu B Protein**—The identity of purified gpB was verified by sequence analysis of the amino terminus. The first 5 residues were found to be MetAsn-Ile-Ser-Asp which is the expected amino acid sequence based upon the nucleotide sequence of the Mu B gene (Miller et al., 1984). This indicates that no preoteolytic processing occurs at the amino terminus. Furthermore, a 95% recovery of the terminal methionine indicates that the N terminus is deformylated.

The amino acid composition of gpB is shown in Table I. Our observed values are in good agreement with the expected composition based upon the nucleotide sequence (Miller et al., 1984). The protein was found to contain 51% polar amino acids which is within the normal range (47 ± 6%) for non-membrane proteins (Capaldi and Vanderkooi, 1972). In addition, the protein contained more basic than acidic residues based upon an isoelectric point of about 9.0 in 8 M urea (data not shown). Based upon the DNA sequence the molecular mass of gpB was calculated to be 34,750 daltons. This is in good agreement with an apparent molecular mass of 33,000 daltons displayed on sodium dodecyl sulfate-polyacrylamide gels (Giphart-Gasser et al., 1981 and Fig. 6).

**TABLE I**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues determined by amino acid composition</th>
<th>Residues predicted from DNA sequences</th>
</tr>
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<tbody>
<tr>
<td>Asx</td>
<td>29.6</td>
<td>30</td>
</tr>
<tr>
<td>Thr</td>
<td>24.1</td>
<td>22</td>
</tr>
<tr>
<td>Ser</td>
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<td>17</td>
</tr>
<tr>
<td>Gix</td>
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<tr>
<td>Pro</td>
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</tr>
<tr>
<td>Gly</td>
<td>20.9</td>
<td>20</td>
</tr>
<tr>
<td>Ala</td>
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<td>27</td>
</tr>
<tr>
<td>Cys</td>
<td>4.0</td>
<td>3</td>
</tr>
<tr>
<td>Val</td>
<td>18.0</td>
<td>18</td>
</tr>
<tr>
<td>Met</td>
<td>8.1</td>
<td>8</td>
</tr>
<tr>
<td>Ile</td>
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<td>19</td>
</tr>
<tr>
<td>Leu</td>
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<td>36</td>
</tr>
<tr>
<td>Tyr</td>
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<td>5</td>
</tr>
<tr>
<td>Phe</td>
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<td>8</td>
</tr>
<tr>
<td>Trp</td>
<td>2.1</td>
<td>4</td>
</tr>
<tr>
<td>Lys</td>
<td>13.9</td>
<td>14</td>
</tr>
<tr>
<td>His</td>
<td>5.3</td>
<td>5</td>
</tr>
<tr>
<td>Arg</td>
<td>27.1</td>
<td>28</td>
</tr>
</tbody>
</table>

* Determined as cysteic acid after performic acid oxidation.
* Determined after hydrolysis with methanesulfonic acid.
The extinction coefficients, ε, at 280 nm were found to be 1.06, 1.15, and 1.25 for a 1 mg/ml solution as determined by dry weight, relative to absorbance at 205 nm, or by alkaline titration of the 5 tyrosine residues at 295–300 nm, respectively. The average of these three values was 1.16.

The purified B protein was stored in 1 M NaCl in order to avoid precipitation. Under conditions where the protein was soluble it had a tendency to aggregate as determined by gel filtration on Bio-Gel A-0.5m or A-1.5m. The unaggregated gpB (usually about 50% of the protein) chromatographed in the position expected for a monomeric form. The protein could be kept in solution at 50 mM NaCl if the solution contained about 1 weight equivalent of DNA. Attempts were made to quantitatively characterize the DNA binding; however, uninterpretable results were obtained, probably due to the precipitation of unbound protein at low salt where DNA binding occurs. The Mu B protein did bind, however, with the same efficiency to both single-stranded and double-stranded DNA cellulose and did not demonstrate a significant preference for Mu DNA binding.

Experiments are now in progress to determine the role of gpB in the transposition process.

Acknowledgments—We would like to thank Drs. Roger McMacken, Martha Howe, Martin Marinus, Martin Rosenberg, and Helios Muralio for providing strains. Amino acid sequence determination was based upon his nucleotide sequence data. The expert assistance of Dale Marsh and Linda Bonis in preparation of figures and typing of the manuscript, respectively, is gratefully acknowledged.

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SUPPLEMENTARY MATERIAL TO
Amplification and Purification of Bacteriophage Mu Encoding 3
Translocation Protein
George Checcone, Greg Cliver, and Mark L. Miller

EXPERIMENTAL PROCEDURES

Media - LB broth was prepared as described by Miller (1972) except that N-2
amino (Scheffield Products, Fracta Inc., Flem, Tenn.) was used as a sub-
situte for Bacto tryptone: LB agar & e# presence of 100 unlXg ampicillin (in liquid) or 100 ug carbenicillin (on
plates).

Bacterial and bacteriophage stocks - The bacterial strain 999 (spercro-
trium, 10% v/v) carrying the donor plasmid strain 196 (McA-) was main-
tained on trypton yeast (Hillier, 1968) in LB broth and plates con-
taining 15% Nitrogen deficient medium and 50 ug ampicillin in liquid
or on plates. The phage strain 794C1 was a Mu transposon insertion mutant
derived from Dr. J. S. Hovland, University of Wisconsin, Madison, WI, 89C1 (89C1, 89C1) carrying a cryptic repressor gene. 

Preparation of bacterial proteins - Bacterial protein extract for use in the
phage transduction and for recA protein preparation and analyses were
harvested by centrifugation in the cold and resuspended in 1X buffer
buffered with 10% (v/v) glycerol and 0.5% (w/v) sodium dodecyl sulfate
(SDS) in 50 mM NaCl. The bacteria were disrupted by sonic oscillation
at low temperature (4°C) and the crude extract was centrifuged at 30,000
g for 30 min at 4°C. The supernatant was dialyzed against 50 ultracentrifugation resulted in the appearance of the protein.

Preparation of nucleic acids - Bacteriophage DNA (Checcone et al., 1988)
and plasmid DNA (Checcone et al., 1989a) were prepared as described
previously. Plasmid DNA was prepared by the method of Birnboim and
Sarkis (1979) and was subsequently digested with HindIII, digested
trough Sephagel 60 filter columns and dialyzed extensively against 30
mM NaCl in buffer B containing 6.2 M urea. Final concentration of DNA was measured by optical
end absorption at 260 nm. Ampliton rich fragments of DNA were
selected in a hydroxyapatite column equilibrated in buffer plus 6.2
mM urea and the purified gpl was resuspended in 10 ml buffer
and stored at -20°C for future use. The absorbance of this
peak fraction was usually 5-7 mg of protein from 1 liter culture
and the entire purification procedure took about 8 hours.

Figure 6. M-Sepharose column chromatography profile. The supernatant from DMB-Sepharose columns was added to
DEAE-Sepharose column. The pooled fractions from hydroxyapatite were collected directly onto 1X 2.5 cm DEAE-
Sephrrose column. The column was washed with 1 ml loading buffer (Buffer B 0.8 M NaCl followed by 17.5 ml buffer B). 8 ml protein was added with buffer B containing 0.8 M NaCl. Fractions containing 1.9 ml were collected and the pooled fractions are indicated as solid circles.

Figure 5. DEAE-Sepharose chromatography profile. The pooled fractions from hydroxy-
apatite were applied directly to a DEAE-Sepharose column equilibrated in buffer B and the fraction was collected at 6.2
M NaCl and pooled as described in Figure 6. The column was run with a pressure head of 36-42 cm. The leading edge of the elution peak was clear but the trailing edge contained contaminating proteins.

Figure 4. Hydroxyapatite column chromatography profile. The supernatant fraction from DMB-Sepharose columns was added to 1 ml DEAE-Sepharose column equilibrated with 1 ml buffer B containing 6.2
M NaCl and applied to a 1 cm DEAE-Sepharose column in buffer B. The column was washed with 1 ml buffer B and the leading edge of the elution peak was clear in the absence of buffer B containing 6.2
M NaCl. The column was run with a pressure head of 36-42 cm. The leading edge of the elution peak was clear but the trailing edge contained contaminating proteins.
Purification of the Mu B Protein

Protein extracts (Fraction II) to provide host factors were prepared from 899 by the method of Miller et al. (1961). Fractions with g34 activity were precipitated from G35 (35 nM carrying g34A) following a 60-minute incubation with 10% normal guanidine-HCl, pH 5.8, followed by centrifugation to remove insoluble g34. The g34 was solubilized in 0.3 M NaCl, 0.01 M Na3EDTA, 0.01 M Tris-HCl, pH 7.4, and assayed by adding 500 ng of g34 to the mixture. The specific activity of g34 was determined by measuring the incorporation of radiolabeled g34 into the extract and into the host cells. The specific activity of g34 was determined by measuring the incorporation of radiolabeled g34 into the extract and into the host cells.

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In vitro complementation reactions - In vitro transposition was performed in 10 ml reactions maintained at 30°C in the presence of 50 units of EcoRI (Khorana et al., 1963). The following conditions were used in our reactions (Belforte et al., 1963). The following conditions were used in our reactions (Belforte et al., 1963). The following conditions were used in our reactions (Belforte et al., 1963).