A Late Exclusion of Bacteriophage T4 Can Be Suppressed by *Escherichia coli* GroEL or Rho

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ABSTRACT

A *litCon* mutation in *Escherichia coli* TU6 results in exclusion of bacteriophage T4 during the late, morphogenetic stage of its development at low temperatures. DNA was synthesized continuously in the infected cells, but less than 10% of the DNA made by 90 min after infection was packaged into DNAase-resistant particles, few viable phage were formed, and the cells lysed poorly. The exclusion could be relieved by conditions leading to elevated levels, determined immunologically, of the *E. coli* Rho protein (believed to be involved in regulation of T4 transcription), or chromosomally encoded *E. coli* GroEL (a chaperone known to be involved in phage assembly), or by supplying GroEL in *trans* from a plasmid. The two suppressing proteins appeared to act independently of each other. GroEL-suppression restored packaging to normal levels, perhaps by preventing GP23 from activating the host Lit protein; in addition DNA synthesis was delayed and reduced and cell lysis enhanced, demonstrating involvement of GroEL in both these processes. Rho suppression was less efficient. Since both transcription-termination-proficient and transcription-termination-deficient Rho suppressed, the results raise the possibility that Rho has a role during T4 development not directly involving transcription regulation.

THE *Escherichia coli* Rho protein is needed continuously during T4 development in *E. coli* (STITT *et al.* 1980), though the reason(s) for this are not well understood. The well known role of this protein is in the regulation of gene expression, affecting transcription termination. Many lines of evidence suggest that it participates in the regulation of T4 early expression (GOLDBERG 1970; RICHARDSON 1970; JAYARAMAN 1972; LINDER and SKÖLD 1980; DAEGELEN *et al.* 1982a,b; HINTON 1989; STITT and MOSIG 1989); the data indicating a similar role for Rho in the regulation of T4 late expression are less clear-cut (STITT *et al.* 1980). Rho mutants are known to have pleiotropic defects, not all of which are correlated to the extent of transcription termination deficiency; our previous investigation (LINDER and CARLSON 1985) suggested the possibility that Rho might have (an)other role(s) during T4 development than transcription regulation.

The *E. coli* strain TU6 is a derivative of W3110 carrying several polar mutations, designed for studies of the effect of *rho* mutations on transcription termination (KORN and YANOFSKY 1976; LINDER and CARLSON 1985). This strain inhibits T4 growth at low temperatures, manifested mainly as premature termination of phage production and poor lysis. Intermediates in the construction series from W3110 to TU6 permit essentially normal growth of T4 (LINDER and CARLSON 1985). A T4 Rid (*Rho* interaction deficient) mutant, which carries a mutation in the lysis gene *t* but induces normal lysis in most genetic backgrounds, completely fails to lyse TU6 (LINDER

and CARLSON 1985). The growth and lysis inhibition could be suppressed either by the presence of a chromosomal *rho* mutation resulting in reduced Rho-dependent transcription termination, or by a multicopy plasmid expressing wild-type Rho (LINDER and CARLSON 1985). To better understand the role of Rho in this suppression we have analyzed the cause of the T4 growth inhibition in this strain and extended the study to another *E. coli* protein, GroEL, known to be involved in T4 morphogenesis (BINKOWSKI and SIMON 1983) and absolutely required for growth of T4 at 37° (GEORGOPOULOS *et al.* 1972) as well as at 44° (WIBERG *et al.* 1988).

GroEL, encoded by the gene *groEL* (also known as *mopA*), belongs to a group of proteins that has been called molecular chaperones, which are involved in the final folding and oligomerization of newly synthesized peptides, in assembly of multiprotein complexes and in peptide transport through membranes (HORWICH *et al.* 1990). GroEL may constitute about 1% of the total cell protein at 30°, and the relative amount may be increased up to 10-fold upon heat-shock (HERENDEEN *et al.* 1979). When present in high amounts GroEL can correct the misfolding of proteins due to amino acid substitutions, either by preventing altered proteins from misfolding or directing misfolded proteins back to their normal configuration (VAN DYK *et al.* 1989). KUMAMOTO (1991) has suggested that GroEL, together with GroES, directs defective proteins which cannot associate properly with other proteins, for example in phage assembly, toward proteolysis. Together with the phage-coded GP31

TABLE 1

Strains

Strain	Parental strain	Relevant genotype	Source and reference
Bacteria			
AG2226	MC4100	<i>F⁻</i> , <i>araD</i> , $\Delta(\text{argF-lac})U169$, <i>rpsL</i> , <i>relA</i> , <i>flbB</i> , <i>deoC</i> , <i>ptsF</i> , <i>rbsR</i> , <i>rpoH⁺</i>	C. GROSS (KUSUKAWA and YURA 1988)
CAG9333	MC4100	As AG2226, $\Delta\text{rpoH30::Kan zhf50::Tn10}$, <i>suhX401</i> ($\Delta\text{pF13-PrpoD}_{\text{hs-lacZ}}$)	C. GROSS (KUSUKAWA and YURA 1988)
CAG18488	MG1655	<i>zjd-2231::Tn10</i> (93.75 min)	C. GROSS (SINGER <i>et al.</i> 1989)
JM101 <i>lit-6</i>		<i>F'</i> , <i>lit(Con)</i>	L. SNYDER (YANNISCH-PERRON <i>et al.</i> 1985)
RS3032		<i>purB58</i> , <i>fadR613::Tn 10</i> (25.75 min), <i>dadR1</i> , <i>trpA62</i> , <i>trpE61</i> , <i>tna-5</i>	B. BACHMANN (SIMONS <i>et al.</i> 1980)
RS3242		<i>zcf-117::Tn10</i> (25.25 min), <i>dadR1</i> , <i>trpA62</i> , <i>trpE61</i> , <i>tna-5</i>	B. BACHMANN
SKB178	K12	<i>sup^o</i> , <i>galE</i> , <i>mcrA</i> , <i>mcrB</i>	B. STITT (STITT <i>et al.</i> 1980)
HDF40.08	SKB178	As SKB178 but <i>rho40.08</i> (HDF)	B. STITT (STITT 1978)
TU29	HDF40.08	As HDF40.08 but all or part of <i>rho</i> from TU6; <i>rho6</i> . P1 transduction from TU6	This work
TU30	HDF40.08	As HDF40.08 but part of <i>rho</i> from TU6 P1 transduction from TU6	This work
OF218	SKB178	<i>galE</i> , <i>recA56</i> , <i>srl::Tn10</i>	C. GEORGIOPOULOS (WIBERG <i>et al.</i> 1988)
W3110	K12	<i>supE</i> , <i>F⁻</i>	C. YANOFSKY (LINDER and CARLSON 1985)
TU6	W3110	<i>trpR</i> , <i>trpE</i> , <i>trpA</i> , <i>lacZU118</i> , <i>val^R</i> , <i>rho6</i> , <i>azi^R</i> , <i>sup^o</i> , <i>F⁻lit106</i>	LINDER and CARLSON, (1985) this study
TU62	TU6	As TU6 but <i>Tn10</i> (25.25 min), <i>lit⁺</i> . GT7 transduction from RS3242	This study
TU63	TU6	As TU6 but <i>Tn10</i> (25.75 min). GT7 transduction from RS3032	This study
TU8	TU6	As TU6 but <i>rho102</i>	LINDER and CARLSON (1985)
TU20	TU6	As TU6 but <i>rho104</i>	LINDER and CARLSON (1985).
B		<i>sup^o</i>	
Bacteriophage			
T4D		Wild type	J. WIBERG
T4 Rid394 $\times$ 4	T4D	<i>t⁻</i> (lysis-defective)	LINDER and CARLSON (1985)
T4 goF1	T4D	<i>goF</i> (altered interaction with <i>E. coli</i> Rho)	B. STITT (STITT <i>et al.</i> 1980)
T4 gol6B	T4D	<i>gol</i> (grows on Lit)	L. SNYDER (COOLEY <i>et al.</i> 1979)
T4 go6	T4D	<i>go6</i> (grows on TU6)	This study
T4 GT7	T4B/D hybrid	<i>42amC87</i> , <i>56amE51</i> , <i>rII-denBANB5060</i> , <i>alcW7</i> ; fails to express T4 endonuclease II	G. WILSON (WILSON <i>et al.</i> 1979)
T6 <i>gt</i>	T6	<i>gt</i>	E. RALEIGH

(which probably substitutes for GroES), GroEL plays a major role in the formation of GP23* (the main phage head protein present in about 960 copies per phage head) from GP23, and in the assembly of new phage particles [reviewed *e.g.* by BINKOWSKI and SIMON (1983) and GEORGIOPOULOS and LINDER (1994)]. In the absence of functional GroEL phage head assembly is impaired (GEORGIOPOULOS *et al.* 1972), and the DNA is not packaged normally (WIBERG *et al.* 1988).

Our present results show that the poor phage production is correlated to a defect in packaging phage DNA into complete (DNase resistant) particles; in addition, as shown before, lysis is poor. These defects are caused by a *litCon* mutation in TU6. Both GroEL and Rho can compensate for the growth deficiency, apparently independently of each other, by suppressing these two defects to varying extents.

MATERIALS AND METHODS

Chemicals and enzymes: [³H]dThd was from Amersham. Nitrocellulose membranes (NCM) 0.45- μ m pore size were from Millipore, Bedford, Massachusetts. Affinity-purified al-

kaline phosphatase-conjugated rabbit anti-mouse immunoglobulin was obtained from Dakopatts, AB, Glostrup, Denmark, DNase I (RNase free), RNase and low molecular weight standard proteins were from Pharmacia, Uppsala, Sweden. Brilliant Blue R was purchased from Sigma. 3-Decynoyl-N-acetyl cysteamine (D-NAC) was a kind gift from Dr. John M. Schwab, West Lafayette, Indiana. Hypoxanthine-aminopterin-thymidine (HAT) medium was from Flow Laboratories Ltd., Irvine, Scotland. All other enzymes and reagents used in this work were commercial products of purest grade available.

Strains and growth media: Strains of bacteria, bacteriophage and plasmids are listed in Tables 1 and 2. Bacteria were grown in Luria broth (LB) or glycerol-casamino acids medium (GCA; FRASER and JERREL 1953). Ampicillin, chloramphenicol or tetracycline were added to plasmid-containing strains at concentrations of 50, 200 or 20 μ g/ml, respectively. Plasmids were handled by conventional methods (SAMBROOK *et al.* 1989). Hybridomas were grown in HAT medium.

Transductions: P1 transductions were carried out as described by MILLER (1992). Transducing T4 lysates were prepared by infecting logarithmically growing cells in LB with T4 GT7 at an moi of 10, lysing the cells with chloroform 90 min later. This transducing lysate was used to infect logarithmically growing recipient cells in LB at an moi < 1. After 30 min of incubation the cells were collected by centrifugation and either spread directly onto selective plates (when transducing to

TABLE 2

Plasmids

Plasmid	Relevant genotype	Source or reference
pJG32	6.9-kbp <i>gppA</i> , <i>mmrA</i> , <i>trxA</i> , <i>rho</i> HindIII fragment cloned in pBR322	D. CALHOUN (GRAY <i>et al.</i> 1981; DANIELS <i>et al.</i> 1992)
pNT29Δ56	2.0-kb <i>rho</i> PvuII-BamHI insert in pBR322 under λ p _L control	K. SHIGESADA (SHIGESADA <i>et al.</i> 1984; DANIELS <i>et al.</i> 1992)
pNT204	λ c1857 in pSC101	K. SHIGESADA (SHIGESADA <i>et al.</i> 1984)
pPMR5	45% of promoter proximal part of <i>rho</i> in a 3.4-kb <i>gppA</i> , <i>mmrA</i> , <i>trxA</i> , <i>PstI</i> -insert into pBR313	M. RUSSEL (RUSSEL and MODEL 1984; DANIELS <i>et al.</i> 1992)
pPMR12	<i>trxA</i> EcoRI-PvuII insert in pGL101 under p _{lacUV5} control	M. RUSSEL (RUSSEL and MODEL 1984)
pMK333	<i>rhIB</i> reading frame cloned in p _{tac} expression vector pKK223-3	M. CASHEL (KALMAN <i>et al.</i> 1991)
pOF14	<i>groEL</i> ⁺ , <i>mopB</i> 314 8.1-kb EcoRI insert into pBR322	J. WIBERG (FAYET <i>et al.</i> 1986)
pOF15	<i>groEL</i> ⁺ , <i>mopB</i> 315, 8.1-kb EcoRI insert into pBR322	J. WIBERG (FAYET <i>et al.</i> 1986)

Plasmid pNT29Δ56 was constructed after Bal31 deletion, the *rho* reading frame is expressed from the λ pL promoter. Plasmids pOF14 and pOF15 both carry the wild type *mopA* (*groEL*) allele but carry different mutations in the *mopB* (*groES*) gene.

strains permissive for growth of this T4 mutant) or washed and resuspended in twice the original volume of LB, whereafter incubation was continued for 2 hr before harvesting by centrifugation and spreading on selective plates.

Antibody preparation: Purified Rho, the generous gift of JOHN P. RICHARDSON, was used as antigen in the production of monoclonal anti-Rho antibodies according to the method of KÖHLER and MILSTEIN (1975). The immunization was performed as described by HOLMDAHL *et al.* (1985), immunizing five Balb/c mice each with 20 μ g of Rho protein in phosphate-buffered saline (PBS) containing 60% Freund's complete adjuvant and 0.22% sodium dodecyl sulfate, and 2 weeks later with the same amount of Rho in incomplete adjuvant. Hybridomas were prepared in a conventional way (KÖHLER and MILSTEIN 1975), and several hybridoma clones producing anti-Rho antibodies were selected, using purified Rho, and tested for their ability to react in enzyme-linked immunosorbent assay (ELISA) and Western immunoblot procedures. Polyclonal antibodies toward GroEL were prepared by WILLIAM WICKNER, Salt Lake City, Utah, and were a kind gift from GUNNAR VON HEIJNE, Huddinge, Sweden.

DNA synthesis and packaging: Cells were grown in GCA at 30° to about 3×10^8 cells/ml and infected with T4 at an moi of 8. Newly synthesized DNA was labeled continuously by adding [³H]dThd to 10 μ Ci/ml and dAdo to 250 μ g/ml 4 min after infection. Samples were removed at intervals for estimation of trichloroacetic acid (TCA)-insoluble material as described by CARLSON and WIBERG (1983). After 90 min the infected cultures were lysed with chloroform. The chloroform was removed 7 min later, a fresh solution of pancreatic DNase was added 98 and 110 min after infection, each time to a final concentration of 2 μ g/ml, and samples were again removed at intervals for estimation of TCA-insoluble material. Only DNA packaged inside mature phage heads resist this DNase treatment (WIBERG *et al.* 1988).

Electrophoresis of proteins: Proteins were precipitated with TCA from 1 ml of cell culture containing about 2×10^8 cells grown in LB, processed essentially as described by CARDILLO *et al.* (1979), and separated on SDS-polyacrylamide gels as described by LINDER and CARLSON (1985). The concentrations of acrylamide and *N,N'*-bismethylene-bisacrylamide in stacking and separating gels were 4.5 and 4.5%, and 14.9 and 1%, respectively. The gels were electrophoresed at 50 V/13 cm until the bromophenol blue dye front reached the bottom of the separating gel. After electrophoresis the proteins were stained with Coomassie Brilliant Blue and dried between cellophane sheets, or transferred to nitrocellulose membranes (NCM) by electroblotting for 3 hr in 50 mM NaH₂PO₄, pH 6.5.

Immunoblotting: Each electroblotted NCM was soaked in

1% bovine serum albumin in PBS (8 g NaCl, 0.2 g KCl, 0.20 g KH₂PO₄ and 1.15 g Na₂HPO₄ per liter) for 3 hr at 37°, to block uncoated sites. After washing three times in TPBS (0.05% Tween 20 in PBS) the membranes were incubated with 50 μ l anti-Rho antibodies (cell culture supernatants diluted 1:1000 in PBS) or anti-GroEL antibodies (antisera diluted 1:2500) in PBS for 2 hr at 37° and washed again. The membranes were incubated for 3 hr with anti-immunoglobulin antibodies conjugated to alkaline phosphatase (diluted in TPBS containing 0.5% bovine serum albumin (BSA) to a concentration sufficient to saturate the primary antibody). A freshly prepared solution of 25 mg naphthol AS-MX phosphate and 10 mg Brilliant Blue R (BB) per 100 ml of Tris-HCl (100 mM pH 9.6) was added and the incubation continued until bands could be seen. The membranes were then washed with water for 15 min and kept at room temperature between Whatman filters until dry.

ELISA assays: Cells were grown in 25 ml GCA at 30° to about 3×10^8 cells/ml, harvested by centrifugation, washed once in a buffer containing 40 mM Tris base, 40 mM boric acid, 55 mM EDTA, 5% β -mercaptoethanol, pH 8.6, centrifuged again and resuspended in 250 μ l of the same buffer. The suspensions were sonicated, and SDS and sucrose were added to 2% (w/v) final concentration each (final volume 300 μ l). The extracts were incubated at 45° 30 min, frozen on dry ice/ethanol and kept at -70° until use. The amount of protein was determined by the method of BRADFORD (1976). Each well of a microtiter plate was coated overnight at 4° with 50 μ l cell extract. Uncoated sites were blocked with 100 μ l 1% BSA in a buffer containing 1.59 g Na₂CO₃, 2.93 g NaHCO₃ and 200 mg NaN₃ per liter. After 3 hr the wells were washed three times with TPBS, 200 μ l/well. Anti-Rho or anti-GroEL antibodies were added to the wells (50 μ l of the same dilutions used for immunoblots) and incubated for 3 hr at 37°. The wells were again washed three times as before. After this, 50 μ l appropriately diluted alkaline phosphatase conjugated rabbit anti-mouse immunoglobulin were added to each well, followed by incubation for 2 hr and washing as before. Finally 50 μ l per well of freshly prepared paranitrophenyl phosphate (1 mg/ml) in a buffer containing per liter 97 ml diethanolamine, 101 mg MgCl₂·6H₂O, and 200 mg NaN₃, pH 9.8, were added. The samples were kept dark at 30° until the absorbance at 405 nm of the standard samples with the highest concentration reached 2.

RESULTS

Suppression of the T4 growth defect in TU6 by Rho: The low temperature inhibition of T4 growth in TU6 is

FIGURE 1.—Map of the *rho* region at 84.5 min of the standard *E. coli* K-12 map (BACHMANN 1990), about coordinate 3740 on the W3110 map (KOHARA *et al.* 1987), based on RUSSEL and MODEL (1984) and DANIELS *et al.* (1992), showing from top to bottom restriction sites, kilobase distance from the leftmost *Pst*I site, genes (open boxes) and regions carried by different plasmids (black boxes). Note that the region cloned in pJG32 extends an additional 2 kbp to the left, into the *rep* gene. P, *Pst*I; Pu, *Pvu*II; Hc, *Hinc*II; Hd, *Hind*III.

suppressed in the presence of a polarity-suppressing *rho* mutation (LINDER and CARLSON 1985), or plasmids that carry the entire *rho* gene, such as pJG32 (LINDER and CARLSON 1985), or pNT29Δ56 (Figure 1, Table 3). Both these plasmids are constructed from strains carrying a wild-type *rho* gene; we confirmed (data not shown) that both plasmids restore polarity in the *trp* operon when introduced into a strain carrying a polar *trpE* mutation and in addition a transcription-termination deficient *rho* allele. In plasmid pNT29Δ56 the *rho* gene is expressed from the λ *p_L* promoter. This promoter is repressed at 30° in the presence of the λ *cI857* repressor (strain TU6·pNT204·pNT29Δ56), but active at 30° in the absence of this repressor (strain TU6·pNT29Δ56). The lack of suppression in the former strain, and the good suppression in the latter (Table 3), argues strongly that suppression is indeed due to Rho. Interestingly, plasmid pPMR5, which contains genes *mmrA* (also known as *rhIB*) and *trxA* in addition to sequences encoding the N-terminal 45% of Rho, restored plaque formation by wild-type T4, though not by the *t* mutant. Plasmids carrying only the *trxA* or *mmrA/rhIB* genes failed to suppress, suggesting that the suppression by pPMR5 is due to the *rho* fragment (Figure 1, Table 3).

T4 growth inhibition in TU6 is not due to decreased levels of Rho or high membrane content of unsaturated fatty acids: Both the polarity-suppressing *rho* mutation and the multicopy plasmids expressing Rho would be expected to increase cellular Rho levels. Thus, it was possible that the level or availability of Rho in TU6 was too low for some essential process during the termination of development (LINDER and CARLSON 1985). The amount of Rho in uninfected cells was quantitated immunologically, and results are shown in Table 4. Although increased Rho levels were observed, as expected, in strains carrying a chromosomal transcription-termination defective allele of *rho* (*rho104* in strain TU20), or a plasmid (pJG32) which carries the wild-type *rho* gene under autogenous control, the nonpermissive strain TU6 had if anything slightly higher levels of Rho than its permissive parental strain W3110. Thus, inhibition of T4 growth in TU6 cannot be due to a low level of Rho *per se*.

The relative membrane content of unsaturated fatty acids is higher at low growth temperatures (MARR and

INGRAHAM 1962; SINENSKY 1971). To test whether this could account for the cold-sensitivity of the T4 exclusion we used D-NAC to inhibit the synthesis of oleic acid, the major unsaturated fatty acid in *E. coli* membranes (KASS 1968). When infection at 30° was carried out in the presence of D-NAC, under which conditions membranes containing relatively more saturated fatty acids are synthesized (YOUNG and KORNBERG 1988), mature phage appeared later and the total yield was lower than in the absence of this inhibitor (data not shown). Thus, it is unlikely that the inhibition of T4 growth at lower temperatures is due to the high content of unsaturated fatty acids at these temperatures.

Restoration of T4 growth in TU6 by heat shock and GroEL: The T4 growth defect in TU6 is manifested quite late; upon infection with T4 wild-type essentially normal gene expression was found up to the last period measured (ending 29 min after infection), including processing of major head proteins (LINDER and CARLSON 1985), but few viable phage were produced, suggesting defect(s) in morphogenesis. Heat-shocking the cells, a treatment expected to induce elevated expression of GroEL, the *E. coli* heat-shock chaperone known to be involved in phage morphogenesis (as well as of other heat-shock proteins) prior to infection at the otherwise non-permissive low temperature, restored growth in TU6 (Figure 2A). The heat-shock was without effect when performed in the presence of chloramphenicol (data not shown), showing that protein synthesis during the heat treatment was essential.

Supplying GroEL from multicopy plasmids (pOF14 and pOF15, both carrying the wild-type *groEL* allele) also suppressed the T4 growth defect (Table 3). Figure 2, C and D, shows that phage production (plating with chloroform) and cell lysis (plating without chloroform) of T4 Rid394x4 in TU6·pOF14 and TU6·pOF15 was as good as or better than that in W3110, where growth of this phage is not defective. Thus, the GroEL plasmids suppressed the T4 growth deficiency in TU6 very efficiently.

In strain CAG9333 the *rpoH*-gene encoding σ^{32} , essential to recognize heat-shock promoters, is deleted. A DNA insertion upstream of the *groEL* gene causes high expression of this gene independently of σ^{32} , while almost no other heat-shock proteins are formed at tem-

TABLE 3
Eop of T4 wild type and Rid394×4

Strain	Relevant plasmid genotype	Phage strain	
		T4 wild type	Rid394×4
W3110		0.8	0.7
TU6		0.2	5×10^{-4}
TU6·pJG32	<i>rho</i> ⁺	1	0.3
TU6·pNT204·pNT29Δ56	<i>rho</i> ⁺ under <i>λcl857</i> control	0.1	2×10^{-3}
TU6·pNT29Δ56	<i>rho</i> ⁺ (constitutive expression)	0.9	0.3
TU6·pPMR5	45% of <i>rho</i>	1	4×10^{-3}
TU6·pPMR12	<i>trxA</i> ⁺	10^{-3}	2×10^{-4}
TU6·pMK333	<i>rhIB</i> ⁺	0.2	$<10^{-5}$
TU6·pOF14	<i>groEL</i> ⁺	0.8	0.9
TU6·pOF15	<i>groEL</i> ⁺	1	1
JM101 <i>lit-6</i>		3×10^{-7}	2×10^{-6}
JM101 <i>lit-6</i> ·pJG32	<i>rho</i> ⁺	0.2	3×10^{-6}
JM101 <i>lit-6</i> ·pNT29Δ56	<i>rho</i> ⁺	0.1	8×10^{-4}
JM101 <i>lit-6</i> ·pOF15	<i>groEL</i> ⁺	0.1	2×10^{-6}

All strains were grown at 37° in LB supplemented with 50 µg/ml ampicillin and/or 20 µg/ml tetracycline as required. The efficiency of plating (eop) on TU6 and its derivatives was determined on tryptone plates at 30°, on JM101*lit-6* and its derivatives at 34° on Luria agar plates, both relative to the eop on *E. coli* B at 37°.

TABLE 4
Amount of Rho in different strains grown at different temperatures

Strain	Chromosomal <i>rho</i>	Plasmid	Temperature	
			30°	45°
W3110	<i>rho</i> ⁺	none	0.6, 0.7	ND
TU6	<i>rho6</i>	none	1.2, 1.5	1.1, 2.1
TU20	<i>rho6</i> , <i>rho104</i>	none	2.9, 3.4	ND
TU6·pJG32	<i>rho6</i>	<i>rho</i> ⁺	1.8, 2.6	ND
TU6·pBR322	<i>rho6</i>	vector	0.9, 1.1	ND
TU6·pOF14	<i>rho6</i>	<i>groEL</i> ⁺	1.7, 1.1	ND

Bacterial strains were grown in GCA with antibiotics as required at 30° to about 3×10^8 cells/ml. Monoclonal antibodies against Rho were used in an ELISA test to determine the amount of Rho, shown as percent of total cellular protein. Purified Rho protein was used as standard at eight different concentrations. The two values are from determinations using two separate cell extracts. For the determination at 45°, cells were grown at 30° and shifted to 45° for 14 min before harvesting. ND, not determined.

peratures from 20° to 40° (KUSUKAWA and YURA 1988; ZHOU *et al.* 1988). In this strain the Rid mutant plates normally even at 30°. The infection yielded about the same number of phage, 100 per cell 60 min after infection, as did infection of its parental strain AG2226, and the infected cells also lysed to about the same extent. Thus, GroEL appears to be the only heat-shock protein that T4 needs in this genetic background.

Determination of the amount of GroEL: To quantify the amount of GroEL in the different strains a polyclonal antibody specific for GroEL (W. WICKNER, personal communication) was used in ELISA tests; we verified that this antibody preparation reacted with only one band in immunoblots of polyacrylamide gels of electrophoresed cell extracts from the *E. coli* strains used in this study (data not shown). The results are presented in Table 5. TU6 actually expressed slightly higher level of this protein than did W3110. Heat treatment,

as expected, raised the amount of GroEL. TU20 (transcription-termination deficient) and TU6·pJG32 (transcription-termination proficient) induced about the same level of GroEL as did W3110, which was somewhat lower than in TU6. This argues against the possibility that Rho regulates GroEL expression by affecting transcription termination. Similarly, Rho levels were unaltered upon heat treatment or introduction of a *groEL*-plasmid (Table 4), indicating that GroEL did not suppress by regulating Rho expression. The two proteins, apparently, suppress independently of each other. We confirmed WIBERG *et al.* (1988) that OF218·pOF15 overexpresses GroEL (more than heat-shocked TU6). Thus, our assay should have picked up overproduction of GroEL from TU6·pOF14 had it occurred. No such overproduction was found, yet the T4 growth in TU6 was restored (Table 3, Figure 2C). The copy number of pOF14 in TU6 was about the same as that of pBR322, as determined by single cell resistance towards benzylpenicillin (UHLIN and NORDSTRÖM 1977). Perhaps expression from this plasmid was reduced due to the genetic background in TU6. These results suggest that the chromosomally encoded GroEL in TU6 cannot support normal T4 development unless overproduced (as after heat-shock), but partial replacement of this protein by a wild-type GroEL in *trans* can restore T4 growth.

Mutation(s) in TU6 causing the T4 growth inhibition:

Rho: Our earlier work had shown that transcription termination at known Rho-dependent terminators (in front of the T4 genes coding for dCTPase and β-glucosyltransferase, (LINDER and SKÖLD 1980), as well as the overall pattern of gene expression throughout infection (LINDER and CARLSON 1985), was essentially normal in TU6. Still, there was a possibility that a *rho* mutation, perhaps of the HDF type (STITT *et al.* 1980), was responsible for growth inhibition in this strain; HDF

FIGURE 2.—Suppression of the T4 growth defect in TU6. (A) TU6 was grown at 30° in LB to about 2×10^8 cells/ml and concentrated 10-fold by centrifugation. The cells were treated at 45° for different times as shown in the figure, diluted 10-fold into prewarmed LB at 30°, and infected at an moi of 10 with the mutant Rid394x4 (t^-). At indicated times samples were withdrawn to test tubes with chloroform and plated after 20 min with *E. coli* B as indicator at 37°. Results are shown as phage produced per infected cell. ●, unheated control; ○, heated for 8 min; ■, 11 min; □, 14 min; or ▲, 17 min prior to infection. (B, C and D) Cells were grown in LB at 30°, plasmid-carrying strains in the presence of 50 µg/ml of ampicillin, to about 2×10^8 , when they were infected at an moi of 10. Samples were withdrawn and plated after chloroform treatment (open symbols) or without chloroform (filled symbols) as described above. (B) ○, ●, T4 goF1 in TU6; (C) T4 Rid394x4: ○, ●, TU6-pOF14; □, ■, TU6-pOF15; (D) T4 Rid394x4: ○, ●, TU6; □, ■ W3110.

TABLE 5

Amount of GroEL in different strains grown at different temperatures

Strain	Chromosomal <i>rho</i>	Plasmid	Temperature	
			30°	45°
W3110	<i>rho</i> ⁺	None	0.016, 0.018	ND
TU6	<i>rho6</i>	None	0.024, 0.025	0.033, 0.032
TU20	<i>rho6</i> , <i>rho104</i>	None	0.019, 0.017	ND
TU6-pJG32	<i>rho6</i>	<i>rho</i> ⁺	0.015, 0.012	ND
TU6-pOF14	<i>rho6</i>	<i>groEL</i>	0.014, 0.016	ND
OF218-pOF15	<i>rho</i> ⁺	<i>groEL</i>	0.093, 0.054	ND

Polyclonal anti-GroEL antibodies were used in an ELISA test to determine the amount of GroEL, shown as A_{405} (alkaline phosphatase activity) per µg protein, using the same cell extracts as for Table 4. The two values are from determination using two separate cell extracts. For the determination at 45°, cells were grown at 30° and shifted to 45° for 14 min before harvesting. For OF218-pOF15, <10% of cells forming colonies in the absence of ampicillin did so also in the presence of ampicillin. ND, not determined.

mutations are known to affect termination differently at different genes and we had only assayed two termination sites directly.

The Rho protein in different strains was analyzed by immunoblots of polyacrylamide gels of cell extracts, using monoclonal antibodies against Rho. Results are shown in Figure 3. These analyses revealed slightly altered mobility of Rho from TU6, and its derivatives TU8

and TU20, as compared to Rho from its parental strain W3110 or from the unrelated strain HDF40.08 or plasmid pJG32. Immunoblots of TU6-pJG32 revealed both Rho bands (data not shown). We have named the *rho* mutation in TU6 *rho6*. It may be a short deletion, or missense; MORI *et al.* (1989) found that Rho from a missense mutant (*rho*^{s-82}) had altered mobility in SDS-polyacrylamide gel electrophoresis (PAGE). The *rho6* allele was probably inadvertently introduced in transducing the precursor of TU6 to Val^R (to be isogenic with *rho*-defective transductants), or arose spontaneously, in the construction of TU6, as it is present in TU6, TU8 and TU20 but not in the other W3110 derivatives (Figure 3).

Phage P1 transduction was used to determine whether the *rho6* mutation in TU6 caused the T4 growth defect. A P1 lysate grown in TU6 was used to transduce the T4-nonpermissive *rho* mutant HDF40.08 to valine resistance (the *ilvG* gene responsible for valine resistance is less than 0.5 min from *rho*, BACHMANN 1990; BREWER 1990). Transductants were screened for their ability to plate T4 wild type and Rid394x4. No transductants inhibited T4 wt or Rid394x4 as TU6 did. About 85% permitted T4 growth, and the remainder inhibited T4 as did the original HDF40.08 strain. Two different types of T4-sensitive transductants were obtained, which are represented by TU29 and TU30. Immunoblot analysis (Figure

FIGURE 3.—Gel analysis and immunoblotting of Rho. Cells were grown in LB at the temperatures indicated, lysed, electrophoresed and immunoblotted using a monoclonal antibody specific against Rho. The bands seen migrating faster than the Rho monomer in panel B may be degradation products of Rho; they are usually much less prominent (*cf.* panel A). W3110*trpR* and W3110*trpREailv* are intermediates in the construction of TU6 from W3110 (C. YANOFSKY, personal communication, see LINDER and CARLSON 1985). (A) From left to right HDF40.08 grown at 37°, HDF40.08-pJG32 grown at 37°, TU6 grown at 30°, TU6 (heat-shock) grown at 45°, TU8 grown at 30°; TU20 grown at 30°, W3110 grown at 30°, W3110 (heat-shock) grown at 45°. (B) From left to right W3110, TU6, W3110*trpR*, W3110*trpREailv*, TU29, TU30 (all grown at 30°), and purified Rho.

TABLE 6

Efficiency of plating of T4 on HDF40.08 derivatives carrying all or parts of rho from TU6

Bacterial strain	Rho mobility	Temp (°C)	Phage strain	
			Wild type	Rid394x4
SKB178	Normal	30	1	0.8
		37	1	0.9
HDF40.08	Normal	30	1	0.02
		37	3×10^{-7}	$<10^{-7}$
TU29	Altered	30	0.9	0.3
		37	0.9	0.4
TU30	Normal	30	1	0.2
		37	1	0.4
TU6	Altered	30	0.7	5×10^{-6}
		37	0.9	10^{-5}

The efficiencies of plating were determined on tryptone plates at 37° relative to the efficiency of plating on *E. coli* B. All indicator strains were grown in LB; SKB178 and HDF40.08 at 30°, while TU6, TU29 and TU30 were grown at 37°. For mobility of Rho, see Figure 3.

3B) showed that TU29 carries the *rho6* allele while TU30 does not. Both transductants permitted good growth of T4 wild type as well as Rid394x4 at low as well as high temperatures, and yielded plating efficiencies only marginally lower than in SKB178, which is the parental strain of HDF40.08 (Table 6). Thus, the *rho6* allele from TU6 supports T4 growth in another genetic background, and is unlikely to be the primary reason why TU6 prevents normal growth of T4.

GroEL: The observation that GroEL restored T4 growth in TU6 raised the possibility that a *groEL* mutation in TU6 was responsible for the growth inhibition. Since P1 transduced the *groEL* region poorly, the T4 transducing strain GT7, which packages about twice as much DNA as P1, was used in transduction experiments with TU6 as recipient. The *groEL*⁺ donor strain (CAG18488; SINGER *et al.* 1989), which supports normal T4 growth, has *Tn10* (conferring resistance to tetracycline) integrated at 93.75 min; the map order in this region is *Tn10* (93.75 min)-*groEL*-*ampC* (BACHMANN 1990), and there is 50% P1 cotransduction between this *Tn10* insert and *ampC* (C. A. GROSS, personal communication, and verified here). Out of 14 tetracycline-resistant transductants tested, all inhibited T4 growth similarly as did the original TU6 strain. Two additional tests support the conclusion that *groEL* is not the critical gene. T4 ϵ mutants (selected for growth in *groEL*-defective bacteria (GEORGOPOULOS *et al.*, 1972), did not grow better than T4 wild-type in TU6, and the T4 *go6* mutant isolated for growth in TU6 (see below) did not grow in *E. coli groEL44* (data not shown). Thus, the growth inhibition in TU6 is not due to a *groEL* mutation.

Lit: Mutations in the *E. coli lit* gene (*lit*Con mutations) that increase its level of expression cause cold-sensitive inhibition of T4 growth, due to cessation of all gene expression late in development (COOLEY *et al.* 1979; KAO and SNYDER 1988; BERGLAND *et al.* 1990). This inhibition is relieved by mutations within a short region,

TABLE 7
Growth of T4 suppressor mutants on TU6 and JM101*lit-6*

Strain	T4 phage (eop) ^a		
	Wild type	<i>gol</i>	<i>go6</i>
TU6	0.2	0.8	0.7
JM101 <i>lit-6</i>	3×10^{-7}	6×10^{-3}	7×10^{-3}

^a The efficiencies of plating were determined as for Table 3.

named *gol*, of the T4 gene encoding the major head protein, gene 23. The *lit* gene resides in the cryptic DNA element *e14*, which also carries the *mcrA* gene encoding a restriction endonuclease cleaving methylated or hydroxymethylated DNA (RALEIGH *et al.* 1989; HIOM and SEDGWICK 1991). Since the T4 growth defect in TU6 is also manifested late in development at low temperatures, we investigated the nature of the *lit* gene in TU6. The *lit*⁺ donors in transduction experiments were RS3242, which carries *Tn10* integrated at 25.25 min, very closely to the left of the *icd* gene, and RS3032, which carries *Tn10* in the *fadR* gene at 25.75 min. The map order in this region is *Tn10* (25.25 min)-*icd*::*e14* [*lit-mcrA*]-*fadR*::*Tn10* (25.75 min) (BACHMANN 1990; HILL *et al.* 1989; HIOM and SEDGWICK 1991; KAO and SNYDER 1988). Both donor strains supported normal T4 growth. Upon transduction, again by T4 GT7, from RS3242 to TU6, all 26 tetracycline-resistant transductants that were tested supported normal T4 growth; TU62 is one such transductant. The same result was obtained when the *lit*(Con) strain JM101*lit-6* was transduced with the same lysate: all tetracycline-resistant transductants tested were *lit*⁺. Upon transduction from RS3032 to TU6 by T4 GT7, 23 out of 24 tetracycline-resistant transductants inhibited T4 growth as did TU6, the 24th permitted normal T4 growth. One of the nonpermissive transductants, named TU63, was used as donor to transduce SKB178 (which is fully permissive for T4 growth but *mcrA*⁻) to tetracycline resistance. Out of 106 Tet^R transductants, 36 (34%) were *mcrA*⁺ (determined by their inability to propagate T6gt phage, REVEL 1983; RglA⁺ = *mcrA*⁺). All but two of these also inhibited T4 growth as did TU6; all strains that inhibited T4 growth were also *mcrA*⁺. These data show a close coupling, but not identity, between the *E. coli* mutation causing inhibition of T4 growth in TU6, and the *mcrA* gene, and support the map order [TU6 mutation]-*mcrA*-*fadR*, suggesting that the mutation causing T4 growth inhibition resides in or very close to *lit*.

Phage mutants growing in TU6: T4 mutations resulting in good growth in TU6 arise with a frequency around 10⁻⁵; *go6* is one of five such mutations that were analyzed. All five mapped close to the *amb17* mutation, which lies in the *gol* region of gene 23, giving less than 1% wild-type recombinants when crossed to a mutant carrying this allele. A T4 *gol* mutant grew in TU6, and the *go6* mutant grew as well as the *gol* mutant in a *Lit* overproducing host (Table 7). We could not isolate secondary mutants from

either *go6* or *gol* phage that grew better than those two in *Lit*(Con) strains; presumably T4 mutations cannot completely compensate for the *Lit* inhibition.

The *goF1* mutant of T4, which is a spontaneous mutant growing in HDF *rho*⁻ strains (STITT *et al.* 1980), also grows better than T4 wild type in TU6 at low temperatures (LINDER and CARLSON 1985). Phage production in infections with this mutant (Figure 2B) was about 5-fold that produced in similar infections with T4 wild-type (which yielded 15–20 plaque-forming units (pfu)/cell plating in the presence of CHCl₃; 2 pfu/cell in the absence of CHCl₃), and the *goF1*-infected cells lysed spontaneously. The level of suppression was lower than suppression due to the multicopy *rho*⁺ plasmid or the chromosomal *rho102* or *rho104* alleles (LINDER and CARLSON 1985), or to GroEL (Figure 2C).

DNA synthesis and packaging, phage production and cell lysis: Our previous study (LINDER and CARLSON 1985) suggested that the quantitative patterns of phage proteins synthesized at different times after T4 infection of TU6 were quite normal, but did not permit any conclusions concerning their functionality. To get a better estimate of this, we monitored phage DNA synthesis (a measure of the functionality of the early proteins, required for DNA replication), and the subsequent packaging of this DNA into mature, DNase-resistant particles (a measure of the functionality of the late, morphogenetic, proteins). In a normal T4 infection, about 50% of the DNA made is packaged this way (WIBERG *et al.* 1988). Results are summarized in Table 8.

In all infected strains except those carrying *groEL* plasmids DNA was synthesized continuously throughout the experiment with kinetics similar to what we showed previously for W3110, TU6 and TU20 (LINDER and CARLSON 1985). In the *groEL*-plasmid-carrying strains TU6-pOF14 or TU6-pOF15 start of DNA synthesis was delayed, and the synthesis proceeded at a slower rate than in the other infections; in JM101*lit-6*-pOF15 DNA synthesis started late, but subsequently proceeded at normal rates (data not shown). This suggests that GroEL may be directly involved in DNA replication of T4, as has been shown for bacterial DNA replication (WADA and ITIKAWA 1984). Also Rho-suppression of the growth defect led to somewhat reduced DNA synthesis as compared to the nonpermissive conditions (Table 8), as noted before for strains TU20 and TU6-pJG32 (LINDER and CARLSON 1985). The altered level or function of Rho in these cells may lead to imbalanced gene expression, and therefore poorer function of the multiprotein DNA replication complex.

Upon infection of the permissive parental strain W3110 with wild-type T4 or the *Rid* mutant at 30° quite normal levels of the newly synthesized DNA was packaged (Table 8 and data not shown). In TU6, and in JM101*lit-6*, which carries a *lit*Con mutation, only 7–11%

TABLE 8
Synthesis and packaging of DNA and phage production

Exp.	Infection	DNA synthesis ^a	DNA packaging ^b	Spontaneously liberated phage per cell ^c	Total phage production per cell
I	Rid394×4 in TU6	0.22	0.08	1	4
	Rid394×4 in TU20	0.20	0.15	2	7
	Rid394×4 in TU6·pOF14	0.04	0.43	14	14
	Rid394×4 in W3110	0.13	0.47	3	46
II	T4 wt in TU6·pPMR5	0.20	0.17	3	19
	T4 wt in TU6·pJG32	0.21	0.21	3	15
	T4 wt in TU6·pNT29Δ56	0.13	0.14	2	10
	T4 wt in JM101 <i>lit-6</i> ·pNT29Δ56	0.25	0.06	4	2
	T4 wt in JM101 <i>lit-6</i> ·pOF15	0.25	0.08	3	3
III	T4 wt in TU6	0.21	0.11	ND	5
	T4 wt in TU62	0.28	0.31	ND	33
	T4 wt in JM101 <i>lit-6</i>	0.39	0.04	ND	~1
	T4 <i>go6</i> in TU6	0.22	0.53	ND	50
	T4 <i>gol6B</i> in TU6	0.08	0.40	ND	50
	T4 <i>go6</i> in JM101 <i>lit-6</i>	0.26	0.04	ND	~1
	T4 <i>gol6B</i> in JM101 <i>lit-6</i>	0.24	0.06	ND	~1

The data are from three separate experiments. ND, not determined.

^a Net DNA synthesis was estimated from the incorporation into trichloroacetic acid (TCA)-insoluble material of [³H]dThd continuously present from 4 min after infection, and calculated as the fraction of total radioactivity added to the culture, normalized to 3×10^8 cells/ml. As this incorporation varies depending on the exact amount of dAdo present, absolute values can be compared within one experiment, but not between different experiments. The data shown are from the incorporation 90 min after infection.

^b The cultures were lysed with chloroform 90 min after infection, and DNase was added. Only DNA that is packaged in mature phage heads resists this treatment. The TCA precipitable activity after 60 min of DNase treatment (beyond 30 min no further solubilization was observed) was calculated as fraction of the total TCA-precipitable activity just before DNase addition, that is, packaged DNA as fraction of total DNA synthesized.

^c The spontaneously released phages, a measure of the extent of spontaneous lysis, were titrated without chloroform treatment, and the total phage production after chloroform lysis; in both cases per cell after subtraction of unadsorbed infecting phage.

of the DNA synthesized 90 min after infection was packaged (Table 8). The *groEL*⁺ plasmid (pOF14), the *rho*⁺ plasmids (pJG32, pNT29Δ56, and the pPMR5 plasmid which carries a truncated *rho* gene) and the polarity-suppressing *rho* mutation (*rho104* in TU20) all suppressed the packaging defect of both T4 wt and the Rid mutant in TU6; in each strain the same level of packaging was seen for both phage (Table 8 and data not shown), and production of viable progeny phage was increased correspondingly. Since the intracellular development of both phage strains, by these criteria, was identical, the failure of pPMR5 to restore plaque formation by the Rid mutant (see Table 3) must be due to lack of suppression of its lysis deficiency. Under these growth conditions, only Rid-infected TU6·pOF14 lysed spontaneously before the conclusion of the experiment (yielding the same phage titer when plated with or without chloroform). In broth TU6·pOF14 cells lysed considerably earlier (Figure 2C), and also TU6·pJG32 and TU20 lysed to some extent (LINDER and CARLSON 1985), while TU6 did not (Figure 2D) (Linder and Carlson 1985). The plasmids did not restore packaging of phage DNA measurably in JM101*lit-6* under these conditions, though phage production was slightly raised; in richer media (Table 3) these plasmids all partially suppressed the T4 growth defect in this strain. Phage carrying the *go6* and *gol6B* mutations, isolated as growing in TU6 and a *lit*(Con) strain respectively, behaved identically to each other in both these hosts: both mutations restored

packaging in TU6, but not in JM101*lit-6*, to essentially normal levels (Table 8). The transduction of the *e14* region into TU6 (Table 8, strain TU62) also restored normal T4 growth. Taken together, these results support the notion that TU6 carries a mutation increasing *lit* expression (a *lit*Con mutation), which causes the T4 growth defect; we have named this mutation *lit106*. Early phage proteins appear to function normally in supporting DNA replication; late proteins, though apparently quantitatively normal, did not support normal assembly of mature phage.

DISCUSSION

In T4-infected *E. coli* TU6 late events affecting phage assembly and cell lysis are deficient at low temperatures. We first noted these defects as failure of a lysis-defective *t* mutant to form plaques, but the intracellular development of wild-type phage is affected similarly, differing from the *t* mutant only in more efficient cell lysis.

GroEL suppression: "GroEL suppression" resulted when GroEL was overproduced due to heat shock, or when the cells carried a plasmid expressing wild-type GroEL (Figure 2, Tables 3 and 8). GroEL suppression was very efficient, supporting packaging of a normal fraction, 50%, of the phage DNA synthesized (Table 8). Phage production was only increased about 4-fold, likely because little DNA was made, and the infected cells lysed early and very efficiently. Thus, lysis inhibition, which is

a normal part of T4 growth in dense bacterial cultures, was not fully established, suggesting a direct role for GroEL in this process. Under suppressing conditions GroEL, as we previously found for Rho, also could sustain prolonged phage production in cells infected with the *t* mutant Rid 394x4. This classic phenotype of *t* mutant T4 (JOSLIN 1970, 1971) apparently requires either GroEL or Rho.

Rho suppression: "Rho suppression" of the same growth defect (Tables 3 and 8), was seen in the presence of plasmids overexpressing wild-type Rho (pJG32 and pNT29Δ56) or in the presence of chromosomal *rho* alleles overproducing Rho proteins deficient in transcription termination (*rho102* or *rho104* in strains TU8 and TU20, respectively) (LINDER and CARLSON 1985). Interestingly, a plasmid encoding part of Rho restored packaging as efficiently as in suppression involving all of Rho, but the lysis defect of the *t* mutant was not suppressed, suggesting that Rho affects these two phenomena somewhat differently.

Rho suppression did not restore the level of DNA packaging into DNase-resistant particles as completely as did GroEL, but still led to 2–4-fold higher production of viable phage in a synthetic medium as compared to TU6 (Table 8), since in this case the infected cells were lysis inhibited.

Failure to grow in TU6: A mutation affecting the *lit* gene, named *lit106*, was found to be responsible for the T4 growth defect. Similar mutations, which result in increased expression of the *lit* gene product and therefore are called *litCon* mutations, have been analyzed by SNYDER and co-workers. These mutations cause inhibition of phage transcription downstream from the *gol* region of gene 23, and inhibition of all cellular translation (BERGSLAND *et al.* 1990). For both effects the *gol* region of gene 23 must be expressed, which is why the inhibition occurs during the late phase of phage development. This is an example of phage exclusion; neither host nor parasite survives. Lit appears to be located in the inner membrane of the cell (KAO and SNYDER 1988). The amino acid sequence of Lit shows similarities to that of known proteases, and cleavage of a cellular protein (EFTu) has been observed upon T4 infection of a *litCon* strain (YU and SNYDER 1994). These observations have led to the suggestion that Lit is a protease activated upon expression of gene 23 after T4 infection (YU and SNYDER 1994).

The late phage expression in TU6 was much less affected than in the *litCon* mutants described by SNYDER and co-workers; we have found essentially normal protein synthesis throughout phage development in TU6 (LINDER and CARLSON 1985). Still, phage suppressor mutants isolated on TU6 or on the *lit-6*-defective mutant grew similarly to each other in both hosts (Tables 7 and 8), and the same plasmids that restored T4 growth in TU6 also restored its growth in JM101*lit-6*, albeit some-

what less efficiently (Tables 3 and 8). We conclude that TU6 carries a *litCon* allele raising Lit production to a lower level than that in the *lit-6* strain. How can this defect be suppressed by GroEL or Rho?

The effects of GroEL suppression are those expected from correction of problems with assembly of phage particles, and GroEL suppression was more efficient than Rho suppression in TU6. We consider it likely that GroEL acts upon the nascent GP23, folding it or facilitating its processing in such a way that it cannot potentiate the activation of the Lit protein. This is a classical chaperone activity. Another possibility is that damage to some of the phage structural proteins introduced by an activated Lit protease can be undone by GroEL. It is unlikely that GroEL suppresses via Rho, although TU6 does carry a mutant Rho which arguably could be re-directed to proper folding by GroEL; this *rho* allele appears to function normally in regulating T4 gene expression in TU6 (LINDER and CARLSON 1985) and supports T4 growth in a different genetic background (Table 6).

The level of GroEL in TU6 was normal, or slightly above normal (Table 5), but the regulation of non-heat-shock GroEL expression appeared perturbed in TU6, since the *groEL* multicopy plasmids did not yield elevated GroEL levels in TU6 at 30°, though they did so in a different W3110 derivative (Table 5). Supplying this level of GroEL at least partially from a plasmid sufficed to relieve the growth inhibition, as did the larger quantities of chromosomally encoded GroEL resulting from a heat-shock prior to T4 infection; thus the normal level of chromosomally encoded GroEL for some reason is unable to protect the phage.

It is more difficult to understand how Rho could suppress this growth inhibition. The same relative amount of GroEL was seen both in the presence of elevated levels of wild-type Rho and in the presence of a polarity-suppressing *rho* allele expected to yield increased expression downstream from Rho-dependent transcription-termination signals (Table 5), conditions expected to have opposite consequences for Rho-dependent transcription termination. This renders it highly improbable that Rho regulates GroEL expression, and suppresses T4 growth inhibition in TU6, via GroEL.

Rho is known to be essential during T4 development, since raising the temperature of a heat-sensitive HDF Rho mutant any time after T4 infection leads to abortion of the infection (TAKAHASHI 1978; STITT *et al.* 1980), but the reasons for this are not well understood. The simplest explanation is that Rho is involved in the regulation of some genes the products of which are essential for phage development. This has been demonstrated conclusively for some early genes required for DNA synthesis (STITT and MOSIG 1989; HINTON 1989), and less straightforwardly also for some late genes (STITT *et al.* 1980). According to this model, the *rho6* allele might be

inadequate for the expression of some essential gene(s), resulting in imbalance and less efficient assembly. Restoring the balance between the different structural proteins may then suppress the defect. The model is rendered unlikely by the normal growth of T4 in other strains carrying this *rho* allele, and by the suppression by transcription-termination-proficient as well as transcription-termination-deficient Rho. The transcription-termination-deficient Rho mutants could restore the transcription downstream from the *gol* region that was inhibited in *lit*Con hosts; the transcription-termination-proficient Rho encoded from plasmids pJG32 and pNT29Δ56 is unlikely to do so, so such a mechanism cannot explain the Rho suppression fully. We consider it likely that Rho here has a role different from transcription regulation.

Rho mutants suffer pleiotropic defects, but the extent of transcription termination proficiency or deficiency is not always correlated to other properties of the mutants (BAUMBERG and LOVETT 1977; DAS *et al.* 1976, 1978; DOMBROSKI and PLATT 1989; GULLETTA *et al.* 1983; GUTERMAN and HOWITT 1979; SUKHODOLETES *et al.* 1982; ZOGRAFF and GINTSBURG 1980). FASSLER *et al.* (1985) noted that *rho15*, *rep* and *rho15*, *ssb* double mutants were inviable, and suggested that Rho may be involved in some general way in the same functions as Rep (a DNA helicase not essential for *E. coli* under normal laboratory conditions) and Ssb (a single-strand-DNA-binding protein required for *E. coli* DNA replication), for example replication and the structural maintenance of DNA. Rho does have helicase activity, at least RNA-DNA helicase (BRENNAN *et al.* 1987) and does bind to both single-stranded and double-stranded nucleic acids (BECKMANN *et al.* 1971; ODA and TAKANAMI 1972; RICHARDSON 1982). A direct role for Rho in DNA replication was suggested also by ZOGRAFF and GINTSBURG (1980), who studied T4 development in a temperature-sensitive *rho15* strain. Phage DNA synthesis was considerably reduced upon T4 infection at 42°. If chloramphenicol was added to the infected cells 9 min after infection, followed by downshift to 30° 1 min later, close to normal DNA synthesis resulted, suggesting that the temperature effect on Rho was reversible, and that Rho has a direct role in T4 DNA replication not mediated via an effect on phage transcription.

A common denominator between the DNA replication discussed above, and our studies of suppression of defects in phage assembly processes, is the multiprotein complexes involved in both cases. If Rho participates directly in the assembly of multiprotein complexes, Rho suppression of T4 growth inhibition in TU6 may be understood in terms of interaction with, or replacement of, GroEL. Elevated levels of Rho, or GroEL, may then suppress defects due to reduced functionality of the other protein. The Psu protein encoded by coliphage P4 has an analogous dual function: it functions as an "antiter-

mination factor" during phage development in permitting readthrough of Rho-dependent termination signals (*psu* = polarity suppression) (LINDEROTH and CALENDAR 1991) and is also a component of the phage head (ISAKSEN *et al.* 1992) where it likely functions to stabilize the hexameric capsomer assembly and prevent undue DNA release from the capsid (DOKLAND *et al.* 1993). The T4 *goF1* mutation has been suggested to alter a phage protein normally interacting with Rho (STITT *et al.* 1980; STITT and MOSIG 1989). This *goF1* mutant grew well in TU6 (Figure 2B). If Rho and GroEL can have similar chaperone function, perhaps the altered phage protein encoded by the *goF1* mutant can interact more efficiently with either or both of these in TU6.

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