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Lysis and Lysis Inhibition in Bacteriophage T4: *rV* Mutations Reside in the Holin *t* Gene

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Upon infecting populations of susceptible host cells, T-even bacteriophages maximize their yield by switching from lysis at about 25 to 35 min at 37°C after infection by a single phage particle to long-delayed lysis (lysis inhibition) under conditions of sequential infection occurring when free phages outnumber host cells. The timing of lysis depends upon gene *t* and upon one or more rapid-lysis (*r*) genes whose inactivation prevents lysis inhibition. *t* encodes a holin that mediates the movement of the T4 endolysin though the inner cell membrane to its target, the cell wall. The rI protein has been proposed to sense superinfection. Of the five reasonably well characterized *r* genes, only two, *rI* and *rV*, are clearly obligatory for lysis inhibition. We show here that *rV* mutations are alleles of *t* that probably render the t protein unable to respond to the lysis inhibition signal. The *tr* alleles cluster in the 5′ third of *t* and produce a strong r phenotype, whereas conditional-lethal *t* alleles produce the classical t phenotype (inability to lyse) and other *t* alleles produce additional, still poorly understood phenotypes. *tr* mutations are dominant to t^+, a result that suggests specific ways to probe T4 holin function.

The T-even bacteriophages employ a powerful strategy to maximize the yield of virus particles when a population of susceptible *Escherichia coli* cells is encountered (7, 14). Cells are initially infected by single phage particles and, at 37°C with aeration, lyse 25 to 35 min later, releasing on the order of 10^2 particles per cell. Eventually, the ratio of released particles to unlysed cells exceeds 1, whereupon cells are infected by one particle and then repeatedly "superinfected" by additional particles. When the interval between infection and superinfection is at least a few minutes, the superinfecting particles trigger lysis inhibition. Lysis is then delayed for up to several hours, and the burst size approaches 10^3 particles per cell.

Lysis inhibition involves several genes (2, 40). *e*, a late gene whose protein-encoding DNA sequence resides at kb 66.493 to 66.985 on the standard map (25), encodes an endolysin (gpe in T4 gene product terminology) that accumulates but does not act until released into the periplasmic space; gpe is a lysozyme that degrades the murein layer of the cell envelope (30). Cells singly infected with *e* mutants cease metabolism after about 30 min at 37°C and synthesize no more phage but neither lyse nor form plaques. *t*, a late gene that resides at kb 160.219 to 160.873, encodes a 218-residue protein (gpt) (28) that acts as a holin, that is, a (regulated) pore allowing the lysozyme access to the murein layer (27, 32, 40). Cells singly infected with the canonical *t* amber mutants accumulate endolysin, but rarely lyse, and form tiny plaques or no plaques at all, depending on the plating medium (references 15 and 16 and this report). Unlike *e* mutants, such *t* mutants continue to synthesize phage particles long after the normal time of lysis, but "*t*" refers not to "timing" or "trigger" but to the unfortunate Tithonus, who was granted immortality but not protection against aging (15, 16).

In rapid-lysis (*r*) mutants, superinfection fails to induce lysis inhibition, resulting in large, sharp-edged plaques instead of the smaller r^+ plaques that are surrounded by a dense haze of superinfected, lysis-inhibited cells (14); however, the larger *r* plaques contain roughly 10-fold-fewer phages than do r^+ plaques (10). In the present context, the key *r* genes are *rI* and *rV*. *rI* is an early gene at kb 59.192 to 59.483, and it has been suggested that gprI senses some primary aspect of superinfection (31). *rV* was first defined by a single allele mapping to the region between *38* and *motA* and producing an r phenotype only at higher temperatures (18, 23); additional *rV* alleles are described in this report. Among the other *r* genes, *rIV* and *rVI* are poorly defined genetically (25, 39) and null mutations in *rIIA*, *rIIB*, and *rIII* produce an r phenotype on some host cells but not on others (2, 31) and thus are not essential for lysis inhibition. These *r* genes are not further considered here.

Here we show that *t* and *rV* mutations are alleles of the same gene. Although *rV* was discovered in 1965 (23) and *t* in 1970 (15), we call the gene *t* because it encodes the holin. We also characterize patterns of lysis and lysis inhibition produced by the two kinds of *t* alleles and suggest how *rI* and *t* alleles may interact.

MATERIALS AND METHODS

T4 strains. The canonical *rV* mutant *rts64* was obtained from Victor Krylov; it produces the r^+ phenotype at 30°C and the r phenotype at 37 to 43°C. *r2* and *r3* were detected in routine screens for r mutants and were tentatively assigned to *rV* because of their close linkage to *rts64* or their r phenotype on BB cells without a sequence change in *rI*. The *t* mutants *tsDH634*, *amtA3*, *amtB5*, *hus19*, and *hus20* were obtained from Dwight Hall, and *Rid394* was obtained from Karin Carlson.

***E. coli* strains.** Bacterial strains were from the laboratory's *E. coli* stock collection. The su^- strain B was used to score *r* plaque morphology. Strain B40su^+II is a B strain in which amber mutations are suppressed by the insertion of glutamine at positions encoded by UAG, and it was used to grow T4 bearing amber mutations in vital genes. On strain BB, *rII* mutants display an r^+ phenotype whereas *rI* and *rV* mutants display an r phenotype. The su^- strain KB is a K-12(λ) lysogen that is nonpermissive for *rII* mutants.

Media and T4 stocks. Our modified Luria-Bertani (LB) broth, M9 buffer, Drake agars, and general plating methods have been described (6), as have the Hershey (H) media (35). LB broth contains, per liter, 10 g of Bacto Tryptone, 5 g of Bacto Yeast Extract, 5 g of NaCl, and 1 g of glucose. Drake bottom agar contains, per liter, 10 g of Bacto Peptone, 1 g of Bacto Yeast Extract, 5 g of NaCl, 0.2 g of glucose, and 10 g of Bacto Agar. Drake top agar contains 65% of the

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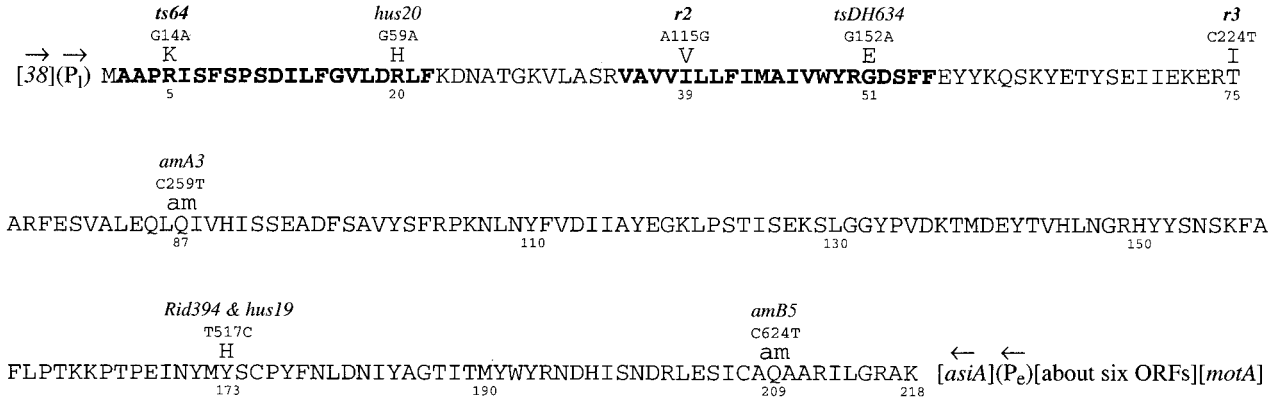

FIG. 1. The *t* gene (GenBank accession no. Y00408), with its derived protein sequence and mutations. *t* is flanked upstream by its late promoter (P_l) and by gene *38* and downstream by *asiA* and its early promoter (P_e); arrows indicate directions of transcription. The two blocks of sequence in boldface are the putative transmembrane residues (32). Numbers below the sequence identify mutated residues or reference residues. Entries above the sequence identify, in order, amino acid changes (am, amber), then base sequence changes in the complementary (noncoding) strand, and, in italics, names of alleles (alleles producing an r phenotype being shown in boldface).

same components. H broth contains, per liter, 8 g of Bacto Nutrient Broth, 5 g of Bacto Peptone, 5 g of NaCl, and 1 g of glucose. Enriched H top agar contains, per liter, 13 g of Bacto Tryptone, 8 g of NaCl, 2 g of sodium citrate dihydrate, 3 g of glucose, and 6.5 g of Bacto Agar; for enriched H bottom agar, the concentration of glucose was reduced to 1.3 g/liter and that of agar was increased to 10 g/liter.

Stocks were grown by stabbing plaques (with sterile paper strips) into 5-ml volumes of LB broth containing host cells (B cells for *ts* mutants, *394*, and *hus* mutants and B40*su*$^+$II cells for *t* amber mutants; 10^8 cells/ml) and incubating them on a rotary shaker for several hours at 37°C. Lysis was completed with chloroform. Phages were concentrated by centrifuging the lysate for 10 min at about 3,000 × *g* to remove debris and then for 1 h at about 50,000 × *g* to sediment phages; pellets were overlaid for several hours or overnight and then resuspended in storage buffer (1 g of NaCl, 0.2 g of $MgCl_2 \cdot 6H_2O$, 1.2 g of Tris buffer, 0.1 g of gelatin, 1,000 ml of distilled water [pH 7]).

PCR and DNA sequencing. A 3,538-base PCR product was prepared (17) by using the primer sequences starting at kb 163.429 (5′-AAAAAGGCCTGGTG GAGAAATCTGG-3′) from the *motA* end (kb 163.429 being the standard map starting kilobase) (25) and at kb 159.914 (5′-CCTCGGTCTTCCTGCTCATTC AA-3′) from the *38* end. The PCR products were purified for sequence analysis with QIAquick PCR purification kits (Qiagen). The ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems) was used to perform fluorescence-based cycle sequencing reactions on PCR products from *rV* and *t* mutants. DNA sequencing was conducted on an ABI 377 DNA Sequencer. Each mutation within *t* was confirmed by sequence analysis in both directions from multiple PCR products. The following primer sequences defined mutations within *t* (numbers in parentheses designating starting kilobases): 5′-CCTCGGTCTTCCTGCTCATTCAA-3′ (159.914), 5′-GTAG TTTATTTCGGGAGTAGG-3′ (160.730), 5′-CTTTCCTTTTCAATAATTTC A-3′ (160.438), 5′-TGAAATTATTGAAAAGGAAAG-3′ (160.418), and 5′-AG AAAATTACACAGACCAGTT-3′ (161.226).

***rts64* backcrosses.** At time zero, *rts64* mutant, T4B, and B cells (the last at 5 × 10^8/ml after mixing) were combined in LB broth at multiplicities of infection (MOIs) of 0.5 for *rts64* and 10 for T4B and were incubated on a rotary shaker at 32°C. At 8 min, the complexes were diluted extensively in LB broth and incubation was continued at 32°C. At 40 min, chloroform was added to complete lysis and the progeny were assayed on B cells at 43°C. Four r plaques were picked and grown into stocks, new backcrosses were conducted on all four lines three more times in a linear sequence, and two mutants displaying a temperature-sensitive r phenotype were selected at random from each line at the end. The *t* regions of the resulting eight r mutants were then sequenced.

One-step growth curves. Single-infection, single-cycle growth curves were obtained by infecting B cells at MOIs of roughly 0.2. At time zero, 0.1 ml of phage (10^9 phages/ml) was added to 0.9 ml of B cells at 5 × 10^8 cells/ml and incubated on a rotary shaker at 37°C. At 4 min, the complexes were diluted to 10^5 cells/ml and incubation was continued without rotation. Starting at 16 min, samples were taken every 2 min and assayed for PFU. The number of PFU per complex was determined by normalizing all titers to the average of the values at 16 and 20 min.

Lysis inhibition assays. These were conducted by using MOIs of about 10. At time zero, 1.5 ml of phage (10^{10} phages/ml) and 1.5 ml of log-phase B cells (10^9 cells/ml) were mixed and incubated at 37°C on a rotary shaker. At 4 min (by which time typically more than 90% of the phage had adsorbed), 2.8 ml of the culture was added to 18.2 ml of broth and again incubated at 37°C on a rotary shaker. At 15 min, 1.5 ml of phage at (10^{10} phages/ml) was added to the culture to achieve superinfection. Starting at 20 min, absorbance readings at 600 nm were taken every 10 min with a Beckman DU 640 spectrophotometer. All values were normalized to the value at 20 min.

RESULTS

Locating *rV*. By using two-factor crosses, the canonical *rV* mutation *rts64* was previously located roughly 22 map units counterclockwise from *rII* (24). Later, Dinh Nguyen, of this laboratory, mapped another *rV* mutant (now called *tr2*) using three-factor crosses involving widely spaced reference markers together with premature lysis (burst size ≈ 1) (9). Based on such crosses, *tr2* was mapped between *38* and *motA* (data not shown).

Because this region contains several open reading frames that are likely to be T4 genes (25), we focused our initial sequencing on these open reading frames. Using PCR, we amplified and sequenced a 2,565-base fragment from *motA* to *t* from three *rV* mutants (*rts64*, *r2*, and *r3*) but observed no mutations. We therefore extended our search into *t*. *t* was not previously sequenced for these mutants because of the marked phenotypic differences between *t* and *rV* mutants: on nonsuppressing cells, amber *t* mutants form either no plaques or small, fuzzy-edged plaques, depending on the medium, while *rV* mutants form large, sharp-edged plaques. We amplified a 3,538-base fragment that extended from inside *motA* through part of *38*. Using primers focused on *t*, we then found a mutation in *rts64* which consisted of a G:C→A:T substitution at nucleotide 14 causing an arginine→lysine replacement at the position encoded by codon 5.

Because *rts64* contained only a conservative missense mutation in *t*, we sought to confirm that the r phenotype was linked to the *t* mutation by extensively backcrossing *rts64* against T4B (see Materials and Methods). These backcrosses failed to separate the r phenotype from the *t* mutation. Subsequent sequencing revealed that two other *rV* mutants (*r2* and *r3*) also contain *t* mutations. Additional sequencing confirmed that none of the three *tr* mutants contain mutations in the distant *rI* gene (whose mutations are phenotypically identical to rV mutations). We next sequenced all of the previously mapped *t* mutants (11, 13, 26), with the results shown in Fig. 1.

t is flanked upstream by gene *38*, a late gene transcribed in

the same direction as *t*. The first *t* codon begins 12 bases 3′ to a late promoter, and *t* is served by no middle or early promoters; the nearest such promoters are about 7 and 10 kb upstream, respectively. Thus, *t* appears to be an exclusively late gene. Downstream, *t* is adjacent to the early gene *asiA*. *asiA* is transcribed in the opposite direction, with no sign of a transcription terminator that would prevent early *asiA* transcription from penetrating into *t* and generating antisense mRNA; however, the function of such a hypothetical antisense RNA remains untested. The three *t* mutations producing the r phenotype reside in the first third of the gene, and none of the other *t* missense mutations have an r phenotype. *hus* mutants grow poorly in the presence of hydroxyurea, while the growth of *Rid394* depends on the allelic state of the host *rho* gene, whose product modulates transcription termination; these traits are considered in Discussion. The locations of most of the *t* mutations agree with previous mapping studies (11, 13, 38), except that *Rid394* does not sequence to the predicted position (26) and the order of *amA3* and *amB5*, while consistent among all later maps, is reversed from that given in the canonical report (15); the amber mutants may have been reversed when originally archived, or the original mapping may have employed too few mutants to secure the order correctly.

Profiles in lysis. The time of lysis depends on the occurrence and timing of superinfection (1, 2, 7). In the absence of superinfection, at 37°C most lysis occurs between 25 and 35 min following either single or multiple infection. To trigger lysis inhibition, superinfection must occur later than a few minutes after the primary infection but at least shortly before lysis begins. Repeated late superinfection may prolong lysis inhibition, but in most cases the system eventually collapses abruptly (3).

Lysis profiles are measured in two different ways. One method is to infect cells with an MOI of <1, dilute the infected cells to a low density, and periodically measure the number of PFU (which may be either infected cells or released phage particles). To trace the course of superinfection-induced lysis inhibition, a high initial MOI (e.g., 10) is applied, followed later by a second high MOI. However, a few cells may lyse near the minimum time; the released phages can then soon outnumber the unlysed cells and tend to obscure their fate. Therefore, lysis inhibition is best measured at a high cell density by monitoring turbidity or absorbance. Because lysis inhibition is usually measured by using multiple infection for the primary infection, dominance tests can also be performed.

We first investigated whether *tr* mutants lyse normally or earlier than 25 to 35 min after infection in single-infection experiments with t^+, *tamA3*, and *tr2* strains. Typical results with H media are shown in Fig. 2A. As demonstrated originally (15, 16), su^- cells infected with *tamA3* show very little lysis but accumulate intracellular phages (and continue to do so even after 30 to 35 min) that can be released by artificial lysis with chloroform. The t^+ and *tr2* lysis profiles are indistinguishable. Therefore, the r phenotype is due not to premature lysis but more probably to an inability to develop lysis inhibition in response to superinfection.

In contrast to the classical phage release profiles obtained with H media, we were surprised to observe considerable spontaneous release of *tamA3* phages with LB broth and Drake agars (Fig. 2B). (The difference in burst sizes shown in Fig. 2A and B is unlikely to be noteworthy because burst sizes often vary among experiments.) Although this dependency on medium has not been explored further, at least three explanations can be entertained, any of which could vary with the medium. First, T4 amber mutants are sometimes leaky (e.g., see reference 34). Second, an additional, inefficient lysis mechanism

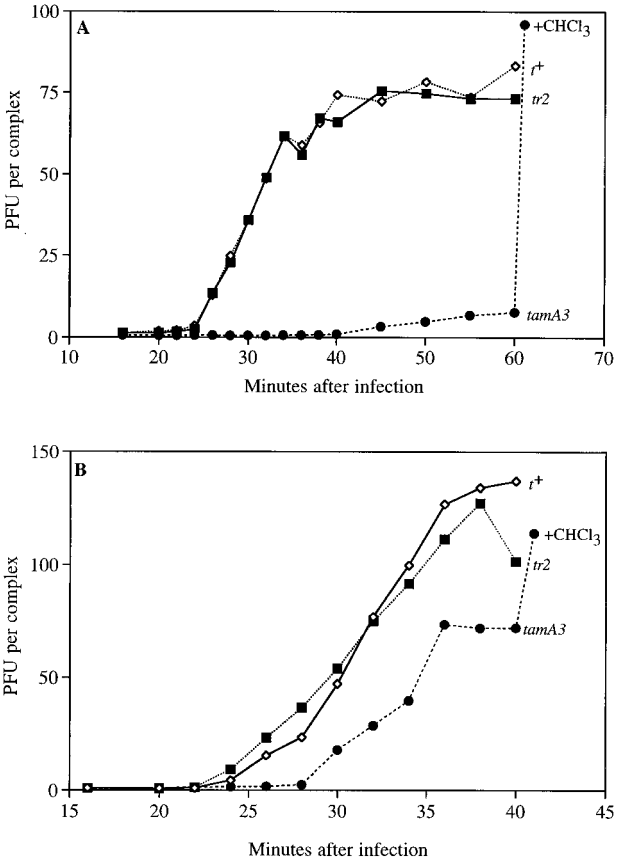

FIG. 2. One-step growth curves of strains grown in H broth and plated with H agars (A) or grown in LB broth and plated with Drake agars (B).

may operate. Third, *tamA3* leaves intact the first 87 amino acids, including the region (discussed below) containing the putative transmembrane residues, and this segment may retain residual holin function.

Because models for *t* function may generate predictions concerning dominance relationships, and in order to confirm the expected phenotype of a *tr* allele under conditions inducing lysis inhibition, we examined lysis profiles with both single-genotype and two-genotype infections. With H media, cells were rapidly infected with an MOI of 10 phage particles (sometimes including two different genotypes); then, at 15 min, the complexes were superinfected with another 10 particles. In our study, lysis profiles varied somewhat among experiments but retained the topological relations among various infecting genotypes quite robustly. Some results are shown in Fig. 3.

Both t^+ and *tamA3* produced the classical profile of long-delayed lysis, and the same result was produced by mixed infection with t^+ plus *tamA3* (data not shown). It is characteristic of such curves that absorbency increases after infection, but this behavior has not been explained to date. At later times (not shown in Fig. 3), the cells eventually lyse regardless of the infecting genotype (3).

As expected from its r phenotype and indicated in Fig. 2, *tr2* showed no lysis inhibition. Mixed infections with t^+ plus *tr2* delayed lysis only slightly compared to *tr2* alone, and similar results were obtained with *trts64* and *tr3* (data not shown). Thus, the t^+ allele is recessive to *tr* alleles. (In experiments whose results are not shown here, *tamA3* was recessive to *tr*, an

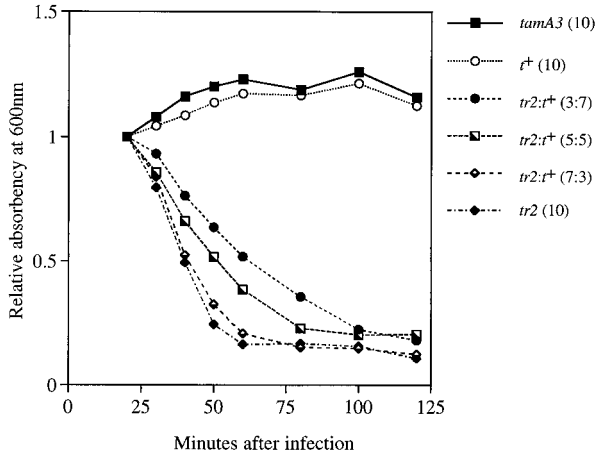

FIG. 3. Lysis profiles determined after primary infection followed by superinfection with phage particles of various genotypes. Values in parentheses are the MOIs (displayed as a ratio of the MOI of the original infection to the MOI of the superinfection).

unsurprising result.) The small lysis delay in infections with t^+ plus *tr2* increased as the t^+-to-*tr* ratio increased from 0.3 through 0.5 to 0.7. Could this result be due to cells that were randomly infected with no *tr2* phages but one or more t^+ phages? The assortment of phages among cells can be described by the Poisson distribution, which predicts that among cells infected with at least one t^+ particle, the fraction infected with no *tr2* particles varies from 0.05 to 0.001 as the *tr2* MOI ratio varies from 3 to 7. Even 5% of such cells is far too few to produce the observed lysis profiles (e.g., 5% of the t^+ value falls well below all of the *tr2* curves).

Anomalous r mutants. Mutations in *rII* but not *rI* or *rIII* were reported to partially suppress *tam* mutations, and *t* revertants were often *t rII* doubles (16, 38). Here, we report several observations in case they may be of interest to other investigators. When stocks of any *t* mutants grown in LB broth are plated on B cells by using Drake agars, roughly 4% of the PFU produce r plaques. We plated a number of such mutants on KB and BB cells. *rI* and *tr* mutants form r plaques on both cells (and on all cells we have tested), while *rII* mutants from r^+ plaques on BB cells but no plaques on K-12(λ) strains such as KB. Of 28 tested r mutants in *t* stocks, 8 produced r phenotypes on BB cells, 7 behaved like *rII* mutants, 2 displayed an r phenotype on KB cells but made no plaques on BB cells, and 11 failed to plate on either BB or KB cells. We sequenced both the *rI* and *t* regions of the 8 mutants producing r plaques on BB cells and found that while they retained their original *t* mutation, no additional mutations were found within either *t* or *rI*. Putative *rI* amber mutants producing a suppressible rI phenotype and linked to but not located within *rI* have also been observed (10, 31). Some of the above-listed mutants presumably reside in additional, less well characterized *r* loci.

DISCUSSION

Our principal findings are as follows. (i) The bacteriophage T4 genes *rV* and *t* are identical. (ii) *t* mutations producing an r phenotype cluster in the first third of the gene. (iii) In lysis inhibition experiments, these *tr* mutations are dominant to t^+.

Although some of the earliest *t* mutants were obtained by enriching specifically for the null t phenotype (15, 38), several of the *t* mutants we sequenced were selected indirectly. First, the *tr* mutants were selected for their r plaque morphology. Second, *tRid394* was selected as a mutant that grows poorly in rho^+ host cells but better on strains with nonlethally mutated versions of *rho* (26). *rho* encodes an mRNA termination factor important in T4 replication (36), but the reason for the growth advantage of *tRid394* on certain *rho* mutants remains unclear. Third, suppressors of *63* mutations are often *t* mutations, and *ttsDH634* was selected as a temperature-sensitive example of such a suppressor (13). *63* encodes a protein with both RNA ligase and tail fiber attachment activities, but the mechanism of suppression has not been worked out. Fourth, *thus19* and *thus20* were selected for their sensitivities to growth inhibition in the presence of hydroxyurea (11), which inhibits ribonucleotide reductase. *hus* mutations map at numerous T4 loci (11, 25), but their appearance in *t* was surprising. The physiology of the *thus* mutants is complex (12). They differ from *tam* mutants in producing few phages when lysis is delayed. A double mutant carrying both *thus19* and a ribonucleotide reductase amber mutation (*nrd*) shows a greater lysis defect than does *thus19* alone, while the single *nrd* mutant shows almost as great a lysis defect as does *thus19* itself. In addition, lysozyme levels are reduced in *nrd* and in *thus19* infections (but not in *thus20* infections), while *nrd* also reduces levels of other, unspecified late proteins. Like *tamA3*, the *thus* mutants are to various degrees codominant with t^+. Unfortunately, this complicated picture does not point very directly at causative mechanisms, although *t* mutations that delay lysis might simply provide extra time to overcome an incomplete genetically or chemically induced inhibition, as already suggested for *Rid394* (40).

One vexing aspect of *t* is its relation to a gene called *stII. st* mutants produce small "star" plaques surrounded by numerous sectors or dots of lysis caused by the growth of suppressor mutations. *stII* mutations map close to *t* on the *asiA* side, recombining with *trts64* at the opposite end of *t* at a frequency of 5.5 to 11% (19, 20). In an intensive mapping study of a large set of now-discarded *t* mutations (38), the two most distal *t* mutations (one being *amB5*) recombined at a frequency of roughly 6%. While this value might be an overestimate if the time of lysis was extended by the *t* mutations, with a resulting increase in recombination (29), 6% is consistent with the estimate that 0.01% recombination corresponds to about 1 bp (29) and thus with the size of *t*, 654 bp (28). Thus, the mapping studies suggest that *stII* is outside of but very close to *t. asiA* is adjacent to *t* and contains 271 coding base pairs (25). Thus, the mapping data suggest that *stII* resides beyond *asiA* but do not exclude the possibility that it is identical either with *asiA* or with *t* itself. Both the proximity of *t* and *stII* mutations and the facts that *stII* mutants display a delayed-lysis phenotype and that *rII* but not *rI* or *rIII* mutations partially suppress both *t* and *stII* mutations (15, 19) led subsequent authors to conclude with ever-increasing certainty that *t* and *stII* were identical (2, 20–22, 38). However, phage is spontaneously released more readily in *stII* than in *t* infections (15, 19), although different experimental conditions were used in the two studies and cells infected with *t* mutants are fragile and can be induced to lyse by apparently gentle manipulations (20, 38). In our studies, *t* mutants did not produce a star phenotype. Therefore, unless the canonical *stII* mutations can be recovered or new ones discovered, the relation between *stII* and *t* is likely to remain unresolved.

We note in passing that *stIII* mutations can suppress *stII* mutations (21, 22), although tests of whether *stIII* mutations suppress *t* mutations have not been reported. In addition, *E. coli* mutants selected as permissive for an *e* null mutation were sometimes also permissive for *tamA3* and *tamB5* mutants (37);

these were not amber suppressors but might suffer some defect in the cell wall or membrane.

Evidence accumulates that gpt is indeed a holin (40). While our BLAST search revealed no homologies between *t* and other holin genes except for the nearly identical *t* gene in phage K3 (33), a BLAST search also revealed no other candidate holins in T4 (32). Although gpt lacks homology to other holins, it localizes in the plasma membrane and can complement a defect in the holin encoded by phage λ *S* (27, 32). Holins characteristically contain transmembrane regions, and *t* probably has two (Fig. 1) (27, 32).

The λ *S* holin and holins in phages P1 and P2 (reviewed in reference 4) provide key insights into holin function. In the S system, both a holin and a holin inhibitor are produced from the same gene (by initiating translation at methionines encoded by either the first or the third codon). The S holin forms oligomers in the plasma membrane and has an intrinsic timing function related to its oligomerization towards an active state. Timing is further modulated by the holin-inhibitor interaction. No such dual-start system is obvious in the T4 *t* gene. However, lysis inhibition in T4 requires the *rI* gene, and gprI has been suggested to be the primary sensor of superinfection (31). (Note, however, the possible involvement of yet another *rI*-like gene [31 and this report].) As is implicit in the recent description of *rI* (31), we explicitly propose the hypothesis that gprI can exist in two states: only gprI accumulates under conditions of single infection, but gprI is converted to gprI* by superinfection. To achieve lysis inhibition, gprI* would then inhibit gpt holin function. In the λ S system, the holin inhibitor stoichiometrically titrates the pool of holin molecules and even a minority of inhibitor molecules is sufficient to delay lysis (5). Whether sufficient hypothetical T4 gprI* accumulates to act stoichiometrically remains unknown. If few gprI* molecules accumulated, then holin inhibition could still occur if a single gprI* molecule could poison a large gpt oligomer.

In addition to its holin function, gpt is linked both with the timing of lysis and with energy metabolism. In addition to the extreme effects of null and *r* mutations, other more subtle mutations in *t* can perturb the time of lysis (32). CN^- induces lysis during the last half of the latent period (8), suggesting a balance between lysis and energy metabolism that is perturbed by CN^-, and cells infected with *tamB5* are largely insusceptible to lysis inducement by CN^- (16). Thus, the rate of gpt oligomerization may be perturbed either by gpt structure or by the plasma membrane potential.

All three *tr* mutations reside in the first third of the gene, two within putative transmembrane domains (Fig. 1). Their amino acid changes are remarkably conservative: R→K (+→+), I→V (two small, nonpolar side chains), and T→I (uncharged polar to nonpolar). (In contrast, two of the other three missense mutations change charges.) It is therefore tempting to anticipate that gprI* interacts precisely with residues in the first third of gpt. It was suggested that gprI is secreted, presumably into the periplasmic space (31). Alternatively, it may have a transmembrane domain: after the initiating methionine residue, 13 of the next 17 residues (or 17 of the next 23) are nonpolar. Thus, gprI might interact with gpt in any of three ways: in the periplasmic space, in the membrane, or in the cytoplasm.

Understanding the dominance of *tr* alleles begins with the assumption that gptr is unable to interact with the hypothetical gprI*. Assuming that *tr* mutations do not affect gene expression, even a 7:3 ratio of gpt to gptr results in very little lysis inhibition. If gprI* simply titrated out gpt, then even the remaining minority of gptr molecules could suffice to form oligomers in time for normal lysis. Clearly, these postulated interactions are subject to direct experimental examination.

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