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Bacteriophage Mu DNA circularizes following infection of *Escherichia coli*

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Mu DNA, isolated from infected cells or minicells, has been shown to be held by proteins in twisted and open circular forms. Circularization does not require protein synthesis in the infected cells. A 64 000-dalton polypeptide is injected into the infected cell with Mu DNA and co-sediments with Mu DNA through sucrose gradients. Circularization of the infecting Mu DNA does not require removal of the *Escherichia coli* DNA sequences which are attached to both ends of the Mu genome in the viral particle.

Key words: Mu infection/DNA-protein complex/circular DNA/minicells/phage proteins

Introduction

The pathway by which Mu DNA proceeds from an infecting viral particle to become a prophage is unknown. It is, however, clear that sequences at both ends of the viral genome, defined as *att*L and *att*R (Kahmann and Kamp, 1979), play integral roles in this process. The most logical mechanism to co-ordinate a reaction requiring the participation of both ends of a linear molecule is *via* a circular intermediate and such intermediates are common to other phage integration systems. Phages such as λ and P2 employ complementary single-stranded cohesive ends to facilitate circularization (Hershey *et al.*, 1963; Ikeda and Tomizawa, 1968), phages of the P1 and P22 types have terminally repetitious sequences which recombine to give circular molecules (Ikeda and Tomizawa, 1968; Thomas *et al.*, 1968). Mu DNA does not, however, have either of these structures and circular intermediates were not detected in experiments designed specifically to identify such intermediates following infection of *Escherichia coli* cells (Ljunquist and Bukhari, 1979). We decided to investigate the structure taken by Mu DNA following infection of anucleate minicells arguing that normally transient intermediates in the process of Mu integration might accumulate in minicells as integration could not possibly occur. Using this procedure we have developed techniques which permit the isolation of circular forms of Mu DNA from both infected minicells and infected nucleated cells.

Results

Sedimentation of Mu DNA through neutral sucrose gradients

Minicells, infected with Mu phage containing radioactively labeled DNA, were lysed by different procedures. The ionic strength, lysozyme concentration and type and concentration of detergent used were varied. Lysates were loaded onto 5-20% sucrose gradients, the gradients centrifuged and fractionated and trichloroacetic acid (TCA) precipitable counts in each fraction determined. A procedure was developed (see Materials and methods) which gave a sedimentation profile in which the infecting Mu DNA formed two distinct peaks (Figure 1A). Mu DNA, phenol-extracted from phage particles, sedimented slightly slower than the slower of the two peaks of DNA (peak II) obtained from infected minicells. Application of this procedure to Mu-infected nucleated cells gave sedimentation profiles essentially the same as those obtained from infected minicells (Figure 1B).

Requirements for formation and disaggregation of the complex

Minicells were infected with radioactively labeled Mu phage in the presence of antibiotics to determine if the sedimentation profile could be modified by inhibition of metabolic processes. Inhibition of protein synthesis by chloramphenicol (Figure 1A, panel 2) and inhibition of RNA synthesis by rifampicin (not shown) had no effect on the observed profile of Mu DNA (Figure 1A). Inhibitors of DNA gyrase (Figure 1A, panels 3 and 4) partially inhibited the formation of peak I.

During the development of the lysis procedure used to obtain the profiles shown in Figure 1, several procedures were used which resulted in profiles in which only peak II was present. It seemed probable that peak I material could be converted to peak II material by procedures which disrupted protein-DNA complexes. This was confirmed by isolation of peak I material and exposure to higher ionic strength environments, proteinase K or increased detergent concentrations (Figure 2). The lysis procedure used by Ljunquist and Bukhari (1979) to lyse Mu-infected cells did not, in our hands, routinely allow the detection of peak I material. Their inability to isolate a Mu DNA:protein complex containing circularized Mu DNA from Mu-infected sensitive cells can therefore be explained as most likely resulting from the lysis technique they used.

Electron microscopic analysis of Mu DNA structures

Mu DNA prepared from peaks I and II was visualized by electron microscopy. Peak I material was composed mostly of tightly twisted, circular DNA structures whereas peak II material was a mixture of open circular forms and linear Mu molecules (Figure 3A and 3B). Many molecules were observed which appeared to be partially untwisted; an example of such a molecule is seen in the lower right hand corner of Figure 3A. Spreading the material from peak I in the absence of added cytochorme c indicated that proteins were bound to the Mu DNA structure to give loops of DNA which appear as domains within the Mu genome (Figure 3C). The procedure used to lyse infected minicells also results in release of DNA from Mu virions. Figure 3D shows that DNA released from phage particles is not circular and, as previously reported (Breepoel *et al.*, 1976), the extruded DNA remains attached at one end to the viral particle.

Mu polypeptides associated with the infecting DNA

The results described above indicate that infecting Mu

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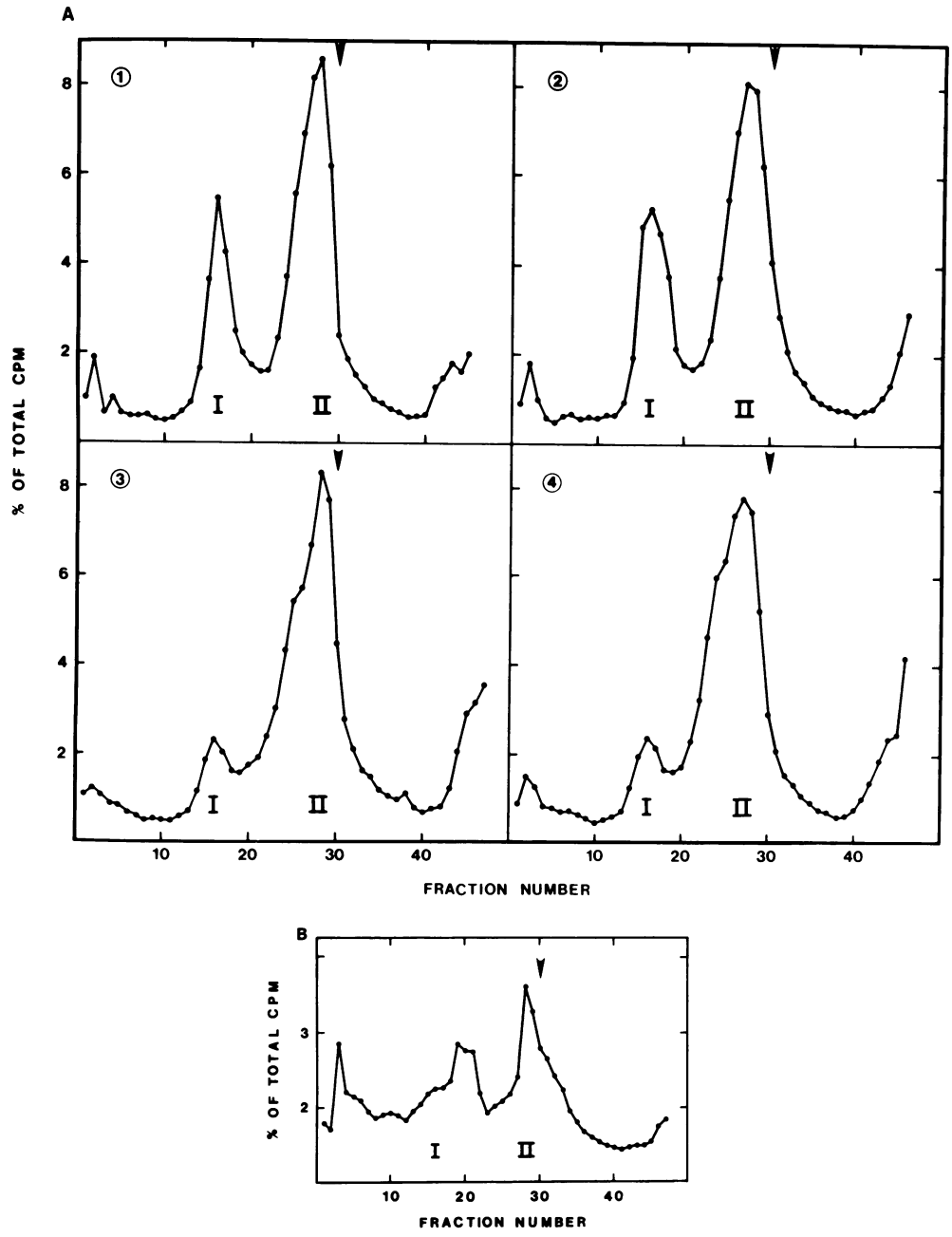

Fig. 1. Sucrose gradient sedimentation profile of Mu DNA isolated from infected minicells or infected cells. **(A)** Minicells were infected with [methyl-^{3}H]-thymidine-labeled Mu*cts62* and, following lysis and sedimentation through a 5–20% w/v sucrose gradient, radioactivity in TCA precipitable material in each fraction was determined. **(Panel 1).** Identical aliquots of minicells were exposed to chloramphenicol (150 μg/ml; **panel 2),** nalidixic acid (120 μg/ml; **panel 3)** or novobiocin (50 μg/ml; **panel 4)** for 15 min before infection and during the infection. Sedimentation was from right to left. Total c.p.m. were: **panel 1,** 43 082; **panel 2,** 33 560; **panel 3,** 28 812 and **panel 4,** 32 478. Peak I, the faster sedimenting material, was composed of twisted, circular molecules and peak II was a mixture of open circular and linear molecules (see Figure 3). Phenol-extracted, linear Mu DNA, prepared from 2-[^{14}C]thymine-labeled phage migrated to the position indicated by the arrow. **(B)** The sedimentation profile of Mu DNA prepared from nucleated cells infected with [methyl-^{3}H]thymidine-labeled phage. Total c.p.m. in Figure 4B was 4311.

DNA enters a protein-DNA complex in which the circular configuration of the DNA is maintained by non-covalent bonds. The possibility that the protein component of this complex contains proteins which enter with the viral DNA was investigated by infecting minicells with [^{35}S]methionine-labeled phage. Infected minicells were lysed and, following sucrose gradient sedimentation, labeled polypeptides in each fraction from the gradient were visualized by fluorography after separation by electrophoresis through a polyacrylamide gel (Reeve and Shaw, 1979). Small amounts of major structural proteins (gpT, gpL and gpS) were found in all fractions, however, the fractions containing Mu DNA were much enriched for a 64 000 mol. wt. polypeptide (Figure 4). Mu is known to encode at least two polypeptides with 64 000 mol. wts. (Giphart-Gassler *et al.*, 1981a, 1981b); one of which is gpH. Two-dimensional electrophoresis was used to show that the 64 000 mol. wt. polypeptide found in the virion does not have the same mobility as gpH during electrofocusing (Figure 4). To confirm that the 64 000 mol. wt. polypeptide was not gpH, use was made of Mu phage carrying amber mutations in gene *H* (*Ham*1043, *Ham*1519, *Ham*1932). These mutations are located close to the carboxyl end of gene *H* (Engler *et al.*,

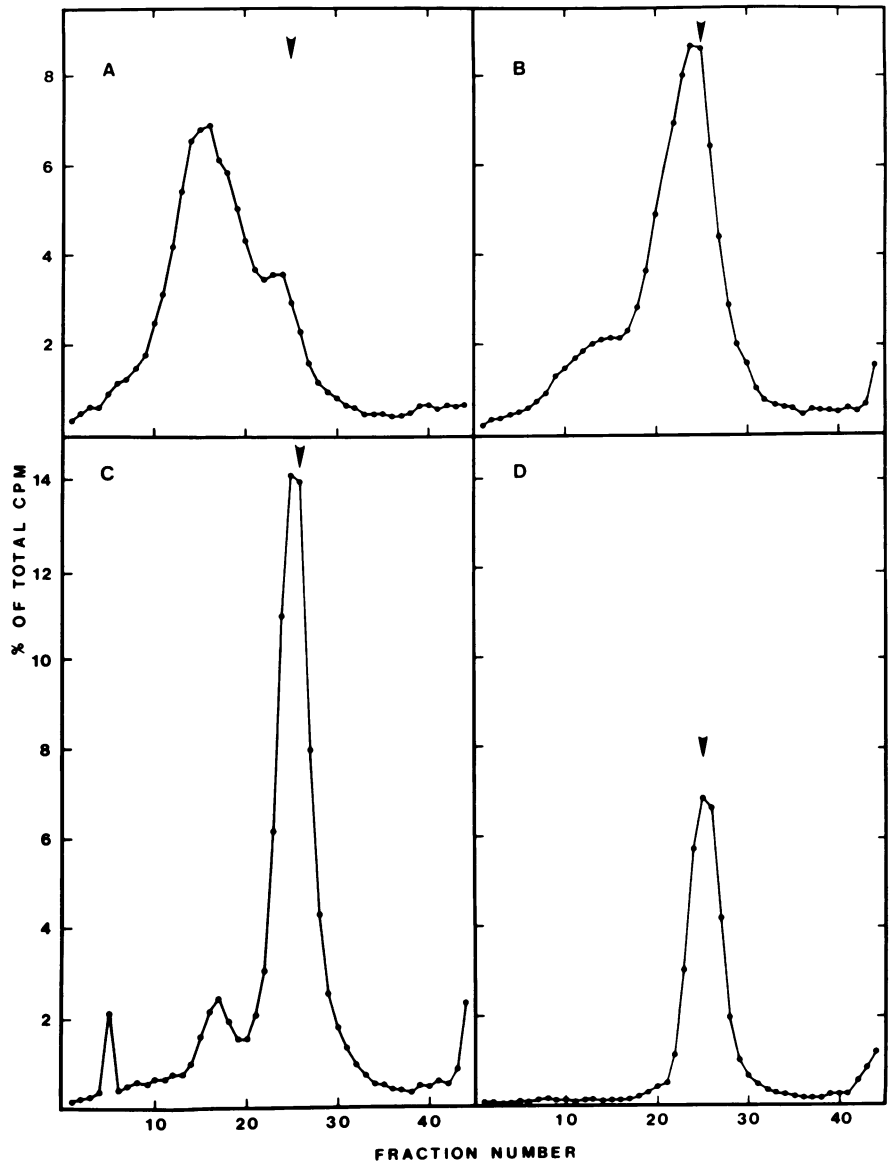

Fig. 2. Sucrose gradient sedimentation profiles of Mu DNA. The material identified as Peak I in Figure 1A, panel 1, was pooled and dialysed against 100 mM Tris-HCl; 1 mM EDTA (pH 8.0). The dialysed material was incubated at 37°C for 1 h (**A**) without additions; (**B**) in the presence of 100 mM NaCl; (**C**) in the presence of 100 μg proteinase K/ml and (**D**) in the presence of 1% w/v SDS. The material was layered onto a 5–20% sucrose gradient and DNA sedimentation profiles determined as in Figure 1. The arrow indicates the position to which phenol-extracted, ^{14}C-labeled Mu DNA sedimented. Total ^{3}H c.p.m. in **A** was 13 648, in **B** was 13 238, in **C** was 14 514 and in **D** was 35 044.

1980) and all give a common 'leaky' phenotype. Induction of these phages from non-suppressing lysogens produces viable phage which, when plated on lawns of non-suppressing cells, form small, very turbid plaques. The polypeptides of the Mu*Ham* particles, obtained by induction of non-suppressing lysogens, are indistinguishable from the polypeptides of Muc*ts*62 particles. A 64 000 mol. wt. polypeptide is present in both Muc*ts*62 and Muc*ts*62*Ham*1043 particles (Figure 4B). Infection of minicells with Muc*ts*62*Ham*1043 phage, obtained from either suppressing or non-suppressing lysogens results, however, in the synthesis of a truncated gpH designated gp*Ham*1043 in Figure 4B. If gpH were a component of the Mu particle then Muc*ts*62*Ham*1043 particles obtained by induction of a non-suppressing lysogen should contain gp*Ham*1043. As this is not the case (Figure 4B) we conclude that gpH is unlikely to be a component of the Mu viral particle.

Role of Mu gene H

It was previously reported, on the basis of electrophoretic mobility, that gpH was probably a component of the Mu head structure (Giphart-Gassler *et al.*, 1981b). Results reported above indicate that this is unlikely to be true. Two results obtained in this study do, however, indicate that gene *H* probably does play some role in the production of normal phage particles. Mutation *Ham*7100 is located approximately in the middle of gene *H* (Engler *et al.*, 1980) and minicells infected with Muc*ts*62*Ham*7100 synthesize a truncated gpH with an apparent mol. wt. of 39 000 (compared with 62 000 for gp*Ham*1043). Induction of non-suppressing *E. coli* strains lysogenic for Muc*ts*62*Ham*7100, unlike induction of lysogens of the 'leaky' *Ham*1043 type, does not produce viable Mu phage. Electron microscopy indicates that lysates produced by induction of such Muc*ts*62*Ham*7100 lysogens,

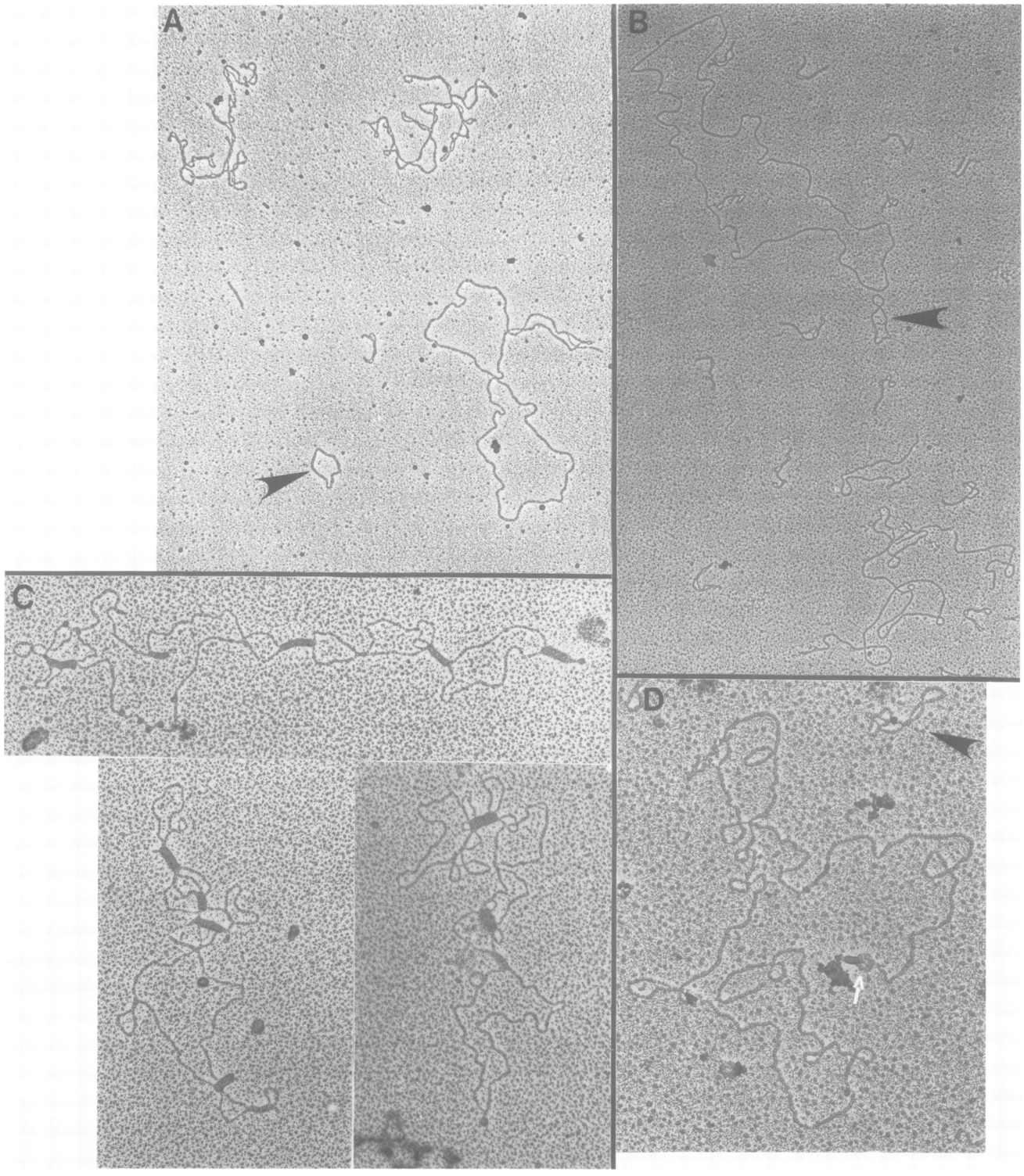

Fig. 3. Electron micrographs of Mu DNA. Material from peak I and peak II of Figure 1A spread in the presence of cytochrome c is shown in **panels A** and **B**, respectively. **Panel C** shows the configuration of Mu DNA from peak I material when spread in the absence of added protein. **Panel D** shows the effect of the lysis procedure on CsCl purified Mu phage. The black arrows indicate pBR322 DNA added as a size marker to the samples. The same types of DNA-protein configurations have been found in peak I and II material isolated from Mu-infected nucleated cells and from cells and minicells infected in the presence of chloramphenicol (results not shown). The white arrow in **panel D** indicates the phage structure still attached to extruded Mu DNA (Breepoel *et al.*, 1976).

contain 'headless' phage tails (results not shown). Thus a 'tight' mutation in gene *H*, which leads to the synthesis of a very defective gpH, exhibits a phenotype suggesting a role for gpH in Mu phage head morphogenesis.

A second observation which indicates that gpH must be active during phage development for production of functionally normal phage is that sedimentation profiles of Mu DNA, isolated from minicells infected with the 'leaky' Mu*cts*62*Ham*1043 phage obtained from a non-suppressing lysogen, do not show the presence of peak I material (Figure

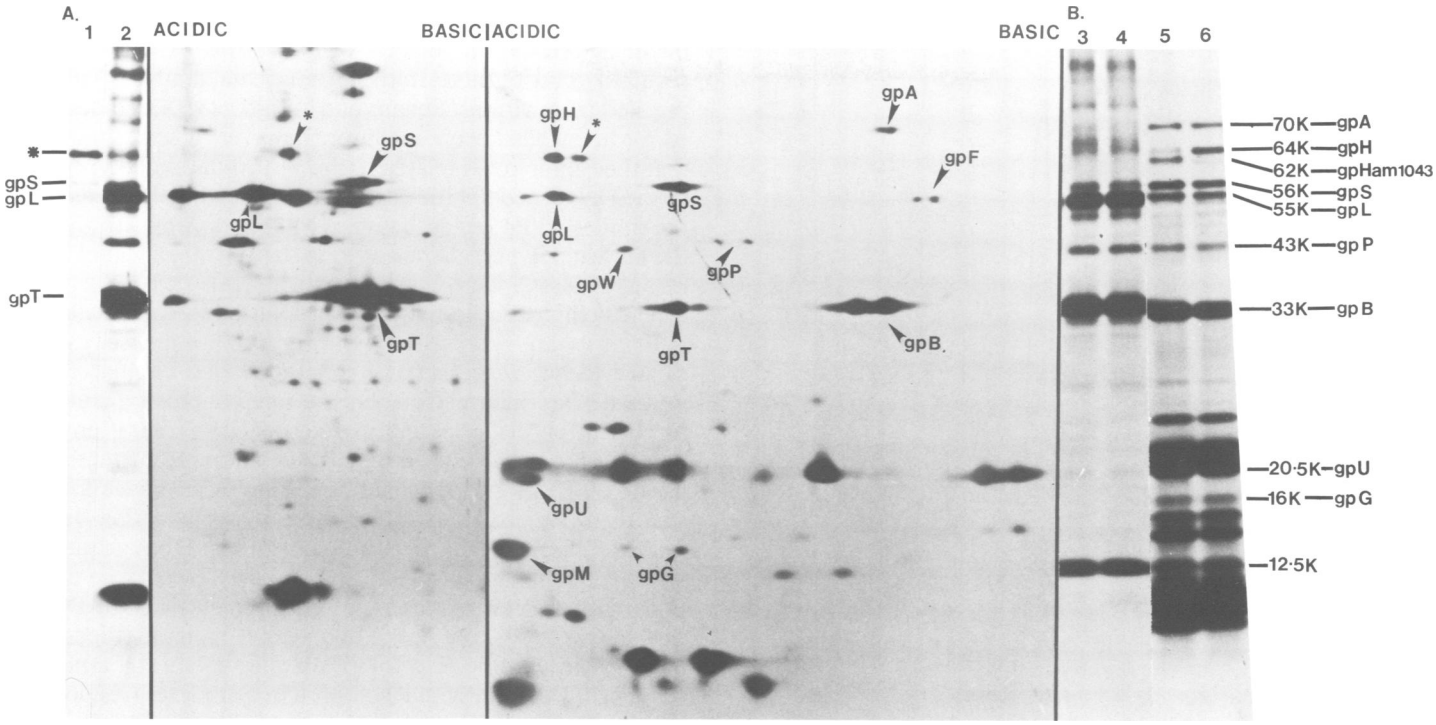

Fig. 4. Comparison of Mu polypeptides synthesized in infected minicells with the polypeptide components of the viral particle. **(A)** Minicells were infected with ^{35}S-labeled Mu*cts*62 phage, washed, lysed and DNA-containing fractions prepared from a sucrose gradient as shown in Figure 1A. The radioactively labeled polypeptides which co-sedimented with Mu DNA were visualized by fluorography following electrophoresis through a 14–20% polyacrylamide gel (**track 1**). **Track 2** shows a one-dimensional separation of the polypeptides which constitute the complete Mu virion. (Giphart-Gassler *et al.*, 1981b) adjacent to a two-dimensional separation of the same virion polypeptides. Mu polypeptides synthesized in Mu*cts*62-infected minicells for 1 h at 37°C in the presence of [^{35}S]methionine are shown in the right-hand two-dimensional separation. The designation that a polypeptide is a specific Mu gene product (gpA, etc.) indicates that suppressor-sensitive mutations in that gene have been found to affect the mobility of that particular polypeptide (Magazin *et al.*, 1978; Giphart-Gassler *et al.*. 1981a, 1981b). Polypeptides gpM, gpU, gpW and gpT (the major head protein) have not previously been so identified and the assignments made in this figure were based on two-dimensional analyses of polypeptides synthesized by minicells infected with Mu*Mam*1114, Mu*Uam*7220, Mu*Wam*1011, and Mu*Tam*7327. Two polypeptides both with apparent mol. wts. of 64 000, are indicated by gpH and * in the separation of Mu polypeptides synthesized in infected minicells. Only the polypeptide designated * can be detected as a component of the Mu particle. Polypeptide gpF is a component of the virion (Giphart-Gassler *et al.*, 1981b) but is not shown in the two-dimensional separation of virion components as the region to which gpF migrates was omitted from the figure to facilitate figure construction. gpF can be seen amongst the polypeptides synthesized in infected minicells. **(B)** The polypeptides which constitute the viral particles resulting from induction of a suppressing (**track 3**) and non-suppressing (**track 4**) lysogen of Mu*cts*62*Ham*1043 are compared with the polypeptides synthesized in minicells infected with Mu*cts*62 (**track 6**) and Mu*cts*62*Ham*1043 (**track 5**). The truncated gpH synthesized in minicells infected by Mu*cts*Ham1043 is indicated as gp*Ham*1043. This truncated polypeptide is not detected in Mu*cts*62*Ham*1043 particles produced by induction of non-suppressing lysogens indicating that the 64 000 mol. wt. polypeptide detected in these particles is not gpH. The polypeptides in **tracks 3–6** were separated by electrophoresis through a 10–20% polyacrylamide gradient gel.

5). In contrast, DNA profiles from minicells infected with Mu*cts*62*Ham*1043 phage prepared from a nonsense mutation suppressing lysogen do show the presence of peak I material. It must therefore be concluded that formation of detectable peak I material in an infected minicell is dependent on fully functional gpH in the lysogen which, on induction, produced the viral particles used in the infection. It should be pointed out that failure to detect peak I material in minicells infected by Mu*cts*62*Ham*1043 produced by a non-suppressing lysogen may only reflect an increased instability of this material and does not necessarily indicate a complete absence of synthesis of peak I material. Open circular forms of Mu*cts*62*Ham*1043 DNA are found in peak II material under the conditions described above where peak I is not detected.

Discussion

The results reported demonstrate that infecting Mu DNA does form a circular structure although the mechanism of its formation is radically different from the mechanisms known to be employed by other temperate phages. Our results demonstrate that the circles of Mu DNA are not covalently closed and that the integrity of these structures requires DNA-protein interactions. Formation of twisted, circular forms is partially dependent on host-cell gyrase but does not require novel protein synthesis within the infected cell. We anticipate that the protein-DNA complexes contain a number of different host proteins and possibly a 64 000-dalton phage-encoded polypeptide which enters the cell with infecting Mu DNA. The Mu gene which encodes this polypeptide remains unknown. We have used two-dimensional electrophoresis to investigate the polypeptides synthesized in minicells infected with different Mu phage and although the effects on polypeptide synthesis of amber mutations in all the known essential genes of Mu have been analyzed, we have not detected an effect on the 64 000-dalton polypeptide. This result may indicate that the polypeptide is encoded either by an unknown essential gene or by a non-essential gene for which amber mutations are unavailable. Alternatively, the result may reflect an experimental limitation (see below). There is a group of non-essential genes, including a *gam* gene, located between genes *B* and *C*. These genes do not however encode polypeptides with mol. wts. of 64 000 (Giphart-Gassler *et al.*, 1981a). The kinetics of expression of the 64 000-dalton poly-

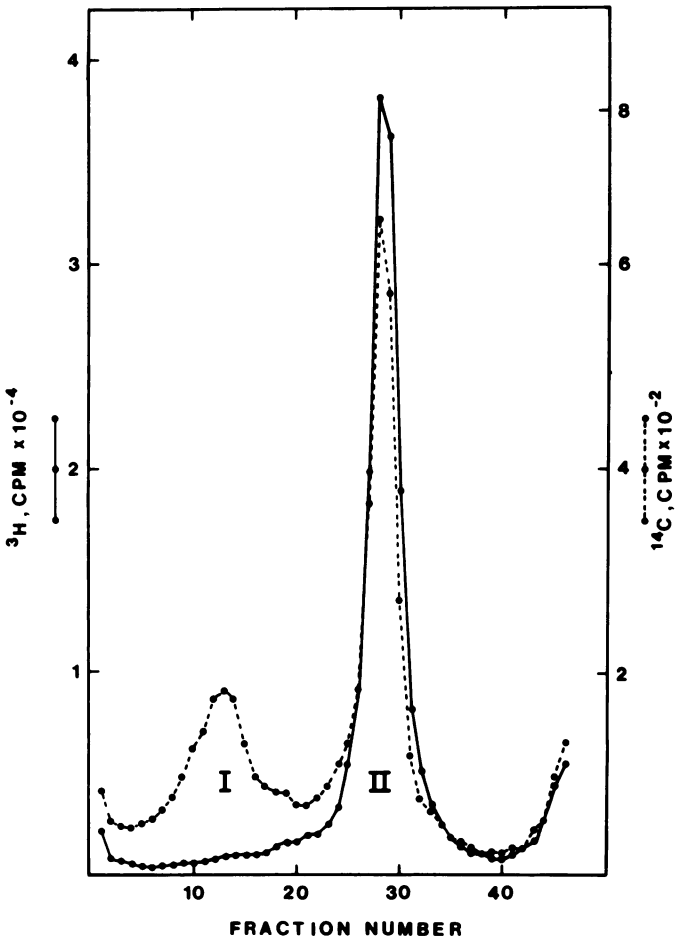

Fig. 5. Sucrose gradient sedimentation profile of Mu*cts*62*Ham*1043 DNA isolated from infected minicells. Minicells were infected with [methyl-^{3}H]-thymidine-labeled Mu*cts*62*Ham*1043 (solid line) induced from a non-suppressing lysogen or with 2-[^{14}C]thymine-labeled Mu*cts*62*Ham*1043 (broken line) induced from a nonsense mutation suppressing lysogen. DNA sedimenation profiles were obtained as described in Figure 1A.

peptide indicate that it is a late gene product and, as the minicell system synthesizes only very limited amounts of Mu late gene products (Magazin *et al.*, 1978; Giphart-Gassler *et al.*, 1981a: note the limited amount of gpT, the major head protein, synthesized in infected minicells as demonstrated in Figure 4), our failure to detect an effect of a mutation on the 64 000-dalton polypeptide may simply be a reflection of the inadequacies of the minicell system for the analysis of Mu late gene expression.

There have been several reports of Mu-encoded activities which may be related to the 64 000-dalton polypeptide described in this report. Chase and Benzinger (1982) demonstrated that a 65 000-dalton polypeptide remained non-covalently bound to Mu DNA following freeze-thaw treatment of Mu particles. The bound polypeptide greatly enhanced transfection apparently by inhibiting *E. coli* exoV activity. It seems likely that the polypeptide shown here to enter the cell with infecting Mu DNA and the exoV inhibitor described by Chase and Benzinger (1982) are the same gene product. It is less clear whether a Mu-encoded exonuclease inhibitor partially purified by Williams and Radding (1981) from induced Mu lysogens is related to the 64 000-dalton polypeptide described here. The properties of this inhibitor, namely a mol. wt. between 40 000 and 80 000 and an ability to bind to the ends of DNA molecules are consistent with the properties of the 64 000-dalton polypeptide but further experiments are required to determine if the exonuclease inhibitor can be purified from virions. Schaus and Wright (1980) also described a Mu-encoded exoV inhibitor; however, it seems unlikely that their inhibitor is related to the 64 000-dalton polypeptide as the DNA encoding this activity was shown to be located in the non-essential region between Mu genes *B* and *C* and all the known polypeptides encoded in this region have mol. wts. of <21 000 (Giphart-Gassler *et al.*, 1981a).

The demonstration that infecting Mu DNA can circularize is an important observation; however, it is not, in itself, sufficient evidence to permit the conclusion that circular DNA molecules are intermediates in Mu integration. Experiments to obtain this evidence and to determine the role, if any, of the 64 000-dalton polypeptide in integration are currently being done. If the circles are intermediates in prophage formation then one additional observation is worth noting. Infecting Mu DNA is flanked by random *E. coli* DNA sequences which are lost during integration. Restriction enzyme digestions and electron microscopic measurements of Mu DNA, phenol extracted from peak I and peak II material (Figure 1), have shown that the *E. coli* DNA sequences are still attached to the Mu DNA in these DNA:protein complexes (results not shown). Thus, if circularization is an intermediate in prophage formation, this step precedes the removal of the *E. coli* DNA sequences.

Materials and methods

Bacterial strains

Strains of *E. coli* used in this study are listed in Table I.

Media and growth conditions

Mu lysogens growing in LB medium (Miller, 1972) at 32°C were shifted, at a cell density of 6 x 10^8 cells/ml, to 43°C for 30 min. Cultures were then shifted to 37°C and shaking continued until lysis occurred. Lysates were filtered by passage through a 0.45 μm cellulose nitrate filter (Gelman Acrodisk, Ann Arbor, MI) and used directly as phage preparations to infect minicells. When more purified or concentrated phage stocks were required, phage were prepared from the lyates by a modification of the CsCl equilibrium centrifugation procedure. CsCl solution [20 g CsCl/12 ml Mu buffer (11.7 g NaCl; 2.4 g Tris-HCl; 0.15 g $CaCl_2$; 9 g $MgSO_4.7H_2O$; 0.1 g gelatin per liter H_2O; pH 7.4)] was slowly mixed with an equal volume of lysate, the mixture centrifuged for 10 min at 12 000 *g*, and the resulting pellet and floating cell debris discarded. The supernatant was centrifuged for 17 h at 32 000 r.p.m. in a Beckman SW50.1 rotor, the phage band which formed during centrifugation was removed and recentrifuged for at least 20 h in the same rotor at 35 000 r.p.m. The phage band was removed and dialyzed against cold (4°C) Mu buffer. Phage stocks (>10^{11} p.f.u./ml) obtained by this technique were stable for several months when stored at 4°C.

Mu phage, containing radioactively labeled DNA, were obtained by removing the growing lysogens from LB medium immediately before the shift to 43°C and resuspending the cells in M9 glucose-minimal salts medium (Miller, 1972) containing 0.2% w/v charcoal treated Difco casamino acids (Difco Lab, Detroit, MI) and 2.5 μg thymine/ml. At 5 min and 10 min after the shift from 43°C to 37°C 20 μCi of [methyl-^{3}H]thymidine (47 Ci/mmol; Amersham Co., Arlington Heights, IL) per ml or 62.5 μCi of [2-^{14}C]thymine (45 mCi/mmol; Research Products International Co., Mount Prospect, IL) per ml were added. Mu phage with radioactively labeled protein components were obtained by resuspending the LB grown lysogens after 15 min at 43°C in M9 minimal salts medium (no glucose) containing 2% v/v LB, 10 mM Tris-HCl (pH 7.5) and 2.5 μg thymine/ml. 10 μl of [^{35}S]methionine per ml (1060 Ci/mmol; New England Nuclear Co., Boston, MA) in methionine assay medium (final concentration 0.1% v/v; Difco Lab, Detroit, MI) were added immediately after the shift from 43° to 37°C. ^{35}S-Labeled phage were purified by at least two sequential CsCl equilibrium density gradient centrifugations before their disaggregation for electrophoresis.

Minicells were prepared from *E. coli* DS410 grown at 37°C in LB as previously described (Reeve, 1979).

Table I. *E. coli* strains used in this study

Name	Genotype	Source/Reference
DS410	*min*A, *min*B, *thi*,λ^s	Reeve, 1979
EB105	△*lac*, △*ara-leu* 498	Bade *et al.*, 1978
QD5003	*sup*F, *mel*, *pro*	O'Day *et al.*, 1979
KMBL1614	*thi*, *gal*K, *trp*::Mu*cts*62	Giphart-Gassler *et al.*, 1981a
MH200	Mu*cts*62*Ham*1043 lysogen of QD5003	O'Day *et al.*, 1979
MH2986	Mu*cts*62*Ham*1519 lysogen of QD5003	O'Day *et al.*, 1979
MH2985	Mu*cts*62*Ham*1932 lysogen of QD5003	O'Day *et al.*, 1979
MH2966	Mu*cts*62*Ham*7100 lysogen of QD5003	O'Day *et al.*, 1979
MH2681	Mu*cts*62*Tam*7327 lysogen of QD5003	O'Day *et al.*, 1979
MH1669	Mu*cts*62*Wam*1011 lysogen of QD5003	O'Day *et al.*, 1979
MH3006	Mu*cts*62*Uam*7220 lysogen of QD5003	O'Day *et al.*, 1979
MH2997	Mu*cts*62*Mam*7251 lysogen of Q1 *thr*, *leu*, *lac*, *ton*A, *sup*E	M.M. Howe
AHP127	*thy*A::Mu*cts*62, *deo* derivative of DS410	This study
AHP134	*thy*A::Mu*cts*62*Ham*1043, *deo* derivative of EB105	This study
AHP137	*thy*A::Mu*cts*62*Ham*1519, *deo* derivative of EB105	This study
AHP138	*thy*A::Mu*cts*62*Ham*7100, *deo* derivative of EB105	This study
AHP142	*thy*A::Mu*cts*62*Ham*1932, *deo* derivative of DS410	This study

*thy*A mutations were selected by resistance to trimethoprim as described by Miller (1972). The *deo* mutation allows growth in the presence of only 2.5 μg thymine/ml.

Minicell and cell infection, lysis and sucrose gradient sedimentation

Minicells (2 x 10^{10}/ml) in LB medium were infected at an input multiplicity of infection (m.o.i.) of 3. Adsorption was allowed for 15 min at 0°C, the infected minicells were then placed at 37°C for 30 min before being exposed to blending (Sorvall Omnimixer; maximum speed setting) for 6 min at 0°C. The blended minicells were washed twice with TES (50 mM Tris-HCl; 5 mM EDTA; 10 mM NaCl, pH 8.0), resuspended at five times the original concentration in TES containing 50 μg lysozyme/ml, frozen and allowed to thaw on ice. Lysis was accomplished by addition of SDS to a final concentration of 0.05% w/v. The lysate was loaded onto a 5–20% w/v neutral sucrose gradient (sucrose dissolved in 0.1 M Tris-HCl; 1 mM EDTA; pH 8 and layered on top of a 0.5 ml shelf of 60% w/v sucrose in the same buffer) and the gradients centrifuged for 2 h at 40 000 r.p.m. (SW50.1 rotor, Beckman Instruments) at 4°C. Fractions (0.1 ml) were collected from the bottom of the gradient on to glass microfiber filters (934-AH, Whatman Inc., Clifton, NJ) which had been soaked in 5% w/v TCA and dried before use. The filters were washed three times with 4 ml of ice-cold 5% w/v TCA, three times with 4 ml of 95% ethanol, dried and the radioactivity retained by the filters measured in a toluene based liquid scintillation counting system.

The above procedure was also used to detect and prepare Mu DNA-protein complexes from infected DS410 cells excepting that infected cells were not blended and the cell lysates were centrifuged (1 min, Eppendorf Model 5412 centrifuge) before being loaded on the sucrose gradients.

Electron microscopy

Fractions from the sucrose gradient were dialysed for 12 h at 0°C against 100 mM Tris-HCl; 1 mm EDTA, pH 8.0 before being spread on grids for electron microscopy. The technique developed by Davis *et al.*, (1971) was used to spread DNA in the presence of formamide and cytochrome c exactly as previously described (Heilmann *et al.*, 1980). DNA spreading in the absence of cytochrome c was accomplished by placing a drop of DNA solution on to a carbon coated grid which was preloaded with a monolayer of cytochrome c (Hartford and Beer, 1972; Abermann and Salpeter, 1979). The cytochrome c monolayer was dry before the DNA solution was placed on the grid. Excess DNA was washed from the grids, the grids floated on a solution of 2% w/v uranyl acetate for 1 min, washed and exposed to shadow casting as previously described (Heilmann *et al.*, 1980). Grids were viewed and photographed using a Zeiss model EM10C electron microscope.

Synthesis and electrophoretic analysis of Mu polypeptides

Mu-infected minicells were allowed to incorporate [^{35}S]methionine and the radioactively labeled polypeptides synthesized in the infected minicells were prepared for electrophoresis exactly as previously described (Giphart-Gassler *et al.*, 1981a). Fluorography and electrophoresis through polyacrylamide gradient gels was as previously described (Reeve and Shaw, 1979). Non-equilibrium isoelectric focusing was used to separate Mu polypeptides for two-dimensional electrophoretic analysis (O'Farrell, 1975). The first dimension was run for 3.5 h at 500 V in 4% w/v acrylamide gels containing ampholytes giving pH ranges 3–10, 5–7 and 6–8 mixed in the ratio 1:3:1.

Mu phage, containing radioactively labeled polypeptides were disaggregated and prepared for electrophoresis as previously described (Giphart-Gassler *et al.*, 1981b).

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References

Abermann,R. and Salpeter,M.M. (1974) *J. Histochem. Cytochem.*, **22**, 845-853.

Bade,E.G., Howe,M.M. and Rawluk,L. (1978) *Mol. Gen. Genet.*, **160**, 89-94.

Breepoel,H., Hoogendorp,J., Mellema,J.E. and Wijffelman,C. (1976) *Virology*, **74**, 279-286.

Chase,C.D. and Benzinger,R.H. (1982) *J. Virol.*, **42**, 176-185.

Davis,R.W., Simon,M. and Davidson,N. (1971) *Methods Enzymol.*, **21**, 413-428.

Engler,J.A., Forgie,R.A. and Howe,M.M. (1980) *Gene*, **10**, 79-83.

Giphart-Gassler,M., Reeve,J.N. and van de Putte,P. (1981a) *J. Mol. Biol.*, **145**, 165-191.

Giphart-Gassler,M., Wijffelman,C. and Reeve,J.N. (1981b) *J. Mol. Biol.*, **145**, 139-163.

Hartford,A.G. and Beer,M. (1972) *J. Mol. Biol.*, **69**, 179-186.

Heilmann,H., Pühler,A., Burkhardt,H.J. and Reeve,J.N. (1980) *J. Mol. Biol.*, **144**, 387-396.

Hershey,A.D., Burgi,E. and Ingraham,L. (1963) *Proc. Natl. Acad. Sci. USA*, **49**, 748-755.

Ikeda,H. and Tomizawa,J. (1968) *Cold Spring Harbor Symp. Quant. Biol.*, **33**, 791-798.

Kahmann,R. and Kamp,D. (1979) *Nature*, **280**, 247-250.

Ljunquist,E. and Bukhari,A.I. (1979) *J. Mol. Biol.*, **133**, 339-357.

Magazin,M., Reeve,J.N., Maynard-Smith,S. and Symonds,N. (1978) *FEMS Microbiol. Lett.*, **4**, 5-9.
Miller,J. (1972) *Experiments in Molecular Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
O'Day,K., Schultz,D., Ericsen,W., Rawluck,L. and Howe,M. (1979) *Virology*, **93**, 320-328.
O'Farrell,P.H. (1975) *J. Biol. Chem.*, **250**, 4007-4021.
Reeve,J.N. (1979) *Methods Enzymol.*, **68**, 493-503.
Reeve,J.N. and Shaw,J.E. (1979) *Mol. Gen. Genet.*, **172**, 271-279.
Schaus,N.A. and Wright,A. (1980) *Virology*, **102**, 214-217.
Thomas,C.A., Kelly,T.J. and Rhoades,M. (1968) *Cold Spring Harbor Symp. Quant. Biol.*, **33**, 417-424.
Williams,J.G.K. and Radding,C.M. (1981) *J. Virol.*, **39**, 548-558.