ESCHERICHIA COLI RHO FACTOR IS INVOLVED IN LYSIS OF BACTERIOPHAGE T4-INFECTED CELLS

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Manuscript received March 20, 1985
Revised copy accepted May 10, 1985

ABSTRACT

A Rid (Rho interaction deficient) phenotype of bacteriophage T4 mutants was defined by cold-sensitive restriction (lack of plaque formation) on *rho*$^+$ hosts carrying additional polar mutations in unrelated genes, coupled to suppression (plaque formation) in otherwise isogenic strains carrying either a polarity-suppressing *rho* or a multicopy plasmid expressing the *rho*$^+$ allele. This suggests that the restriction may be due to lower levels of Rho than what is available to T4 in the suppressing strains.—Rid394×4 was isolated upon hydroxylamine mutagenesis and mapped in the *t* gene; other *t* mutants (and *mot*, as well as *dda dexA* double mutants) also showed a Rid phenotype. In liquid culture in strains that restricted plaque formation Rid394×4 showed strong lysis inhibition (a known *t*$^-$ phenotype) but no prolonged phage production (another well-known *t*$^-$ phenotype). This implies that when Rho is limiting the *t* mutant shuts off phage production at the normal time. Lysis inhibition was partially relieved, and phage production prolonged to varying extents depending on growth conditions in strains that allowed plaque formation. No significant effects on early gene expression were found. Apparently, both mutant (polarity-suppressing) and wild-type Rho can function in prolonging phage production and partially relieving lysis inhibition of Rid394×4 when present at a sufficiently high level, and Rho may play other role(s) in T4 development than in early gene regulation.

THE Rho protein of *Escherichia coli*, which is essential for cell growth (DAS, COURT and ADHYA 1976; GULLETTA, DAS and ADHYA 1983), catalyzes an NTP-dependent (HOWARD and DE CROMBRUGGHE 1976; SHARP and PLATT 1984) termination of transcription at specific sites on the DNA template *in vitro* (ROBERTS 1969; RICHARDSON 1970) and *in vivo* (KORN and YANOFSKY 1976a,b), probably by causing release of nascent RNA (DARLIX and HORAIST 1975; SHIGESADA and WU 1980), and, at least *in vitro*, allowing recycling of RNA polymerase to initiate new transcripts (FULLER and PLATT 1978). The termination activity probably requires direct protein-protein interaction between Rho and RNA polymerase (GUARENTE 1979), but the precise mechanism of action is not known. *rho* mutations may cause reduced transcription termi-

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nation (RICHARDSON, GRIMLEY and LOWERY 1975; DAS, COURT and ADHYA 1976; KORN and YANOFSKY 1976b; GUARENTE, MITCHELL and BECKWITH 1977) and a wide variety of additional phenotypes (BAUMBERG and LOVETT 1977; DAS, COURT and ADHYA 1979; GULLETTA, DAS and ADHYA 1983; GUTERMAN and HOWITT 1979a,b; SIMON *et al.* 1979; SUKHODOLETS, MIRONOV and LINKOVA 1982). Rho acts in transcription termination during the development of bacteriophage T4, probably by limiting RNA synthesis to immediate-early (IE) species (GOLDBERG 1970; RICHARDSON 1970; JAYARAMAN 1972), originally defined as those synthesized *in vivo* in the presence of the protein synthesis inhibitor chloramphenicol (GRASSO and BUCHANAN 1969; SALSER, BOLLE and EPSTEIN 1970). Delayed-early (DE) RNA is synthesized *in vivo* when phage protein synthesis is permitted after infection, before DNA replication starts, either in a process antagonizing Rho factor (DE I) (DAEGELEN and BRODY 1976; LINDER and SKÖLD 1980; DAEGELEN, D'AUBENTON-CARAFA and BRODY 1982a; THERMES and BRODY 1984) or by activation of new promoters dependent upon the phage *mot* gene product (DE II or middle mode transcription) (MATTSON 1974; MATTSON, VAN HOUWE and EPSTEIN 1978; LINDER and SKÖLD 1980). The DE I mode may involve both phage antitermination factor(s) antagonizing Rho in *trans* (LINDER and SKÖLD 1977; 1980, STITT *et al.* 1980; GOLDFARB and MALIK 1984) and an attenuation-like mechanism not requiring synthesis of functional phage proteins (THERMES and BRODY 1984). The mechanisms behind antitermination in either case are poorly understood. Many T4 genes appear to be expressed in both the DE I mode and the DE II mode (PULITZER, COPPO and CARUSO 1979; DAEGELEN, D'AUBENTON-CARAFA and BRODY 1982b).

Several bacterial strains which were isolated independently as defective in supporting T4 growth (hd590, SIMON, SNOVER and DOERMANN 1974; *tabC*, TAKAHASHI 1978; HDF, REVEL *et al.* 1980; STITT *et al.* 1980) were found to carry mutations, probably within the *rho* gene (SIMON *et al.* 1979; STITT *et al.* 1980; DAS *et al.* 1983), that were responsible for this restriction. Also, *rho ts15*, which is permissive for T4 at intermediate temperatures where its transcription termination is defective, restricts T4 growth at high temperature (ZOGRAFF and GINTSBURG 1980). The *rho*$^-$ strains that fail to support T4 growth will be referred to in the following as host-defective (Hdf) *rho* mutants, in contrast to the many polarity-suppressing (Psu) *rho* mutants that do support T4 growth. It is not clear whether the restriction of T4 in these Hdf strains can be accounted for by defects in transcription termination or antitermination.

To understand better the mechanism of action of Rho protein and its role in T4 development, we have sought phage mutants defective in interaction with *E. coli* Rho factor. This communication describes the isolation and partial characterization of a Rid (Rho interaction deficient) mutant of T4, isolated on the basis of lack of growth in strains carrying wild-type Rho factors but growing in otherwise isogenic strains carrying mutated Rho factors. This Rid mutant was found to be defective in *t* expression. Our results suggest that this mutant requires elevated levels of a wild-type or polarity-suppressing Rho for growth.

TABLE 1

Strains of bacteria

Strain	Parental strain	Relevant genotype	Source and reference
CR63	K12	*supD*	W. SZYBALSKI
W3110	K12	*supE*, F^-	C. YANOFSKY
W3110*trpR*	W3110	*trpR*, *sup°*, F^-	C. YANOFSKY
W3110*trpREAilv*	W3110	*trpR*, *trpE*, *trpA*, *ilv*, *sup°*, F^-	C. YANOFSKY
TU6	W3110	*trpR*, *trpE*, *trpA*, *lac-ZU118*, val^R, azi^R, *sup°*, F^-	C. YANOFSKY, cured from its F-factor here
TU8	W3110	As TU6 but *rho102*	C. YANOFSKY; KORN and YANOFSKY (1976a,b), cured from its F-factor here
TU20	W3110	As TU6 but *rho104*	C. YANOFSKY; KORN and YANOFSKY (1976a,b), cured from its F-factor here
AD1766	K12	*his*, *galam57*, str^R, val^R, *sup°*, F^-	A. DAS; DAS, COURT and ADHYA (1976)
AD1704	AD1766	As AD1766 but *rhots15*	A. DAS; DAS, COURT and ADHYA (1976)
SKB178	K12	*sup°*, *galE*, *rglA*, *rglB*	B. STITT; STITT *et al.* (1980)
HDF40.08	SKB178	As SKB178 but HDF	B. STITT; STITT *et al.* (1980)
B		*sup°*	
CTr5x	"Hospital strain"	*sup*	T. J. SNOPEK; DEPEW and COZZARELLI (1974)
HR42	PA610	*thi*, *argH*, *his*, *leu*, *lys*, *thr*, *ara*, *gal*, *lacY*, *malA*, *mtl*, *xyl*, str^R, *tonA*, *sup°*	C. RICHARDSON; SAITO and RICHARDSON (1981); GAUSS, DOHERTY and GOLD (1983)
HR44	PA610	As HR42 but *optA*	C. RICHARDSON; SAITO and RICHARDSON (1981); GAUSS, DOHERTY and GOLD (1983)
DW319	RV	*thi*, *lacZ*::IS*1MS319*, *bgl*, *ilv340*, F^-	S. GUTERMAN; GUTERMAN and HOWITT (1979b)
ML1	RV	*thi*, *lacZ*::IS*1MS319*, *bgl*, *ilvC462*, *rho-115*	D. CALHOUN; GRAY, PATIN and CALHOUN (1981)

MATERIALS AND METHODS

Bacterial strains: The strains of bacteria employed and their properties are summarized in Table 1. All are *E. coli* strains, and all but B and CTr5x are K12 derivatives. Strains TU6, TU8 and TU20 were obtained from isogenic F^+ strains by treatment with acridine orange (MILLER 1972), scoring for simultaneous appearance of resistance to phage M13 (male specific) and sensitivity to phage T7 (restricted in the presence of F). HDF40.08, obtained from B. STITT, was isolated by ethyl methanesulfonate mutagenesis (B. STITT, personal communication) and is similar to the Hdf strains described by STITT *et al.* (1980).

Bacteriophage strains: Wild-type T4D and its mutants *tamA3*, *tamB5* (JOSSLIN 1970), *motfarP85*

(JOHNSON and HALL 1973), *38amB262×1* and *52amH17* were obtained from J. WIBERG. Mutants *38amH41* and *52amNG576* were from W. B. WOOD, *pseT* (DEPHEW and COZZARELLI 1974) from T. J. SNOPEK and *goF1* (STITT *et al.* 1980) from B. STITT. The mutant *39-56del12, r1589* (HOMYK and WEIL 1974) was from G. MOSIG and backcrossed here to yield *39-56 del12×2*. Mutant *ddaL148* (BEHME and EBIZUSAKI 1975) (which is also *dexA*$^-$, P. GAUSS and L. GOLD, personal communication) was from M. BEHME. Multiply mutant strains were constructed and verified as described by WIBERG *et al.* (1977), except that complementation tests often were performed by direct toothpicking. Rid mutants were obtained by treating a freshly prepared stock of T4D with hydroxylamine (1 ml of phage 4×10^{11} plaque-forming units (pfu)/ml + 9 ml of 0.05 M Na_2SO_4, 0.5 M NH_2OH, 0.001 M Na_2 ethylenediaminetetraacetic acid (EDTA), pH 6.0, 37°, 24–72 hr; TESSMAN 1968) and plating on a Rho-deficient strain of *E. coli* (W3110 *rho102*, or AD1704, which is *rhots15*). Plaques were then replicated onto the Rho-deficient strain and its isogenic *rho*$^+$ parent. Isolates forming plaques on the former but not on the latter were retained as possible Rid mutants. About 1500 plaques were scored, among which 17 Rid candidates were found. Isolates were then genetically purified by repeated backcrosses to T4 wild type. The number of backcrosses is indicated by the ×2, ×4, etc. in the designation of these and other strains.

Growth of strains: Bacteria and phage were plated on tryptone agar (TBA) containing per liter 10 g Bacto tryptone, 5 g NaCl, 1.2 g $MgSO_4$ and 11 g Bacto agar, pH 7.2. The efficiency of plating (eop) of Rid mutants was very sensitive to plating conditions. Plating indicators were grown in LB (per liter: 10 g Bacto tryptone, 5 g Difco yeast extract, 1.1 g glucose, 10 g NaCl) at 37° (except HDF40.08 which was grown at 30°) with aeration to 3×10^8, determined by cell counting. Per plate was used 0.2 ml of this culture + 3 ml of soft agar (TTA: 10 g Bacto tryptone, 8 g NaCl, 1.1 g glucose, 7 g Bacto agar/liter, pH 7.2) + 0.1 ml of phage dilution. Plaques were scored after 18 hr of incubation at appropriate temperatures. The eop is calculated relative to the eop on *E. coli* B (CR63 for amber mutants) at 37°. Data within one table were obtained in the same experiment, or series of experiments, and are comparable. Data reported in different tables may have been obtained at different times and may show a two-fold variation in eop.

Plasmids: To isolate plasmids, cells were grown to about 2×10^8 cells/ml in LB at 37°, supplemented with uridine or cytidine to 1 mg/ml (NORGARD, EMIGHOLZ and MONAHAN 1979) and further incubated until the cell density was about 1×10^9, when chloramphenicol to 0.2 mg/ml was added. The culture was then incubated overnight (about 18 hr) at 37° with aeration. Plasmids were isolated and transformed as described by MANIATIS, FRITSCH and SAMBROOK (1982). Cells were cured from their plasmids by the method of MILLER (1972), using 25–150 μg/ml of acridine orange.

DNA synthesis and enzyme measurements: DNA synthesis was estimated by incorporation of [^{3}H]-thymidine (dThd) or [^{14}C]dThd (Amersham no. TRK 418 and CFA 532) in the glycerol casamino acids (GCA) medium of FRASER and JERREL (1953) into trichloroacetic acid (TCA)-insoluble material as described by KUTTER and WIBERG (1968). Deoxycytidine triphosphatase (EC 3.6.1.12), β-glucosyltransferase (EC 2.4.1.27) and deoxynucleotide kinase (EC 2.7.4.13) activites were determined as described by LINDER and SKÖLD (1977, 1980).

Radioactive labeling of T4 proteins: T4 proteins were labeled largely as described by CARDILLO, LANDRY and WIBERG (1979). Bacterial cultures in M9 (KELLENBERGER and SÉCHAUD 1957) supplemented with 0.5% glucose, 100 μg/ml of casamino acids (KEBO, Stockholm, Sweden, no. 8.0230-454) and 20 μg/ml of tryptophane were grown to about 3×10^8 cells/ml at 37°, transferred to 30° and infected at a multiplicity of infection (moi) of 10. At different times samples were pulse-labeled for 4 min, using 2 μCi of uniformly labeled L-[^{14}C]casamino acids (Amersham CFB 104) and 13 μg of cold casamino acids/ml. Incorporation was stopped by the addition of an equal volume of ice-cold 20% TCA, and samples were then processed as described by CARDILLO, LANDRY and WIBERG (1979).

Electrophoresis of proteins: The procedure for discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was essentially that of JERGIL and OHLSSON (1974), which is a slight modification of the procedure of NEVILLE (1971), and was carried out according to a manual developed by K.-E. JOHANSSON (Department of Biochemistry, BMC, University of Uppsala, personal communication). The gels were 15 × 15 × 0.15 cm, concentrations of acrylamide and *N,N'*-bismethylene-bisacrylamide in stacking and separating gels, respectively, 4.5 and 4.5%, and 15 and

1%, and electrophoresis was first at 50 V for 30 min, then at 65 V for 18 hr or until the dye front (bromphenol blue) reached the bottom of the gel. The gel was then dried between sheets of cellophane as described by WALLEVIK *et al.* (1982) and autoradiographed.

RESULTS

Isolation and mapping of Rid 394×4: The isolate Rid39 was obtained by screening mutagenized phage at 37° in strain AD1766 (which is *rho*$^+$ and restricted the phage) and strain AD1704 (which is *rhots15* and allowed plaque formation). Rid39 and its derivatives showed the same pattern of restriction and growth on the pairs TU6 (which is *rho*$^+$) and TU8 (which is *rho102*), and DW319 (which is *rho*$^+$) and ML1 (which is *rho115*) (see Table 2). This pattern, together with growth in the *rho*$^+$ strain in the presence of the multicopy *rho*$^+$ plasmid pJG32 (Table 3), defines the Rid phenotype. Strain Rid394×4 was obtained from Rid39 by repeated backcrosses to T4 wild type. Mapping results shown in Figure 1 indicated a map position for Rid394×4 between *tamB5* and *motfarP85*. The Rid394×4 mutant failed to complement the two *t* mutants *amA3* and *amB5* for plaque formation under restrictive conditions, suggesting a position within the *t* gene, a defect in *t* regulation or inhibition of *t* activity. As seen in Table 4, a *t am* mutant showed a Rid phenotype.

Growth of Rid strains on different hosts: Table 2 summarizes the plating properties and phenotypes of the Rid strain in comparison to T4 wild type and a *goF* mutant of T4. In this table lines 1–6 form a series starting with a "wild-type" K12 strain (W3110 in line 1), into which defects in the *trp* repressor gene (line 2), two genes in the *trp* operon and one in the *ilv* operon (line 3) and finally in *lacZ* (line 4) are introduced. Into this *trpREAilvlacZ* mutant polarity-suppressing *rho* mutations *rho102* (line 5) or *rho104* (line 6) are introduced (KORN and YANOFSKY 1976a,b; C. YANOFSKY, personal communication). W3110 alone in this series carries an amber suppressor; however, Rid394×4 grew equally well in the *sup*° wild-type K12 strain SKB178 (line 9) and thus probably is not a nonsense mutant. DW319 and ML1 (lines 7 and 8) are isogenic except for the *rho* gene (and different *ilv* mutations). SKB178 (line 9) is the parental strain of HDF40.08 (line 10), which is a heat-sensitive Hdf *rho* mutant which restricts T4 at temperatures greater than 30° (STITT *et al.* 1980). *goF1* is a T4 mutation that compensates for Hdf restriction (STITT *et al.* 1980).

Rid394×4 grew well in wild-type strains of *E. coli* B and *E. coli* K12 but showed decreasing plating efficiency as additional, unrelated mutations were introduced into the *rho*$^+$ bacterial strains. The eop was somewhat reduced on the W3110 derivatives W3110*trpR* and W3110*trpREAilv* and severely reduced in the W3110 derivative TU6 which contains several polar mutations in the *trp* and *lac* operons. In strains isogenic to TU6, except for the polarity-suppressing *rho* mutations *rho102* or *rho104*, growth was restored. Restriction in DW319 was less severe than in TU6 but here also growth was restored by a polarity-suppressing *rho* allele (*rho115* in ML1). There was no growth in HDF40.08, showing that the Rid$^-$ phage was not *goF* (STITT *et al.* 1980). The *goF1* mutant, moreover, grew exceedingly well (better than T4 wild type) in the most restrictive host, TU6.

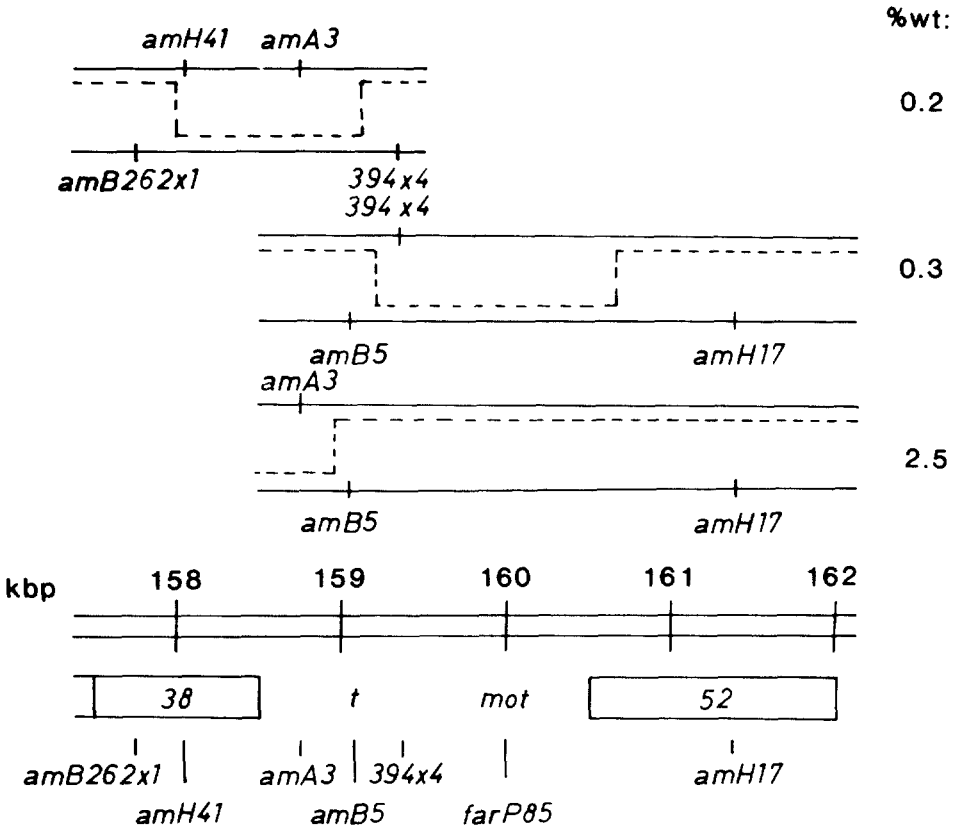


FIGURE 1.—Mapping of Rid394×4. Standard phage crosses were carried out in *E. coli* CR63 grown to 3 × 10^8 cells/ml in LB (moi 3 each phage, 37°, cells diluted 10^4-fold 4 min after infection). Total progeny was estimated by plating on CR63 and wild-type recombinants by plating on HR44 at 30°, where none of the mutants employed here plate. The scale indicates approximate distances in kilobase pairs. Below this is the approximate positions of the genes (KUTTER and RÜGER 1983; MOSIG 1983) and above that the configurations of mutations in three- and four-factor crosses. % wt is the percent wild type obtained in each cross. The order of the *t* mutations *amA3* and *amB5* is in agreement with that found by THIEL and ASTRACHAN (1977); see this article for a discussion of mapping artifacts in this region. Absolute map distances between these markers are difficult to determine due to varying degree of lysis inhibition and continued phage production in crosses involving *t* mutants. The order shown was confirmed by two-factor crosses involving all markers shown in the figure. These crosses gave about 2% wild-type recombinants between Rid394×4 and *tamB5* and 6% between Rid394×4 and *motfarP85*.

Analysis of the rho *allele in TU6*: The different levels of restriction of Rid^- phage by W3110 and its derivatives could conceivable be due to a mutation in the *rho* gene accidentally introduced during strain construction. To test this possibility P1 transduction experiments were carried out using TU6 (which is Val^R) as donor and HDF40.08 (which is Val^S) as recipient. The *rho* gene maps close to the *ilvGEDA* operon and cotransduces with *ilv* markers with high frequency (MORSE and GUERTIN 1972; DAS, COURT and ADHYA 1976). Valine resistance in *E. coli* depends upon the expression of the *ilvG* gene (FAVRE *et*

TABLE 2

Eop of T4 strains on rho$^+$ *and* rho$^-$ *strains of* E. coli

Bacterial strain	*rho* genotype	Phage strain		
		T4 wild type	Rid394×4	*goF1*
W3110	+	0.8	0.7s	0.8
W3110*trpR*	+	0.9	0.2s	0.8
W3110*trpREAilv*	+	0.9	0.1s	1.0
TU6	+	0.5	10^{-5}	0.8L
TU8	*rho102*	0.5	0.1	0.7
TU20	*rho104*	0.7	0.1	0.7
DW319	+	0.8	0.4s	0.7
ML1	*rho115*	1.1	1.0	0.7
SKB178	+	0.9	0.8s	0.8
HDF40.08	*rho40.08*	10^{-6}	2×10^{-6}	0.6

All plates were incubated at 37°. "s" and "L" indicate, respectively, plaques significantly smaller or larger than plaques of wild-type phage.

al. 1976; UZAN *et al.* 1981), which provides a convenient selection method. Among 100 Val^R transductants tested, 89 were heat resistant; 20 of these were tested for T4 growth and were found to permit growth of T4 wild type and Rid394×4 to about the same extent at 37° as did SKB178, the *rho*$^+$ parental strain of HDF40.08. These results show that TU6 harbors a wild-type *rho* gene.

Growth of a Rid mutant in the presence of a multicopy plasmid carrying the wild-type allele of the K12 rho *gene*: The plasmid pJG32 (GRAY, PATIN and CALHOUN 1981) contains 6.8 kilobase pairs of bacterial DNA from the *ilv-rho-cya* region cloned in pBR322. It codes for a protein of the same size as Rho factor. It was obtained from a bacterial strain carrying no known *rho* mutation and suppresses the rifampicin sensitivity of *rho115* (GRAY, PATIN and CALHOUN 1981). Whereas strain TU20, as expected (KORN and YANOFSKY 1976a), grows in minimal medium in the presence of 7-azatryptophane (1 μg/ml) and indole (5 μg/ml), neither TU6 nor TU6·pJG32 does. Thus, pJG32 likely carries and expresses a wild-type *rho* gene. The plasmid was introduced into several *E. coli* strains, and plating of T4 wild type and Rid 394×4 was tested. Results are shown in Table 3. The plasmid permitted better growth of Rid 394×4 in TU6 and also permitted better growth of T4 in HDF40.08. The simplest explanation for these results is that a wild-type *rho* gene can be expressed from the plasmid pJG32 and that such expression facilitates growth of Rid394×4 in TU6 and growth of T4 in HDF40.08.

Growth of Rid$^-$ phage at different temperatures: Table 4 shows the eop of different phage strains at different temperatures. Growth of Rid394×4 was markedly cold sensitive in the multiply mutant *rho*$^+$ host TU6. Also, plating of T4 wild type in this strain was somewhat cold sensitive: although the eop was not drastically reduced at 30°, plaques were small. Wild-type phages T5, T7, P1, P2, λ, φ80 or Mu were not similarly inhibited in TU6 (data not shown). Mutations *rho102* (strain TU8), *rho104* (strain TU20) and *rho115*

TABLE 3

Effect of plasmid pJG32 in plating of Rid394×4

Host strain	Phage strain	
	T4 wild type	Rid394×4
B	1.0	1.0
TU6	0.3	10^{-5}
TU6·pJG32	0.8	0.2
TU6·pJG32 cured[a]	0.5	10^{-5}
TU8	0.6	0.2
TU8·pJG32	0.7	0.1
TU8·pJG32 cured	0.7	0.3
SKB178	1.0	1.1
SKB178·pJG32	1.0	1.0
HDF40.08	4×10^{-7}	2×10^{-6}
HDF40.08·pJG32	1.0	0.9
HDF40.08·pJG32 cured[a]	10^{-7}	10^{-7}

Bacterial strains carrying plasmids were grown in the presence of ampicillin, 50 μg/ml. No antibiotics were added to the plates. All plates were incubated at 37°.

[a] TU6·pBR322 and HDF40.08·pBR322, respectively, gave eop results similar to these.

TABLE 4

Eop of phage strains on different hosts at different temperatures

Host strain		Phage strain		
		T4 wild type	Rid394×4	*tamB5*
TU6	30°	0.2	10^{-6}	$<10^{-8}$
	37°	0.3	4×10^{-6}	$<10^{-6}$
	42°	0.6	0.03	0.02
TU8	30°	0.4	10^{-5}	$<10^{-8}$
	37°	0.5	0.06	0.01
	42°	0.6	0.3	0.05
TU20	30°	0.7	0.1	0.04
	37°	0.7	0.1	0.06
	42°	0.8	0.1	0.04
TU6·pJG32	30°	0.7	0.09	0.1
	37°	0.8	0.1	0.1
	42°	0.7	0.2	0.2

(strain ML1, data not shown) suppressed the cold-sensitive plating defects of T4. Growth on the *rho104* strain was not temperature dependent, whereas some cold sensitivity remained on the *rho102* (and *rho115*, data not shown) strains. Also the rho^+ multicopy plasmid pJG32 suppressed the cold sensitivity.

Rid394×4 was restricted also in the "hospital" strain CTr5x, previously

shown to restrict T4 mutants defective in genes *pseT* (DEPEW and COZZARELLI 1974), *pla*CTr5x (HOMYK and WEIL 1974), *mot* (HALL and SNYDER 1981) and *63* (RUNNELS *et al.* 1982) and in the *optA*$^-$ strain HR44 at high temperatures (data not shown). At low temperatures both HR44 and its *optA*$^+$ parental strain HR42 restricted Rid394×4 to about the same extent as other multiply defective *rho*$^+$ strains. Introduction of pJG32 into HR44 did not significantly improve its ability to support growth of Rid394×4 at high temperatures. A mutation in the *optA* gene in *E. coli* leads to restriction of growth of bacteriophage T7 carrying a mutation in the gene *1.2* (SAITO and RICHARDSON 1981). GAUSS, DOHERTY and GOLD (1983) have shown that an *optA*$^-$ strain of *E. coli* at high temperatures restricts certain T4 mutants carrying mutations in genes *43* (DNA polymerase). Restriction of T4 strain L148 in *optA*$^-$ bacteria, also observed by GAUSS, DOHERTY and GOLD (1983), was recently shown to be due to a *dexA* (exonuclease A, WARNER *et al.* 1972) mutation present in addition to a *dda* (DNA helicase/DNA-dependent ATPase, BEHME and EBIZUSAKI 1975; KUHN, ABDEL-MONEM and HOFFMANN-BERLING 1978) mutation in strain L148 (P. GAUSS and L. GOLD, personal communication).

Some previously characterized T4 mutants displayed plating properties similar to those of Rid394×4 (data not shown). Mutants *motfarP85, tamB5, tamA3, dda dexA* L148 and *39-56del12×2* all showed a Rid phenotype: cold-sensitive restriction in the *rho*$^+$ strain TU6 and relief of this restriction in the *rho102* or *rho104* derivatives of TU6 and in TU6·pJG32. They were also restricted in CTr5x. A *pseT*$^-$ mutant grew in TU6 at all temperatures, although it was cold sensitive in CTr5x, whereas *63amM69* was restricted in both TU6 and CTr5x at all temperatures. Amber mutants in T4 genes *30, 32, 39, 40, 41, 42, 43, 44, 45, 46, 52, 56, 59, 60, 63* or the λ gene *N* did not form plaques in TU8, TU20 or TU6·pJG32.

Reversion of the Rid mutation: Large plaques of Rid394×4 arose with a frequency of 10^{-4} to 10^{-6} on the different restricting hosts. Phage from such plaques invariably showed wild-type phenotype under all plating conditions (data not shown), suggesting that all mutant phenotypes of Rid394×4 were due to a single defect in the phage. We have not determined whether these are true revertants or pseudorevertants.

Growth of the Rid mutant in liquid culture: Figure 2 shows SDS-polyacrylamide gel electrophoresis of proteins synthesized in TU6 (*rho*$^+$) and TU20 (*rho104*) infected with either T4 wild type or Rid394×4 at 30°. The kinetics of appearance of different T4 proteins appeared quite similar in cells infected with wild type as compared to Rid394×4 phage, and in TU6 as compared to TU20. Some minor differences were seen in the *rho*$^-$ strain, resembling but less pronounced than the differences seen by STITT *et al.* (1980) in T4 wild type infection of the Hdf *rho*$^-$ strain HDF0.26. The synthesis of many proteins ceased earlier in cells infected with Rid394×4 than in cells infected with T4 wild type. Minor deviations in protein synthesis may contribute to the lower phage production in TU20 as compared to TU6 (see below), whereas the early cessation of protein synthesis in Rid394×4-infected cells correlates with the early termination of phage development in these cells (see below).

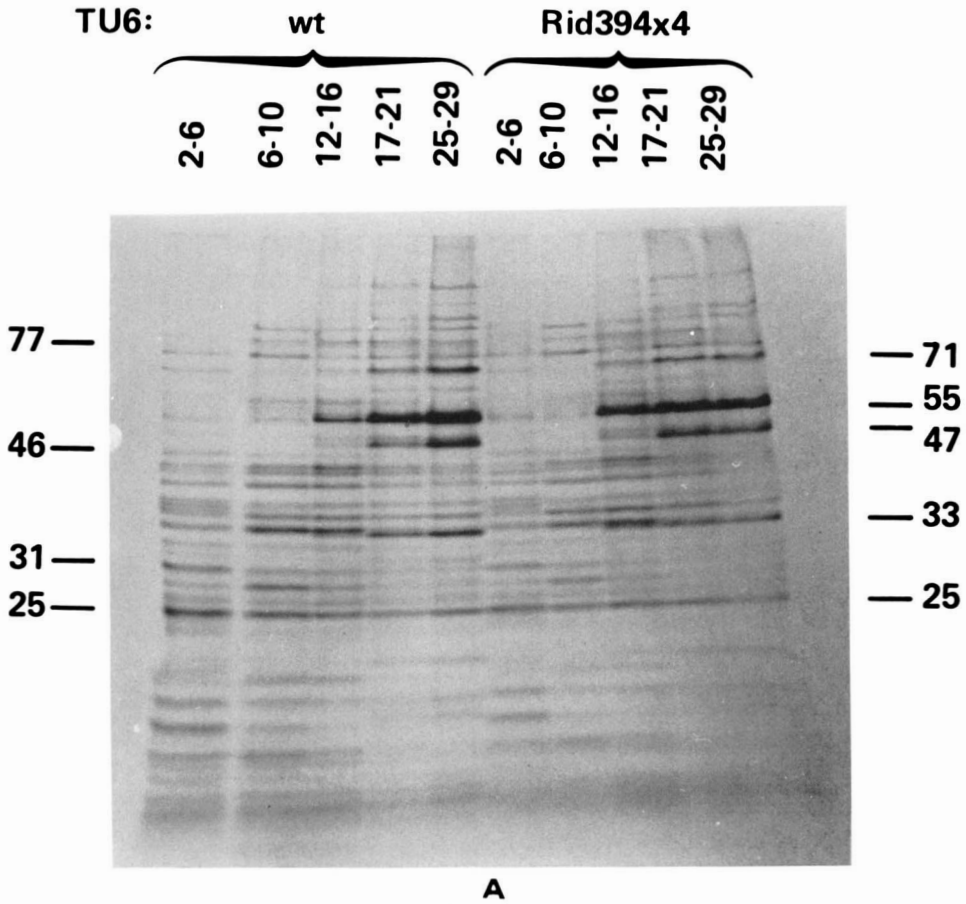
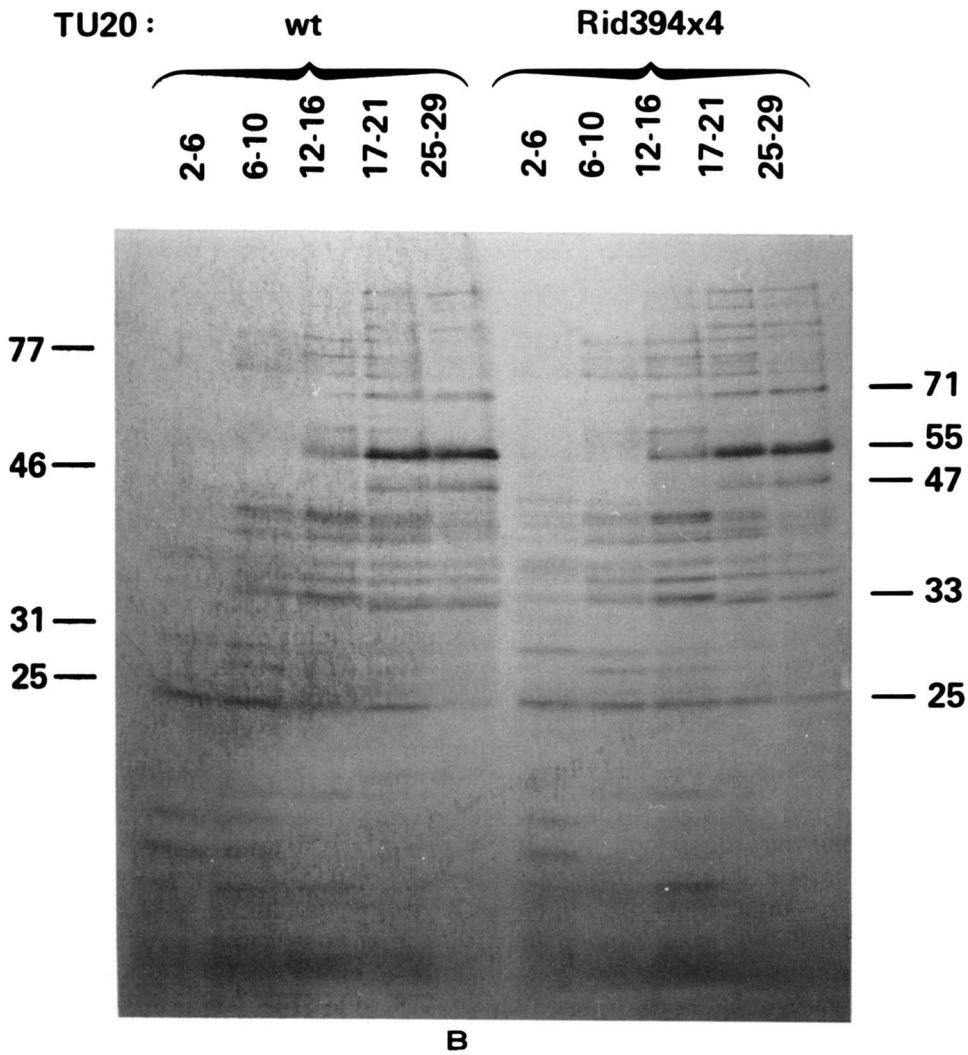


FIGURE 2.—Protein synthesis in TU6 and TU20 infected with T4 wild type and Rid394×4. Infected cells were pulse-labeled with [^{14}C]amino acids at 30° for the indicated periods. All samples in one panel are in the original order and alignment. Polypeptide molecular weights [kilodaltons (kd)] were estimated from plots of molecular weight *vs.* the inverse of distance migrated; this plot was approximately linear in the size range from 94 to 14 kd. The estimated molecular weights for some prominent bands are shown to the left and right of each panel.

The synthesis of the enzymes dCTPase (DE I), β-glucosyl transferase (DE I) and deoxynucleotide kinase (DE II) at 30° was not significantly affected by the Rid394×4 mutation, since kinetics of induction and amount of enzyme synthesis were quite similar in TU6 infected with T4 wild type or Rid394×4 (data not shown).

Figure 3 shows that the kinetics of DNA synthesis at 30° were quite similar after infection with Rid394×4 or T4 wild type but for both phage were delayed in TU6 as compared to W3110. The net synthesis was also lower in TU6 than in W3110 but, nevertheless, amounted to >100 phage-equivalent units/infected cell 90 min after infection (estimated by uptake of $^{32}P_i$, data not shown). DNA synthesis in TU20 was not significantly different from synthesis in TU6. DNA synthesis was not affected in HR44 at 42° (kinetics being similar



B

after infection of *E. coli* B, HR42 or HR44 at 42° with T4 wild type or Rid394×4, data not shown).

Maturation and cell lysis at 30° were somewhat differently affected after infection at low moi/low cell density, or high moi/high cell density (Figures 4 and 5). In low moi infections, maturation of Rid394×4 started 20–30 min after infection and continued until about 90 min after infection in the permissive *rho*$^+$ strain W3110 (Figure 4A); lysis started 40–50 min after infection and was essentially complete 110 min after infection. In TU6, maturation started at about the same time (Figure 4B) but ceased about 50 min after infection, and lysis was considerably delayed, not starting until 70–80 min after infection. In strains TU20 and TU6·pJG32, maturation started later (30–40 min after infection, Figure 4A and B) and yielded lower phage production than in TU6, but lysis was more efficient, especially in TU6·pJG32. Infection

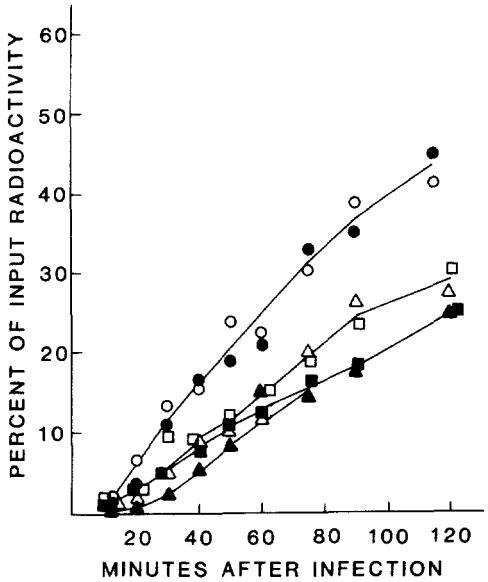


FIGURE 3.—DNA synthesis. *E. coli* W3110, TU6 and TU20 were grown in GCA to 3×10^8 cells/ml at 37°, transferred to 30° and infected with T4 wild type or Rid394×4 at moi 8. Seven minutes after infection [^{3}H]dThd (Amersham TRK.418) and cold deoxyadenosine (dAdo) were added to yield 1 μCi/ml (6×10^{-9} g/liter or 2.4×10^{-8} M) and 250 μg/ml, respectively. Aliquots of 30 μl were withdrawn at different times, and DNA synthesis was estimated. The figure shows percent of added radioactivity (100% equals 4.8×10^5 cpm/ml) incorporated into TCA-precipitable material. Infective centers and phage production were similar to those shown in Figure 4. ●, W3110 infected with T4 wild type; ○, W3110 infected with Rid394×4; ▲, TU6 infected with T4 wild type; △, TU6 infected with Rid394×4; ■, TU20 infected with T4 wild type; □, TU20 infected with Rid394×4.

of TU6 or TU20 with T4 wild type (Figure 4C) resulted in normal single-step growth, but here also phage production was lower in TU20.

After infection at high moi (Figure 5) onset and termination of maturation and total phage production per infected cell of Rid394×4 in TU6 were about the same as in the low moi experiment, but lysis was less efficient (Figure 5A). In TU20, TU6·pJG32 and W3110, phage production continued unabated for the duration of the experiment (2.5 hr). Lysis was somewhat more efficient than in TU6, but none of the cultures lysed visibly, as determined by turbidimetry (Figure 5B).

DISCUSSION

This communication describes a new phenotype of T4 *t*-mutants, Rid, and suggests a role for *E. coli* Rho factor in maturation and lysis of T4. Plating of Rid mutants was cold sensitive in most *rho*$^+$ strains of *E. coli* but was restored by polarity-suppressing *rho* mutations (Table 2) or by the presence of a multicopy plasmid carrying a wild-type allele of the *rho* gene (Tables 3 and 4). The suppression of the plating defect was correlated with prolonged phage production and suppression of lysis deficiency in liquid culture (Figures 4 and 5).

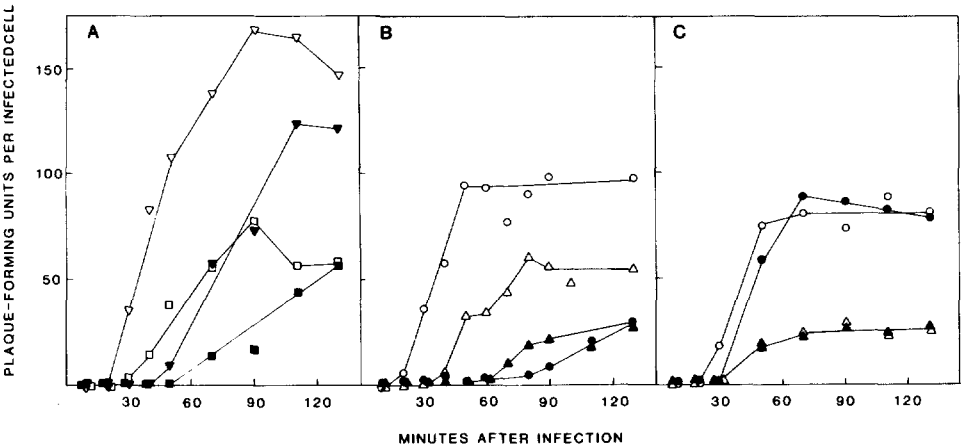


FIGURE 4.—Maturation and lysis in *rho*$^+$ and *rho*$^-$ cells infected with Rid394×4 or T4 wild type, respectively, at low moi. *E. coli* W3110 and TU6·pJG32 (A) and TU6 and TU20 (B and C) were grown in LB (LB + ampicillin for TU6·pJG32) at 37° to 3 × 10^8 cells/ml, transferred to 30° and infected with Rid394×4 (A and B) or T4 wild type (C) at moi 0.1. Five minutes after infection the infected cells were diluted 10^4-fold in prewarmed LB (without ampicillin). Samples were removed at different times and plated without (closed symbols) or with (open symbols) treatment with chloroform. All plating was on *E. coli* B at 30°, except that viable cells at the time of infection and survivors 5 min after infection were plated at 37°. To calculate the yield, the number of infected cells was approximated by the number of infecting phage, since survivors could not be accurately estimated at this low moi, and all infective centers did not plate with 100% efficiency. A, ▼ and ▽: W3110 infected with Rid394×4 (infective centers: 10%); ■ and □: TU6·pJG32 infected with Rid394×4 (infective centers: 8%). B, ● and ○: TU6 infected with Rid394×4 (infective centers: 5%); ▲ and △: TU20 infected with Rid394×4 (infective centers: 10%). C, ● and ○: TU6 infected with T4 wild type (infective centers: 6%); ▲ and △: TU20 infected with T4 wild type (infective centers: 9%).

Restriction in rho$^+$ *strains*: Since expression of the *rho* gene is regulated by attenuation (BROWN *et al.* 1982; KUNG *et al.* 1984), it is likely that all polarity-suppressing mutants overproduce Rho factor. This has been shown directly for *rho115* and some other polarity-suppressing *rho* alleles (RATNER 1976; IMAI and SHIGESADA 1978). Thus, cells carrying a polarity-suppressing *rho* allele will likely contain more copies of Rho factor than a cell with only a chromosomal copy of a wild-type *rho* allele. Also, a cell carrying a multicopy plasmid derived from pBR322 expressing a wild-type *rho* gene will likely overproduce Rho factor, by a factor of at least two (CHRISTIANSEN and PEDERSEN 1981) compared to a wild-type cell lacking such a plasmid. Strains that allow growth of Rid394×4 are thus likely to contain normal (wild-type strains) or high (Psu or multicopy *rho*$^+$ plasmid carrying strains) levels of wild-type or polarity-suppressing Rho. Therefore, we suggest that the reason for restriction of the Rid mutant in certain *rho*$^+$ hosts is that these strains contain less available Rho factor. The cold sensitivity of the restriction may indicate that a membrane-associated function or a highly energy-requiring step is involved or that Rho factor is unnecessary, or more available, at high temperature.

The reason(s) for host strain-specific variation in restriction ("unavailability" of Rho factor only in certain *rho*$^+$ strains) is not well understood. A transduc-

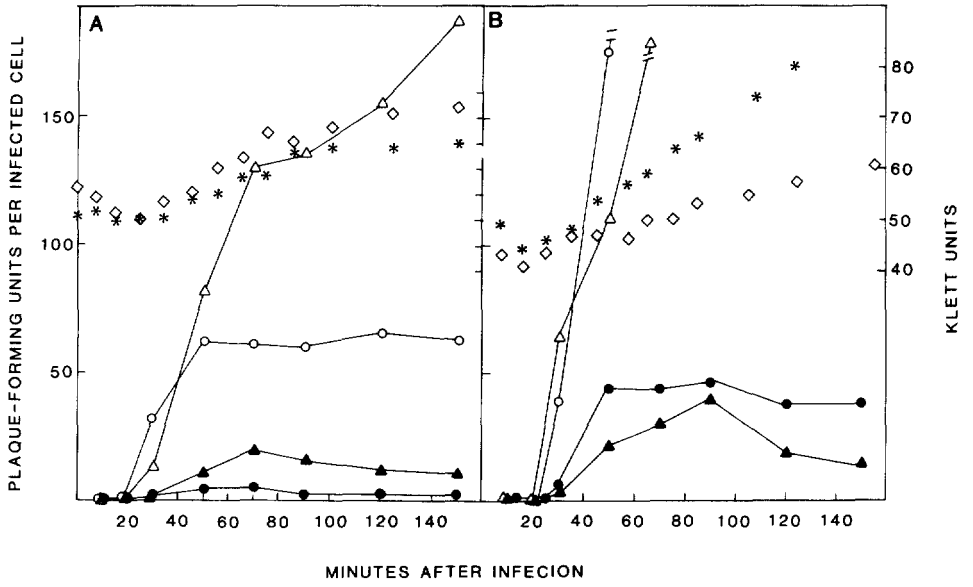


FIGURE 5.—Maturation and lysis in *rho*$^+$ and *rho*$^-$ cells infected at high moi with Rid394×4. *E. coli* cells were grown as for Figure 4 and infected at moi 10 with Rid394×4. Cells were not diluted after infection. Samples were removed at different times and plated without (closed symbols) or with (open symbols) treatment with chloroform. Plating was as for Figure 4. The number of infected cells was considered equal to the number of viable cells at the time of infection (2.5×10^8 cells/ml), since ≤1% of the cells survived as colony formers 5 min after infection. A, TU6 (infective centers: 16%): ● and ○, pfu; *, turbidity. TU20 (infective centers: 57%): ▲ and △, pfu; ◇, turbidity. B, W3110 (infective centers: 58%): ● and ○, pfu; *, turbidity: TU6·pJG32 (infective centers: 39%): ▲ and △, pfu, ◇, turbidity. The total yield 150 min after infection was 675 pfu/cell in TU6·pJG32 and 430 pfu/cell in W3110. Turbidity of the infected cultures was measured in a Klett spectrophotometer.

tion experiment verified that the most restricting strain, TU6, indeed carries a wild-type *rho* gene that can support normal T4 growth in a different genetic background. We note that strongly restricting strains invariably carry a large number of additional, but not necessarily related, mutations (Tables 2 and 4; C. H. LINDER and K. CARLSON, unpublished observations) and suggest that the cold-sensitive restriction of Rid$^-$ phage is correlated somehow with the occurrence of polarity and polar mutations in the bacterial genome. The possibility that polarity reduces the availability of Rho factor is currently being tested by introducing and removing polar mutations by transduction in these strains.

Restriction of Rid$^-$ phage in other strains of E. coli: Bacterial mutations that restrict viral growth have served to identify several areas where interactions between phage and host components are essential to phage development. The nature of the interactions are not always easily understood, whether or not the functions of the protein involved are well known. In fact, many restrictive systems may be understood only in retrospect, when more information has been gathered concerning the phage and host components. The Rid phenotypes of the *dda dexA* double mutant, *(39-56)del12*, and *mot* mutants and the restriction of Rid394×4 in CTr5x and in the *optA*$^-$ host cannot be explained

from the present data and may be due to direct or indirect effects of the phage and host mutations. However, all phenotypic defects of Rid394×4 are likely caused by one single mutation, since all phenotypic defects revert in one step.

T4 protein(s) interacting with Rho: We had designed our selection scheme to yield T4 mutants defective in antitermination, since our previous work (LINDER and SKÖLD 1980) had shown that phage-coded proteins may transactivate certain DE genes. A mutation in a gene coding for such a transactivating protein regulating an essential gene would result in a mutant unable to grow in a *rho*$^+$ host but able to grow in a polarity-suppressing strain where antitermination was not essential for expression of the affected gene. No such mutants were found, however, which suggests that few, if any, essential genes depend solely upon such transactivation for their expression.

Host-defective mutations probably within the *rho* gene (SIMON, SNOVER and DOERMANN 1974; TAKAHASHI 1978; STITT *et al.* 1980) lead to abortion of T4 infection. Although the possibility that host defectiveness is caused by a secondary mutation close to, but outside, *rho* has not been completely ruled out, it appears unlikely in view of the available data concerning these strains. We shall assume in this discussion that host defectiveness is due to a *rho* mutation. In these Hdf strains under restrictive conditions, early and late phage protein synthesis is aberrant, phage DNA synthesis depressed and maturation and lysis delayed and reduced. Compensatory mutations *comC-α* (growing in *tabC*; TAKAHASHI 1978; TAKAHASHI and YOSHIKAWA 1979) and *goF1* (growing in HDF strains, STITT *et al.* 1980) map clockwise of gene *39*, close to each other and to the mutation *go9H* isolated by JENSEN and SUSMAN (1980), which compensates for an uncharacterized host defect, preventing growth of T4 wild-type phage. T4 mutations compensating for these host defects have been isolated also in gene *45* (TAKAHASHI 1978), near gene *e* (STITT *et al.* 1980), and between genes *31* and *33* (STITT *et al.* 1980). The restriction of phage λ in an Hdf strain, which is accompanied by lack of antitermination at tR2 but normal *N*-dependent antitermination elsewhere (DAS *et al.* 1983), is considerably weaker than restriction of T4 in these strains. PULITZER and coworkers (CARUSO *et al.* 1979; PULITZER, COPPO and CARUSO 1979) and STITT *et al.* (1980) suggest that Hdf mutants produce a Rho factor unable to interact with T4 antitermination protein(s), and that *goF1* and *comC-α* mutations alter this antitermination function so that it may again interact with the altered Rho. To account for the observed requirement for a functional Rho also at late times, STITT *et al.* (1980) suggested that some late expression may depend upon readthrough of termination signals due to T4 modulation of Rho activity, although some late defects could be secondary results of earlier alterations in DNA synthesis or gene expression. The wild-type allele of the postulated anti-terminator gene is not essential, since deletion of the *goF1* and *comC-α* sites, as in *39-56del12*, does not prevent phage production in *rho*$^+$ strains, although the burst size is somewhat reduced. Presumably essential DE genes may be transcribed via the alternate *mot*-dependent pathway (PULITZER, COPPO and CARUSO 1979).

This model does not explain why the Hdf defect reduces T4 growth several

orders of magnitude more than a deletion of the *goF1* site (STITT *et al.* 1980). Possibly, Rho has additional role(s) in T4 development, in addition to a role in regulating transcription which is nonessential under laboratory conditions. In fact, work by ZOGRAFF and GINTSBURG (1980) suggests that the reduced DNA synthesis observed in a T4-infected Hdf strain (*rhots15* at 42°) is not mediated via an effect on early phage transcription.

Our results suggest *t* as another gene or region involved in interaction with Rho, directly or indirectly. The *t* gene product has been suggested by JOSSLIN (1970, 1971) to disrupt the inner membrane of the host cell, giving lysozyme access to the peptidoglycan, but its mechanism of action is not understood. JOSSLIN (1970, 1971) isolated *t* mutants by a different selection procedure and showed that in single-step growth experiments they continue phage production beyond the normal cessation time and are lysis inhibited. *t* mutants also suppress mutations in genes *rII* (JOSSLIN 1971) and *63* (HALL *et al.* 1980). Two *t* mutants isolated by Josslin showed similar plating properties as Rid394×4 in our study.

Our data indicate that normal or above-normal levels of Rho factor were essential for the prolonged phage production (Figures 4 and 5). Lysis inhibition, on the other hand, was most pronounced when Rho appeared undersupplied. Thus, the phenotypes of *t* mutants described by JOSSLIN (1970, 1971) are intermediate results obtained at normal (intermediate) cellular Rho levels.

The restriction of Rid394×4 on solid media was correlated with cessation of phage production in liquid culture 40–50 min after infection and with severe lysis inhibition, whereas plaque formation was correlated with varying degrees of relief of these restrictions. Continued phage production and slow lysis are normal features of T4 growth in dense cultures infected with high moi, where early-released progeny is believed to superinfect remaining cells and cause these phenomena in a poorly understood fashion. At low cell densities (Figure 4) such superinfection is unlikely. Here, our results most likely reflect a requirement for Rho, both for continued phage production and for lysis of Rid394×4-infected cells. This, in turn, suggests that Rho, directly or indirectly, can replace or boost T activity in causing these phenomena.

We found no evidence for significant differences in regulation of expression of T4 genes in TU6 which could be attributed to the T4 *t* mutation Rid394×4, except an earlier turn-off of protein synthesis. This correlates with the early cessation of phage development in Rid394×4-infected cells. Since both mutant (Psu) and wild-type Rho could support Rid 394×4 growth, this function may not involve Rho transcription termination activity. Overall kinetics of protein synthesis and induction of specific DE enzymes in TU6 closely resembled what has been observed by us and others in wild-type *E. coli* strains. Thus, apparently enough Rho is available for normal transcription regulation. Perhaps the more stringent Rho requirement in continued maturation and cell lysis reflects a structural, rather than catalytic, Rho function in these processes.

Rho in DNA synthesis and phage maturation: The polarity-suppressing *rho104* strain TU20 gave delayed and reduced DNA synthesis, and delayed and (in single-step growth) reduced phage production, as compared to its wild-type

rho$^+$ parental strain W3110 (Figures 3–5). Some minor alterations in protein synthesis patterns were observed in the *rho* mutant strain (Figure 2). As mentioned above, Hdf strains give similar results (STITT *et al.* 1980; ZOGRAFF and GINTSBURG 1980), although here restriction of T4 is much more severe.

Oversupply of wild-type Rho, in strain TU6·pJG32, had similar effects as the *rho104* mutation on both DNA synthesis and phage production, whereas undersupply of wild-type Rho, in strain TU6, delayed only DNA synthesis. These Rho effects were seen in cells infected with T4 wild type to the same extent as in cells infected with Rid394×4 and, thus, do not involve the *t* gene product. These effects on protein and DNA synthesis and on maturation apparently are not severe enough to lead to reduced plaque-forming ability of the phage in these strains.

Overproduction of wild-type Rho would be expected to have opposite consequences for transcription termination as compared to a polarity-suppressing mutant Rho, although both may adversely affect the balance in transcription. These Rho effects of phage DNA synthesis and maturation, therefore, are more likely to result from either unbalanced transcription or unbalanced supply of Rho itself rather than from deficiencies in Rho transcription termination activity.

Role(s) of Rho in T4 development: The preceding discussion supports a somewhat broader model for the T4 phenotype in Hdf strains: the mutated Rho factor is defective in essential interaction(s) with T4 protein(s). One such interaction may be with the *t* gene product. Another may be with proteins in the DNA replication apparatus. We note that a *goF1* mutant, selected for ability to grow in an Hdf strain, grew much better than T4 wild type in TU6 (Table 2), and we were unable to isolate a *goF1* × Rid394×4 recombinant combining both the GoF and the Rid phenotypes. Perhaps the *goF1* mutation permits the use of alternate, Rho-independent pathways in DNA synthesis, phage production and lysis.

Our results do not allow us to discriminate between direct and indirect role(s) of Rho in the events governed by the *t* gene product. A direct mechanism would imply protein-protein interaction between Rho and the *t* gene product; indirect mechanisms could involve Rho control of a T4 protein interacting with *t* protein or a Rho-mediated modification of the cell membrane rendering it more or less susceptible to the action of the T4 *t* gene product. A membrane-associated effect is, of course, an attractive hypothesis since it permits a simplified model for Rho involvement: a modification of membrane properties which, in turn, affects initiation of DNA synthesis, onset of phage maturation and gene product *t* action in termination of phage production and cell lysis (all of these events are known to involve the cell membrane). One of several possible explanations for the observed cold sensitivity of the Rid mutants is that a membrane function is involved. Additional evidence for Rho effects on the cell membrane comes from the observations that restriction of T4 in hd590 (an Hdf strain) was partially relieved in the presence of EDTA (SIMON, SNOVER and DOERMANN 1974) and that Rho probably controls, or forms part of, a membrane-bound ATPase (DAS, COURT and ADHYA 1979).

We are grateful to M. BEHME, D. CALHOUN, A. DAS, S. GUTERMAN, E. KUTTER, G. MOSIG, C. C. RICHARDSON, T. J. SNOPEK, B. STITT, J. WIBERG and C. YANOFSKY for gifts of strains, to K.-E. JOHANSSON for invaluable help with protein gels, and to A. ØVERVATN and C. SJUNNESKOG for technical assistance. Several of the experiments described here originated as student exercises in molecular genetics in Tromsø and in pharmaceutical microbiology in Uppsala. We thank all of the students involved, especially INGELA ABELIN, KERSTIN BACKMAN, GUNILLA HEDSTRÖM and ANNIKA and INGER NORDÉN, for their interest and contributions. This investigation was supported in part by a grant from the Norwegian Research Council for Science and the Humanities (to K.C.), from the Swedish Medical Research Council (to C.L.) and from the Swedish Pharmaceutical Society and Sven Söderlundhs Minnesfond (to C.L.).

LITERATURE CITED

BAUMBERG, S. and M. G. LOVETT, 1977 Reduced recovery of plasmid transconjugants in crosses with *Escherichia coli rho* mutant recipients. Plasmid **1:** 118–122.

BEHME, M. T. and K. EBIZUSAKI, 1975 Characterization of a bacteriophage T4 mutant lacking DNA-dependent ATPase. J. Virol. **15:** 50–54.

BROWN, S., B. ALBRECHTSEN, S. PEDERSEN and P. KLEMM, 1982 Localization and regulation of the structural gene for transcription termination factor rho of Escherichia coli. J. Mol. Biol. **162:** 283–298.

CARDILLO, T. S., E. F. LANDRY and J. S. WIBERG, 1979 *regA* protein of bacteriophage T4D: identification, schedule of synthesis, and autogenous regulation. J. Virol. **32:** 905–916.

CARUSO, M., A. COPPO, A. MANZI and J. F. PULITZER, 1979 Host-virus interactions in the control of T4 prereplicative transcription I. *tabC* (*rho*) mutants. J. Mol. Biol. **135:** 959–977.

CHRISTIANSEN, L. and S. PEDERSEN, 1981 Cloning, restriction endonuclease mapping and post-transcriptional regulation of *rpsA*, the structural gene for ribosomal protein S1. Mol. Gen. Genet. **181:** 548–551.

DAEGELEN, P. and E. BRODY, 1976 Early bacteriophage T4 transcription: a diffusible product controls *r*IIA and *r*IIB RNA synthesis. J. Mol. Biol. **103:** 127–142.

DAEGELEN, P., Y. D'AUBENTON-CARAFA and E. BRODY, 1982a The role of *rho* in bacteriophage T4 development. I. Control of growth and polarity. Virology **117:** 105–120.

DAEGELEN, P., Y. D'AUBENTON-CARAFA and E. BRODY, 1982b The role of *rho* in bacteriophage T4 development. II. *mot*-dependent (middle mode) RNA synthesis. Virology **117:** 121–134.

DARLIX, J. L. and M. HORAIST, 1975 Existence and possible roles of transcriptional barriers in T7 DNA early region as shown by electron microscopy. Nature **256:** 288–292.

DAS, A., D. COURT and S. ADHYA, 1976 Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in transcription termination factor rho. Proc. Natl. Acad. Sci. USA **73:** 1959–1963.

DAS, A, D. COURT and S. ADHYA, 1979 Pleiotropic effect of *rho* mutation in *Escherichia coli*. pp. 459–467. In: *Molecular Basis of Host-Virus Interaction*, Edited by M. CHAKRAVORTY. Science Press, Princeton, New Jersey.

DAS, A., M. E. GOTTESMAN, J. WARDWELL, P. TRISLER and S. GOTTESMAN, 1983 A mutation in the *Escherichia coli rho* gene that inhibits the N protein activity of phage λ. Proc. Natl. Acad. Sci. USA **80:** 5530–5534.

DEPEW, R. E. and N. COZZARELLI, 1974 Genetics and physiology of bacteriophage T4 3′-phosphatase: evidence for the involvement of the enzyme in T4 DNA metabolism. J. Virol. **13:** 888–897.

FAVRE, R., A. WIATER, S. PUPPO and M. IACCARINO, 1976 Expression of a valine-resistant acetolactate synthase activity mediated by the *ilvO* and *ilvG* genes of *Escherichia coli* K12 Mol. Gen. Genet. **143:** 243–252.

FRASER, D. and E. A. JERREL, 1953 Amino acid composition of T3 bacteriophage. J. Biol. Chem. **205:** 291–295.

FULLER, R. S. and T. PLATT, 1978 The attenuator of the tryptophan operon in *E. coli*: rho-mediated release of RNA polymerase from a transcription termination complex *in vitro*. Nucleic Acids Res. **5:** 4613–4623.

GAUSS, P., D. H. DOHERTY and L. GOLD, 1983 Bacterial and phage mutations that reveal helix-unwinding activities required for bacteriophage T4 DNA replication. Proc. Natl. Acad. Sci. USA **80:** 1669–1673.

GOLDBERG, A. R., 1970 Termination of *in vitro* RNA synthesis by ρ factor. Cold Spring Harbor Symp. Quant. Biol. **35:** 157–161.

GOLDFARB, A. and S. MALIK, 1984 Changed promoter specificity and antitermination properties displayed *in vitro* by bacteriophage T4-modified RNA polymerase. J. Mol. Biol. **177:** 87–105.

GRASSO, R. J. and J. M. BUCHANAN, 1969 Synthesis of early RNA in bacteriophage T4-infected *Escherichia coli* B. Nature **244:** 882–885.

GRAY, J. E., D. W. PATIN and D. H. CALHOUN, 1981 Identification of the protein products of the *rrnC, ilv, rho* region of the *Escherichia coli* K12 chromosome. Mol. Gen. Genet. **183:** 428–436.

GUARENTE, L., 1979 Restoration of termination by RNA polymerase mutations is *rho* allele-specific. J. Mol. Biol. **129:** 295–304.

GUARENTE, L. P., D. H. MITCHELL and J. BECKWITH, 1977 Transcription termination at the end of the tryptophan operon. J. Mol. Biol. **112:** 423–436.

GULLETTA, E., A. DAS and S. ADHYA, 1983 The pleiotropic *ts15* mutation of *E. coli* is an IS*1* insertion in the *rho* structural gene. Genetics **105:** 265–280.

GUTERMAN, S. K. and C. L. HOWITT, 1979a Rifampicin supersensitivity of *rho*$^-$ strains of *E. coli* and suppression by *sur* mutation. Mol. Gen. Genet. **169:** 27–34.

GUTERMAN, S. K. and C. L. HOWITT, 1979b Rho and ribosome mutation interaction: lethality of *rho-15* in *rpsL* or *rpsE* strains, and *rho-15* methionine auxotrophy in *rps*$^+$ strains of *Escherichia coli*. Genetics **93:** 353–360.

HALL, D. H., R. G. SARGENT, K. F. TROFATTER and D. L. RUSSELL, 1980 Suppressors of mutations in the bacteriophage T4 gene coding for both RNA ligase and tail fiber attachment activities. J. Virol. **36:** 103–108.

HALL, D. H. and R. D. SNYDER, 1981 Suppressors of mutations in the rII gene of bacteriophage T4 affect promoter utilization. Genetics **97:** 1–9.

HOMYK, T. and J. WEIL, 1974 Deletion analysis of two nonessential regions of the T4 genome. Virology **61:** 505–523.

HOWARD, B. H. and B. DE CROMBRUGGHE, 1976 ATPase activity required for termination of transcription by the *Escherichia coli* protein factor ρ. J. Biol. Chem. **251:** 2520–2524.

IMAI, M. and K. SHIGESADA, 1978 Studies on the altered rho factor in *nitA* mutants of *Escherichia coli* defective in transcription termination. I. Characterization and quantitative determination of rho in cell extracts. J. Mol. Biol. **120:** 451–466.

JAYARAMAN, R., 1972 Transcription of bacteriophage T4 DNA by *Escherichia coli* RNA polymerase *in vitro*: identification of some immediate-early and delayed-early genes. J. Mol. Biol. **70:** 253–263.

JENSEN, J. L. and M. SUSMAN, 1980 A mutant of *E. coli* that restricts growth of bacteriophage T4 at elevated temperatures. Genetics **94:** 301–325.

JERGIL, B. and R. OHLSSON, 1974 Phosphorylation of proteins in rat liver: endogenous phosphorylation and dephosphorylation of proteins from smooth and rough endoplasmic reticulum and free ribosomes. Eur. J. Biochem. **46:** 13–25.

JOHNSON, J. R. and D. HALL, 1973 Isolation and characterization of mutants of bacteriophage T4 resistant to folate analogs. Virology **53:** 413–426.

JOSSLIN, R., 1970 The lysis mechanism of phage T4: mutants affecting lysis. Virology **40:** 719–726.

JOSSLIN, R., 1971 Physiological studies on the *t* gene defect in T4 infected *Escherichia coli*. Virology **44:** 101–107.

KELLENBERGER, E. and J. SÉCHAUD, 1957 Electron microscopical studies of phage multiplication. II. Production of phage-related structures during multiplication of phages T2 and T4. Virology **3:** 256–274.

KORN, L. J. and C. YANOFSKY, 1976a Polarity suppressors increase expression of the wild-type tryptophan operon of *Escherichia coli*. J. Mol. Biol. **103:** 395–409.

KORN, L. J. and C. YANOFSKY, 1976b Polarity suppressors defective in transcription termination at the attenuator of the tryptophan operon of *Escherichia coli* have altered rho factor. J. Mol. Biol. **106:** 231–241.

KUHN, B., M. ABDEL-MONEM and H. HOFFMANN-BERLING, 1978 DNA helicases. Cold Spring Harbor Symp. Quant. Biol. **43:** 63–67.

KUNG, H.-F., E. BEKESI, S. K. GUTERMAN, J. E. GRAY, L. TRAUB and D. H. CALHOUN, 1984 Autoregulation of the *rho* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **193:** 210–213.

KUTTER, E. and W. RÜGER, 1983 Map of the T4 genome and its transcription control sites. pp. 277–290. In: *Bacteriophage T4*, Edited by C. K. MATHEWS, E. M. KUTTER, G. MOSIG and P. B. BERGET. American Society for Microbiology, Washington, D. C.

KUTTER, E. M. and J. S. WIBERG, 1968 Degradation of cytosine-containing bacterial and bacteriophage DNA after infection of *E. coli* B with bacteriophage T4D wild type and with mutants defective in genes *46, 47* and *56*. J. Mol. Biol. **38:** 395–411.

LINDER, C. H. and O. SKÖLD, 1977 Evidence for a diffusible T4 bacteriophage protein governing the initiation of delayed early RNA synthesis. J. Virol. **21:** 7–15.

LINDER, C. H. and O. SKÖLD, 1980 Control of early gene expression of bacteriophage T4: involvement of the host rho factor and the *mot* gene of the bacteriophage. J. Virol. **33:** 724–732.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MATTSON, T., 1974 Mutant of bacteriophage T4D affecting expression of many early genes. Nature **250:** 48–50.

MATTSON, T., G. VAN HOUWE and R. H. EPSTEIN, 1978 Isolation and characterization of conditional lethal mutations in the *mot* gene of bacteriophage T4. J. Mol. Biol. **126:** 551–570.

MILLER, J. H., 1972 Curing of episomes from *E. coli* strains with acridine orange. pp. 104–106. In: *Experiments in Molecular Genetics*, Edited by J. H. MILLER. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MORSE, D. E. and M. GUERTIN, 1972 Amber *su*A mutations which relieve polarity. J. Mol. Biol. **63:** 605–608.

MOSIG, G., 1983 T4 genes and gene products. pp. 362–374. In: *Bacteriophage T4*, Edited by C. K. MATHEWS, E. M. KUTTER, G. MOSIG and P. B. BERGET. American Society for Microbiology, Washington, D. C.

NEVILLE, D. M., JR., 1971 Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. J. Biol. Chem. **246:** 6328–6334.

NORGARD, M. V., K. EMIGHOLZ and J. MONAHAN, 1979 Increased amplification of pBR322

plasmid deoxyribonucleic acid in *Escherichia coli* K12 strains RR1 and χ1776 grown in the presence of high concentrations of nucleoside. J. Bacteriol. **138:** 270–272.

PULITZER, J. F., A. COPPO and M. CARUSO, 1979 Host-virus interactions in the control of T4 prereplicative transcription. II. Interaction between *tab*C (*rho*)mutants and T4 *mot* mutants. J. Mol. Biol. **135:** 979–997.

RATNER, D., 1976 Evidence that mutations in the *suA* polarity suppressing gene directly affect termination factor rho. Nature **259:** 151–153.

REVEL, H. R., B. L. STITT, I. LIELAUSIS, and W. B. WOOD, 1980 Role of the host cell in bacteriophage T4 development. I. Characterization of host mutants that block T4 head assembly. J. Virol. **33:** 366–376.

RICHARDSON, J. P., 1970 Rho factor function in T4 RNA transcription. Cold Spring Harbor Symp. Quant. Biol. **35:** 127–133.

RICHARDSON, J. P., C. GRIMLEY and C. LOWERY, 1975 Transcription termination factor rho activity is altered in *Escherichia coli* with *su*A gene mutations. Proc. Natl. Acad. Sci. USA **72:** 1725–1728.

ROBERTS, J. W., 1969 Termination factor for RNA synthesis. Nature **224:** 1168–1174.

RUNNELS, J. M., D. SOLTIS, T. HEY and L. SNYDER, 1982 Genetic and physiological studies of the RNA ligase of bacteriophage T4. J. Mol. Biol. **154:** 273–286.

SAITO, H. and C. C. RICHARDSON, 1981 Genetic analysis of gene 1.2 of bacteriophage T7: isolation of a mutant of *Escherichia coli* unable to support the growth of T7 gene 1.2 mutants. J. Virol. **37:** 343–351.

SALSER, W., A. BOLLE and R. EPSTEIN, 1970 Transcription during bacteriophage T4 development: a demonstration that distinct subclasses of the "early" RNA appear at different times and that some are "turned off" at late times. J. Mol. Biol. **49:** 271–295.

SHARP, J. A. and T. PLATT, 1984 Rho-dependent termination and concomitant NTPase activity requires a specific, intact RNA region, J. Biol. Chem. **259:** 2268–2273.

SHIGESADA, K. and C. W. WU, 1980 Studies of RNA release reaction catalyzed by *E. coli* transcription termination factor rho using isolated ternary transcription complexes. Nucleic Acids Res. **8:** 3355–3369.

SIMON, L. D., M. GOTTESMAN, K. TOMCZAK and S. GOTTESMAN, 1979 Hyperdegradation of proteins in *Escherichia coli rho* mutants. Proc. Natl. Acad. Sci. USA **76:** 1623–1627.

SIMON, L. D., D. SNOVER and A. H. DOERMANN, 1974 Bacterial mutation affecting T4 phage DNA synthesis and tail production. Nature **252:** 451–455.

STITT, B. L., H. R. REVEL, I. LIELAUSIS and W. B. WOOD, 1980 Role of the host cell in bacteriophage T4 development. II. Characterization of host mutants that have pleiotropic effects on T4 growth. J. Virol. **35:** 775–789.

SUKHODOLETS, V. V., A. S. MIRONOV and E. V. LINKOVA, 1982 Influence of the *rho-15* temperature-sensitive (ts) mutation on the expression of the *deo*-operon in *Escherichia coli*. Mol. Gen. Genet. **187:** 157–161.

TAKAHASHI, H., 1978 Genetic and physiological characterization of *Escherichia coli* K12 mutants (*tab*C) which induce abortive infection of bacteriophage T4. Virology **87:** 256–265.

TAKAHASHI, H. and H. YOSHIKAWA, 1979 Genetic study of a new early gene, *com*C-α of bacteriophage T4. Virology **95:** 215–217.

TESSMAN, I., 1968 Mutagenic treatment of double- and single-stranded DNA phages T4 and S13 with hydroxylamine. Virology **35:** 330–333.

THERMES, C. and E. BRODY, 1984 T4-induced antipolarity: temporal heterogeneity in response of early transcription units. J. Virol. **50:** 191–201.

THIEL, T. and L. ASTRACHAN, 1977 Isolation and mapping of *t* gene mutants of bacteriophage T4D. J. Virol. **24:** 518–524.

UZAN, M., R. FAVRE, E. GALLAY and L. CARO, 1981 Genetical and structural analysis of a group of λ*ilv* and λ*rho* transducing phages. Mol. Gen. Genet. **182:** 462–470.

WALLEVIK, K., J. C. JENSENIUS, I. ANDERSEN and A. M. POULSEN, 1982 A simple and reliable method for the drying of polyacrylamide slab gels. J. Biochem. Biophys. Methods **6:** 17–21.

WARNER, H. R., D. P. SNUSTAD, J. F. KOERNER and J. D. CHILDS, 1972 Identification and genetic characterization of mutants of bacteriophage T4 defective in the ability to induce exonuclease A. J. Virol. **9:** 399–407.

WIBERG, J. S., S. L. MENDELSOHN, V. WARNER, C. ALDRICH AND T. S. CARDILLO, 1977 Genetic mapping of *regA* mutants of bacteriophage T4D. J. Virol. **22:** 742–749.

ZOGRAFF, Y. N. and A. L. GINTSBURG, 1980 Transcription termination factor rho and T-even phage development. Mol. Gen. Genet. **177:** 699–705.

Communicating editor: G. MOSIG