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Participation of the Host Protein(s) in the Morphogenesis of Bacteriophage P22

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Summary. Spontaneous mutants of *S. typhimurium* resistant to thiolutin are conditionally non-permissive for phage P22 development (Joshi and Chakravorty 1979). At 40° C non-infective phage particles are produced. Phage development in two non-permissive hosts (18/MC4 and 153/MC4) has been studied in detail. The steps at which the phage morphogenesis is interfered with differ in the two mutants. The electron micrograph of the particles produced in the mutant 18/MC4 reveals the presence of normal-looking particles; these particles contain phage DNA, adsorb to the permissive host but fail to inject their DNA. The particles produced in the mutant 153/MC4 which fail to adsorb to the host are found to be tail fibre-less. These observations indicate the involvement of host protein(s) in phage P22 morphogenesis.

Introduction

Bacteriophages having limited genetic content have to depend partially on the host machinery for their development. Conditionally non-permissive host mutants are the best tools to study the involvement of host functions in phage development. Thiolutin-resistant mutants of *Salmonella typhimurium* (isolated in our laboratory) that support poorly phage P22 development at 40° C are thus quite suitable for the purpose. Although the phage DNA and phage specific enzyme, lysozyme, are synthesised normally at a non-permissive temperature in such mutants, the lysate contained very few infectious particles (Joshi and Chakravorty 1979). Of all the thiolutin-resistant mutants isolated, 18/MC4 and 153/MC4 were found to be most defective. The present report deals with the detailed studies carried out with these two mutants.

Materials and Methods

Bacteriophage and Bacterial Strains. The clear plaque-forming mutant (C_1) of phage P22 and its host *S. typhimurium* (strain Nos. 18 and 153) were originally obtained from M. Levine of the Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA. The thiolutin-resistant mutants, 18/MC4 and 153/MC4, were siolated in our laboratory from the wild strain 18 and the histidine requiring mutant strain 153, respectively (Joshi and Chakravorty 1979). For convenience the defective particles produced in 18/MC4 and 153/MC4 will be referred as 18/MC4 and 153/MC4 particles respectively.

Growth Media. The compositions of the minimal media (MM) and Luria broth (LB) were as described by Chakravorty (1970) and Levine (1957) respectively. While growing 153/MC4 in MM the medium was supplemented with 20 μg per ml histidine.

Determination of Phage Titre. Plaque forming units (PFU) were determined by the agar overlay method. Infected cells were treated with chloroform before determining phage titre. Unless otherwise stated, *S. typhimurium* (Strain No. 18) was used as the plating bacterium and incubation was at 37° C.

Preparation of Phage Lysate. Salmonella typhimurium strain 18, a derivative of LT2 was grown in M9CAA (Smith and Levine 1965) up to 2.6×10^8 cells/ml at the desired temperature. Phage were added at a multiplicity of infection (m.o.i.) of 0.10, and incubated with aeration till lysis was complete. Finally, chloroform was added to lyse any unlysed cells.

Purification of the Phage. Bacterial debri were removed from phage lysates by centrifugation at 9,000 r.p.m. for 20 min in a GS3 rotor of the RC5B Sorval centrifuge. The lysate was concentrated by a two phase system using 6.5% polyethylene glycol, 0.2% dextran sulphate and 0.3 M NaCl (Philipson et al. 1960). The phage were finally purified in a stepwise gradient of CsCl (1.4–1.6 gm/cm^3). Centrifugation was done at 38,000 r.p.m. for 60 min (using a SW 50.1 rotor) in the Beckman preparative ultracentrifuge. The phage band was removed by puncturing the tube from the bottom and was dialysed overnight against 0.01 M Tris-HCl, pH 7.4.

Isolation of Phage DNA. To the purified phage (1×10^{14} particles/ml) an equal volume of $0.1 \times$ SSV ($1 \times$ SSV is 0.015 M NaCl, 0.03 M EDTA, pH 8.0) was added and these particles were extracted twice with SSV-saturated phenol. The aqueous layer was collected and the last trace of phenol removed by washing twice with chloroform. DNA was precipitated by adding double the volume of ice-cold 95% ethanol. Ethanol was removed by drying the tube in a vacuum desiccator. The DNA was dissolved in $0.01 \times$ SSV.

EcoRI Endonuclease Cleavage. EndoR *Eco*RI was purified according to the method of Thomas and Davis (1975). The preparation was free of contaminating endonuclease activity as checked

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by assaying for a longer period with excess of enzyme. *Eco*RI digestion was carried out at 37° C for 2 h in a total volume of 20 µl containing 0.5 µg DNA, 100 mM Tris-HCl, pH 7.5, 50 mM NaCl and 5 mM $MgCl_2$. The reaction was terminated by the addition of 10 µl 0.1 M EDTA.

Agarose Gel Electrophoresis. Electrophoresis was carried out in horizontal slab gels of 1% agarose (w/v) prepared in electrophoresis buffer containing 40 mM Tris-HCl, 1 mM EDTA and, 5 mM sodium acetate (pH 8.2) and 0.5 µg/ml ethidium bromide. DNA samples were mixed with glycerol (final concentration 10%) and bromophenol blue (0.005%) and then layered under buffer into the slots of the gels. Just before layering, samples were heated for 5 min at 70° C to disrupt hydrogen bonding between short cohesive ends. Electrophoresis was performed at 100 V (12 mA) for 4 h at room temperature (30° C). The bands were visualised after placing the gels over a U.V. light source (Aaij and Borst 1972, Sharp et al. 1973). The gels were photographed with a polaroid land camera using an orange filter and polaroid type 667 coaterless black and white land films.

Electron Microscopy. The samples were negatively stained with uranyl acetate. Micrographs were taken in a Philips EM200 with an operating voltage of 60 kV at the University of Leeds, England.

Preparation of ^{32}P-Labelled Phage Particles at 40° C (non-permissive temperature). ^{32}P-labelled phage were prepared according to the method of Botstein (1968) with the modification that the cells were grown at 40° C and 2 µCi $^{32}PO_4^{---}$ were added per ml. The phage particles present in the lysate were concentrated by high speed centrifugation and purified through a caesium chloride gradient.

Preparation of ^{14}C-Labelled Phage Particles. Cells were grown in MM at 40° C from an overnight culture. Exponentially growing cells at a density of 2.6×10^8 cells per ml were infected with phage P22 C_1 at an m.o.i. of 10. Five minutes after infection ^{14}C-chlorella hydrolysate (42 mCi per atom of C) was added to the growth medium (2 µCi per ml). Casamino acids (0.15%) were added simultaneously. After lysis the phage particles were concentrated and purified as mentioned above.

Measurement of the Rates of Incorporation of ^{14}C-Uridine into Trichloroacetic Acid-Insoluble Fraction. The measurement was done as described earlier (Chakravorty and Bhattacharya 1971).

Results

Antisera Neutralising Capacity of the Lysates Produced at Non-Permissive Temperatures

It was reported earlier that the lysates obtained by infecting the mutants 18/MC4 and 153/MC4 with P22 contain mostly non-infectious particles (Joshi and Chakravorty 1979). To check whether the particles produced by these mutants are tail fibre-less and hence non-infectious, the antisera neutralising capacities of the lysates prepared from the wild and the mutant strains were compared. The results presented in Table 1 indicate that the antisera neutralising capacity of the lysate prepared from the P22-infected 18/MC4 is quite comparable to that of the parent strain 18 whereas the antisera neutralising capacity of the lysate obtained from 153/MC4 is comparatively much less. At 30° C the antisera neutralising capacities of the four different types of lysate are comparable (data not presented). Thus it appears that tail fibre synthesis is reduced in the host 153/MC4 at 40° C.

Table 1. Antisera neutralising capacity of the lysates prepared at non-permissive temperature (40° C)

Lysate prepared from	Number of tester phage present
–	1.7×10^6
18	8.5×10^7
18/MC4	7.5×10^7
153	8.0×10^7
153/MC4	4.5×10^6

Lysates were obtained by infecting cells growing exponentially in L.B. at 40° C, with phage P22 (C_1) at an m.o.i. of 10. To 0.9 ml P22 antiserum (K = 2) was added 0.1 ml various lysates to be tested. In control experiments no lysate was added. The mixture was incubated at 37° C for 15 min. The mixtures were then tested for the residual antiserum activity by adding 0.1 ml tester phage (P22 C^+). The number of tester phage added was 2.5×10^9 PFU), phage P22 C^+ was used as the tester phage as it produces turbid plaques and can be distinguished from the phage (clear mutants) produced in the lysates to be tested. The extent of inactivation of P22 antisera by the lysates obtained can be compared by measuring the number of tester phages remaining at the end of the second incubation.

When lysates prepared from all these strains at 30° C were used in similar experiments the number of tester phage present varied from 7.5×10^7 to 8.2×10^7

Electron Micrographs of the Defective Particles

To characterise the particles produced in 18/MC4 at 40° C, their morphology was studied under an electron microscope. The particles produced from 18/MC4 under non-permissive conditions appear normal (Fig. 1). The tail fibres are distinctly visible as spike-like protrusions. In contrast, the particles produced in 153/MC4 mostly lack tail fibres (Fig. 2). They look like the tail fibre-less particles produced by P22 9^- phage (Berget and Poteete 1980). An electron microscopic survey of the particles produced in 153/MC4 indicated that less than 5% particles possessed tail fibres. Figure 3 shows defective particles as well as normal particles. The difference in morphology is distinctly visible.

Adsorption of the Defective Particles

It may be argued upon that apparently normal-looking particles produced in 18/MC4 host may be defective in one or more tail fibre proteins and thus do not adsorb to the host and fail to carry out the infection. So the extent of adsorption of such particles to the permissive host was studied (Table 2). ^{32}P as well as ^{14}C-labelled 18/MC4 and 153/MC4 particles were prepared separately by infecting the requisite strains at a non-permissive temperature. These particles were individually added to the permissive host (Strain 18) at an m.o.i. of 10. After 10 min the infected cells were collected on millipore filters, washed with medium, and the radioactivity associated with the cells determined on the filters. The results indicate that the defective particles produced in 18/MC4 adsorb to the host to the same extent as the normal phage P22 whereas those produced in 153/MC4 adsorb to a much less extent.

EcoRI Cleavage Fragments of DNA Isolated from Normal and Detective Phage Particles

To ascertain whether the defective particles contain phage or bacterial DNA, the DNAs isolated from such particles were

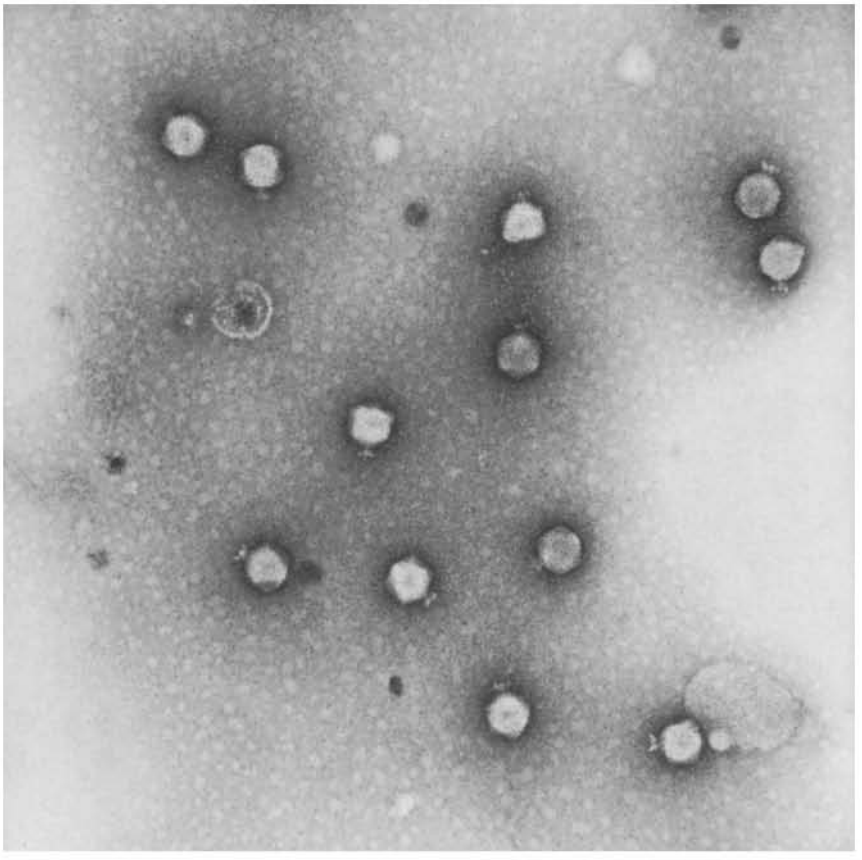

Fig. 1. Electron micrograph of particles produced in the strain 18/MC4. Magnification ×160,000

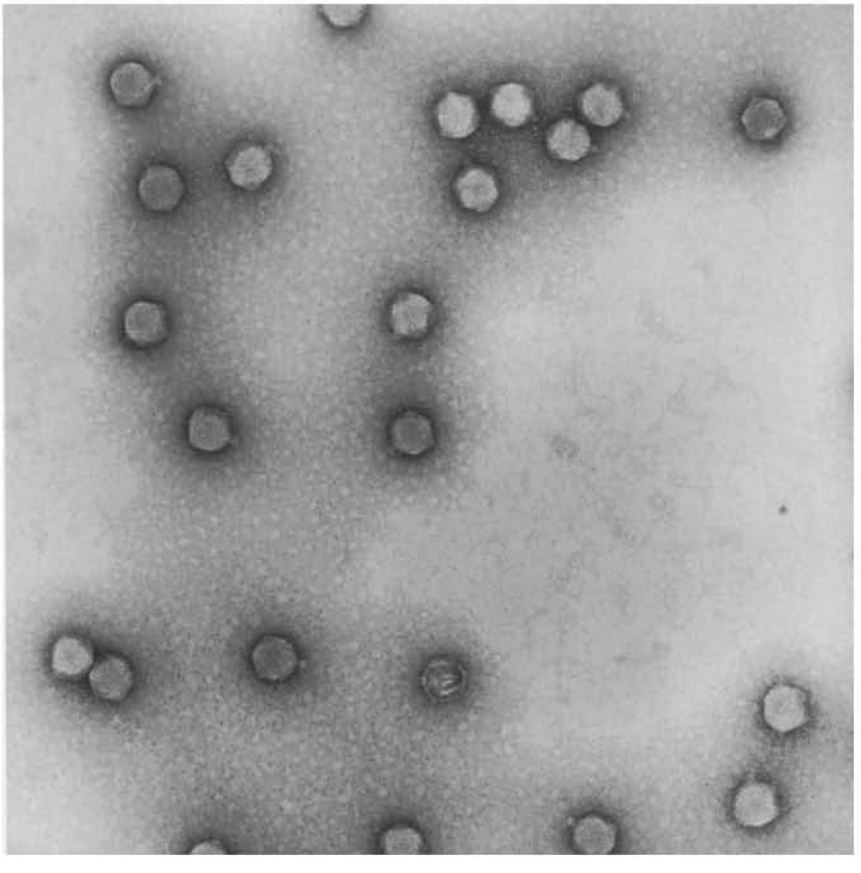

Fig. 2. Electron micrograph of particles produced in the strain 153/MC4. Magnification ×160,000

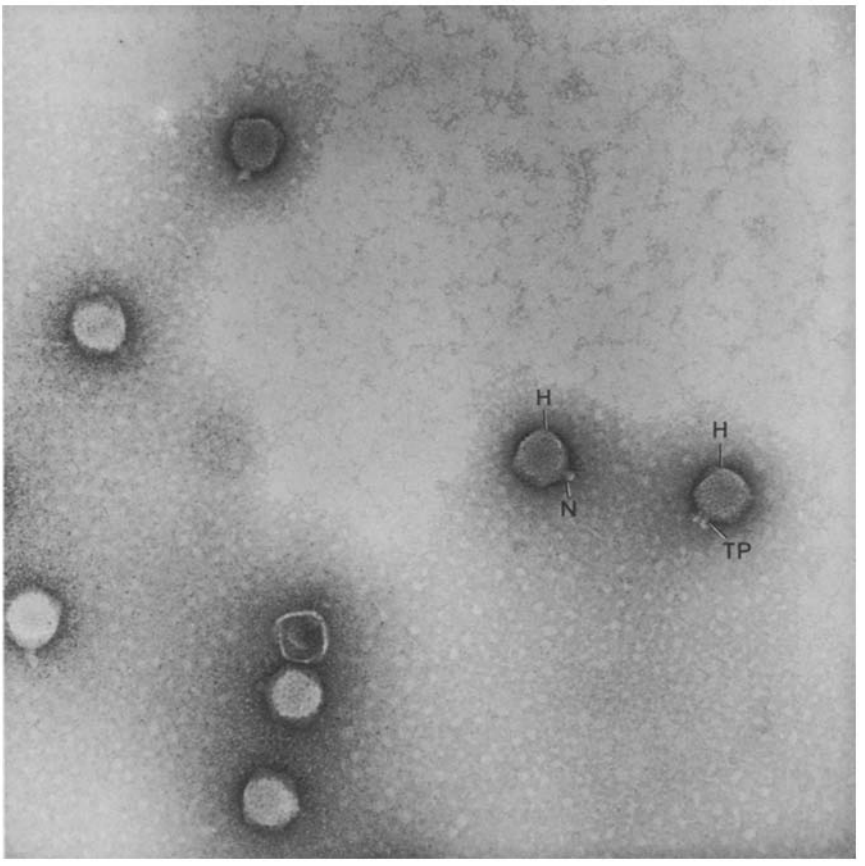

Fig. 3. Normal and tail fibre-less particles of phage P22. Magnification ×200,000. *H* Head, *n* neck, *TP* tail protein

Table 2. Extent of adsorption of the particles produced in the strains 18/MC4 and 153/MC4 at non-permissive temperature

Particles produced from strain	Counts added		Counts adsorbed to the cells		% of counts adsorbed	
	^{32}P	^{14}C	^{32}P	^{14}C	^{32}P	^{14}C
18	6.6×10^4	2.1×10^3	4.4×10^4	1.5×10^3	65	70
18/MC4	2.6×10^4	1.0×10^3	1.6×10^4	1.2×10^3	61	63
153	3.0×10^4	2.0×10^3	1.8×10^4	1.3×10^3	60	65
153/MC4	2.7×10^4	1.8×10^3	4.7×10^3	2.8×10^2	17	15

Permissive strain 18 was infected (m.o.i. = 10) with different types of particles as indicated above. Details of the experiments are described in the text

treated with the restriction endonuclease, *Eco*RI. The digestion pattern was compared with the *Eco*RI digestion fragments produced from normal (infective) P22 phage DNA. The results presented in Fig. 4 clearly indicate that the digestion fragments of phage P22 DNA and the DNA obtained from the defective particles are identical. The fragments produced are as reported by Helling et al. (1974).

Do the Normal Looking 18/MC4 Defective Particles Inject Their DNA into the Host?

The next step in phage development after adsorption to the host is the injection of the phage genetic material into the host. The 18/MC4 particles possess tail fibres, adsorb to the host and contain phage DNA. So it was determined whether or not these particles inject their DNA. As phage P22 does not have a tail it is rather difficult to remove the empty phage capsids adsorbed to the host after the DNA is injected. An indirect method was adopted to find out whether the defective particles inject their DNA or not. It has been demonstrated in our laboratory that a successful injection of phage P22 DNA results in transient depression of cellular transport processes (Bandyopadhyay et al. 1979). Therefore the rate of uptake of extracellular uridine by strain 18 infected with defective particles 18/MC4 was followed. The particles were added at a very high m.o.i. to detect the effect of infection, if any. Even at an m.o.i. of 40 there was no inhibition in the rate of uptake (Fig. 5). The results clearly demonstrate that the cellular transport process is not affected when cells are infected with the defective particles. Thus it appears that although these particles adsorb to the host yet their DNA is not injected. As 153/MC4 particles are mostly fail fibre-less such experiments were not carried out with 153/MC4 particles.

Discussion

As mentioned already, thiolutin-resistant mutants of *S. typhimurium* are conditionally non-permissive hosts for phage P22. The growth of P22 in these mutants at a non-permissive temperature (40° C) results in the release of a nearly normal burst of non-infectious particles (Chakravorty and Joshi 1979). The defects in these mutants lie in morphogenesis, although the blockages are at different steps. As all the thiolutin resistant mutants isolated in a random fashion from different sets of experiments are conditionally non-permissive hosts (Joshi and Chakravorty

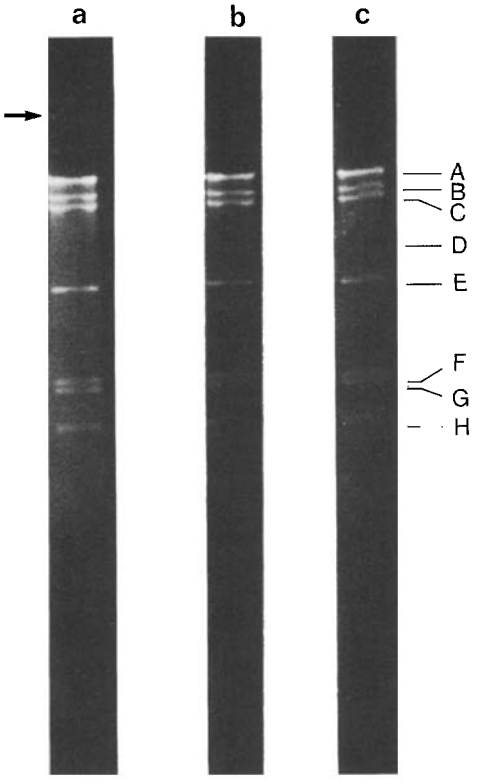

Fig. 4. Agarose gel electrophoresis of *Eco*RI endonuclease cleavage products of (a) P22 (b) 18/MC4 (c) 153/MC4 DNAs. Agarose gel electrohporesis was performed as described in Materials and Method. The bands are labelled by capital letters in order of increasing electrophoretic mobility according to Smith and Nathans (1973). The arrow indicates the point of application of the samples

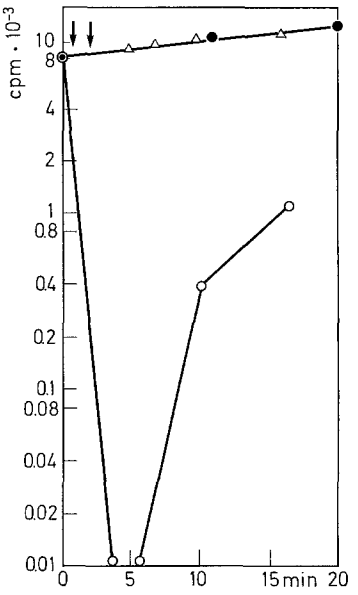

Fig. 5. Rate of incorporation of exogenous uridine into acid precipitable material by cells after infection with defective particles produced in 18/MC4. Cells (2.6×10^8 per ml) growing exponentially in MM were divided into three batches. One batch was infected with the defective particles at an m.o.i. of 40, and another batch was infected with normal phage at an m.o.i. of 10. The third batch was kept as an uninfected control. Symbols, (●) uninfected; (△), infected with defective particles; (○), infected with normal particles. Arrows indicate the times of infection. The left arrow denotes the time of infection with normal particles while the right one indicates the time of infection with defective particles. At every time incorporation was carried out for 1 min only

1979), it appears that thiolutin resistance leads to an alteration in host protein(s) which can not function efficiently at a higher temperature. Moreover, such proteins do not interfere with the growth of the bacteria (as they can grow at 40° C) but are essential for phage morphogenesis. It may also be quite possible that at a higher temperature host proteins which are involved in phage morphogenesis, like the heat shock proteins reported by Neihardt and Van Bogelene (1981) are defective in these mutants. At 40° C the mutant 153/MC4 produces mostly tail fibre-less particles which are consequently non-infectious. These capsids are not empty but contain phage DNA. The particles produced in the other mutant 18/MC4 are still more interesting. These particles are, however, morphologically normal, they contain phage DNA, can adsorb to the host but cannot inject the DNA and are thus non-infectious. Thiolutin-resistant mutants of *Escherichia coli* have been reported (Sivasubramanian and Jayaraman 1976). These mutants map at two loci near *dnaZ* and *rho*. The presence of both mutations in the same cell makes the cell thiolutin resistant in minimal medium. Either of them alone confers conditional auxotrophy in the presence of the drug (Sivasubramanian and Jayaraman 1980). The map position of the thiolutin-resistant mutations of *Salmonella typhimurium* so far reported (Joshi and Chakravorty 1979) is not known. Like the *E. coli* mutants harbouring a single mutation, the *Salmonella typhimurium* mutants used in this study exhibit conditional auxotrophy in the presence of thiolutin. Unfortunately, the mode of action of thiolutin is not definitely known (Kachatourians and Tipper 1974; Sivasubramanian and Jayaraman 1976). Although it was postulated that thiolutin interferes with RNA synthesis in *E. coli*, the inhibition of *E. coli* RNA synthesis by thiolutin could not be demonstrated in vitro. Experiments carried out in our laboratory indicate that the drug has multiple sites of action. It interferes with the cellular transport process(es). It also preferentially inhibits phage gene expression (data not presented). The mutant 153/MC4 is most probably a permeability mutant, the resistance being due to impermeability towards thiolutin. The nature of the mutation in 18/MC4 is not known at present.

E. coli mutants incapable of supporting phage development at higher temperatures have been reported (Pulitzer and Yanagida 1971; Coppo et al. 1973; Georgopoules et al. 1973; Engelberg Kulka et al. 1979). Such mutants of *Salmonella typhimurium* apart from the present one have not been reported. These mutants do not resemble the mutants of *E. coli*. The results presented in this paper indicate the involvement of some host cell proteins in phage morphogenesis. In these mutants such proteins may not be synthesised at their normal high frequency at 40° C or may be inactive.

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