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Isolation, Characterization, and Mapping of Temperature-Sensitive Mutations in the Genes Essential for Lysogenic and Lytic Growth of the Mycobacteriophage L1

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Forty temperature-sensitive mutations affecting lytic growth and eight affecting both establishment and maintenance of lysogeny of the temperate mycobacteriophage L1 have been isolated. All of the latter mutations form one complementation group and map within a very short region around the 15% coordinate of the L1 genome; these affect a single gene, *cI*, coding for the L1 repressor. The former 40 mutations form 28 complementation groups, identifying 28 different genes, *G1–G28*, essential for the lytic growth of L1. These genes have been mapped using the *Gts* mutations. Of the 28 *Gts* mutants, 14 are defective in host lysis at 42° but not at 32° while the other 14 can lyse the host at both temperatures. Among the former 14 *Gts* mutants, 6 are also defective in L1 DNA synthesis at 42°, and they map in two different clusters, 4 around 65% and 2 around 84% of the L1 genome.

INTRODUCTION

The molecular biology, genetics, and pathogenesis of mycobacteria are not well understood (Grange, 1983; Greenberg and Woodley, 1985). One reason is the lack of well-defined gene transfer agents in these bacteria. Recently, investigators have begun to develop both plasmid- (Ranes *et al.*, 1990; Radford and Hodson, 1991; Goto *et al.*, 1991; Hinshelwood and Stoker, 1992) and bacteriophage- (Jacobs *et al.*, 1987; Snapper *et al.*, 1988) based vectors to study mycobacterial systems at the molecular level. By analogy to the lysogenic phage λ–*Escherichia coli* system (Murray, 1983), it seems likely that an extensive molecular biological analysis of a mycobacterial lysogenic phage could aid not only in understanding gene regulation in mycobacteria but also in providing the foundation for construction of phage-derived vectors.

L1 is a temperate phage and its natural host is an unspeciated *Mycobacterium* (ATCC 27199; Doke, 1960). The lysogen of this phage is quite stable but is induced spontaneously as well as by exposure to uv (Tokunaga *et al.*, 1964). L1 has a 50-kb double-stranded DNA genome and it can lysogenize *M. smegmatis* (Snapper *et al.*, 1988). Although a large portion of L1 DNA has been used to construct a shuttle phasmid vector, phAE15, which replicates as a plasmid in *E. coli* and shows both lytic and lysogenic growth similar to a phage in *M. smegmatis* (Snapper *et al.*, 1988), nothing is known about the physiology and developmental regulation of this phage. In this communication, we describe the isolation and mapping of temperature-sensitive mutations of L1 showing defects either in lytic growth or in lysogeny at nonpermissive temperatures. We have also established the functional identity of some of these genes.

MATERIALS AND METHODS

Materials

Chemicals and enzymes. All chemicals were purchased from Sigma Chemical Co. (USA). Restriction enzymes were obtained from Bethesda Research Laboratories (Gaithersberg, USA).

Media and solutions. The compositions of different media are Middlebrook 7H9 broth: $(NH_4)_2SO_4$, 0.05%; sodium glutamate (l), 0.05%; trisodium citrate, 0.01%; pyridoxine hydrochloride, 0.0001%; biotin, 0.00005%; Na_2HPO_4, 0.25%; KH_2PO_4, 0.1%; ferric ammonium citrate, 0.004%; $MgSO_4$, $7H_2O$, 0.005%; $CaCl_2$, $2H_2O$, 0.00005%; $ZnSO_4$, $7H_2O$, 0.0001%; $CuSO_4$, $5H_2O$, 0.0001%; Tween 80, 0.05% (V/V); and glycerol, 0.4% (V/V). Enriched 7H9 broth: Bovine serum albumin, 0.25% and dextrose, 0.2% in 7H9 broth. Phage dilution medium: NaCl, 0.5%; $MgCl_2$, 0.2%; CaCl2, 2m*M*, and tryptone, 0.1%. Hard agar: 1.5% agar in 7H9 broth. Soft agar: 0.7% agar, 2 m*M* $CaCl_2$, and 10 m*M* $MgCl_2$ in 7H9 broth. The compositions of SM buffer and TE buffer are given in Sambrook *et al.* (1989).

Bacteria and phage strains. The bacterial strain, *M. smegmatis* mc^26 and the phage L1 as lysogen in mc^26 were obtained from B. Bloom. The former bacterium was used for phage growth and assay.

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Methods

Growth of bacteria and bacteriophage L1. Mycobacteria culture was routinely grown with shaking at 37° in enriched 7H9 broth. Growth was monitored by measuring the OD at 590 nm. For the growth of cultures for phage infections, Tween 80 was omitted and 2 m*M* $CaCl_2$ was included in the medium.

Wild-type phage L1 used in this work is a variant derived from the supernatant of an L1 lysogen that gives turbid plaques with equal efficiency at 32 and 42°. L1 lysogens were isolated from the turbid plaques and purified. Phage lysates were routinely prepared by infection of mc^26 culture in enriched 7H9 broth or by the plate lysate method and quantitated by standard plaque assay on mc^26 host.

Isolation of clear plaque mutant of L1. From the supernatant of a culture of mc^26(L1) grown at 32°, a spontaneous L1 mutant forming clear plaques at 32 and 42° on mc^26, but no plaques on an mc^26(L1) lysogen, was isolated and purified. This mutant, L1*c*l*c*4, was called L1*c*l$^-$ (*c*I symbolizes the gene coding for repressor).

Isolation of L1 mutants with ts defects in lysogeny at 41°. The mc^26(L1) lysogen was mutagenized by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to 50% survival according to Miller (1972) and grown at 32° for 6 hr in enriched 7H9 broth. The cells were collected by centrifugation, washed several times to remove free phage, and grown again in the above medium at 32° to saturation. This was then subcultured in 7H9 broth, grown to 1 OD at 32°, and induced for 30 min at 41° followed by growth at 38° with shaking. After 6 hr, the culture was chilled, chloroformed (0.5%), kept for 1 hr, and centrifuged. The phages in the supernatant were assayed at 41°. From these, the phages, which formed clear plaques at 41° but turbid plaques at 32° and whose lysogens formed at 32° were killed at 41°, were selected as the putative mutants with *ts* defects in lysogenic growth and purified.

Isolation of L1 mutants with ts defects in lytic growth at 42°. These mutants were isolated from L1*c*l$^-$ and L1*c*l*ts*339 parents. A phage lysate at around 10^{10} PFU/ml was mutagenized with hydroxylamine to a survival of 0.1 to 1.0% by the method of Silhavy *et al.* (1984). The surviving phages were plated on mc^26. After incubation at 32° for 16 hr when tiny plaques appeared, the plates were shifted to 42°. The plaques which remained tiny after 12 hr or more at 42° were selected. From these, the phages forming plaques at 32° but not at 42° were taken as L1 mutants with *ts* defects in lytic growth and purified.

Complementation. Complementation analyses were routinely done on plates by spotting around 10^4 PFU of each of the two *ts* phages to be tested at 42° on an mc^26 lawn. Sometimes, complementation was also done in broth culture using the test phages at 5 m.o.i. of each at 42°.

Isolation of mycobacterial DNA. The procedure of Mizuguchi and Tokunaga (1970) was modified. A fresh culture of mc^26 grown to around 1 OD was made 0.15 *M* with glycine and incubated at 37° for 90 min with shaking. The cells were harvested by centrifugation and suspended in 0.2 vol of a buffer containing 25% sucrose, 0.25 *M* EDTA, and 0.15 *M* Tris–HCl, pH 8.0. To this were added pronase and lysozyme to 0.5 mg and 1 mg/ml, respectively, and mixed well. The mixture was incubated at 37° for 3 hr and then SDS was added to 1%, mixed, and incubated at 65° for 15 min. The DNA was isolated from this lysate by a standard method (Sambrook *et al.*, 1989).

Preparation of L1 DNA. L1 lysate was prepared by induction of mc^26(L1*c*l*ts*391) at 42° for 30 min or by infection of mc^26 with L1*c*l$^-$ followed by growth at 38° for 6–8 hr. Phages were concentrated by pelleting at 25,000 *g* for 90 min and purified by CsCl banding. The phage suspension was dialyzed and then digested with 0.5 mg/ml of pronase in the presence of 0.5% SDS and 0.25 *M* EDTA at 37° for 1 hr. The DNA was then isolated by a standard procedure (Sambrook *et al.*, 1989).

Phage cross. Phage cross was done by infecting mc^26 by the two L1 mutant parents at 5 m.o.i. each and growing at 32° until lysis.

RESULTS

Isolation of L1 mutants producing temperature-sensitive repressor

Eight *ts* mutants of L1 defective in both establishment and maintenance of lysogeny at 41° but not at 32° were isolated as described under Materials and Methods. Their lysogens in mc^26 showed 1 to 3 log killing within 1 hr of shifting their cultures to 41° (Fig. 1; results for six such lysogens are shown). These eight mutations failed to complement for stable lysogeny at 40° and mapped very close to one another (see below). These results suggest that all eight mutations have affected only one gene which codes for the L1 repressor. These mutants were called L1*c*l*ts*7, L1*c*l*ts*29, L1*c*l*ts*339, L1*c*l*ts*391, L1*c*l*ts*442, L1*c*l*ts*502, L1*c*l*ts*578, and L1*c*l*ts*789. The latent period of this phage determined by heat induction of mc^26(L1*c*l*ts*391) lysogen was around 100 min and the average burst 30. The burst of L1 determined earlier by uv induction was reported to be 3 by Tokunaga *et al.* (1964).

Isolation of L1 mutants with defects in lytic development at 42°

Forty *ts* mutants of L1 (28 from L1*c*l$^-$ and 12 from L1*c*l*ts*339 parents) forming plaques at 32° but not at

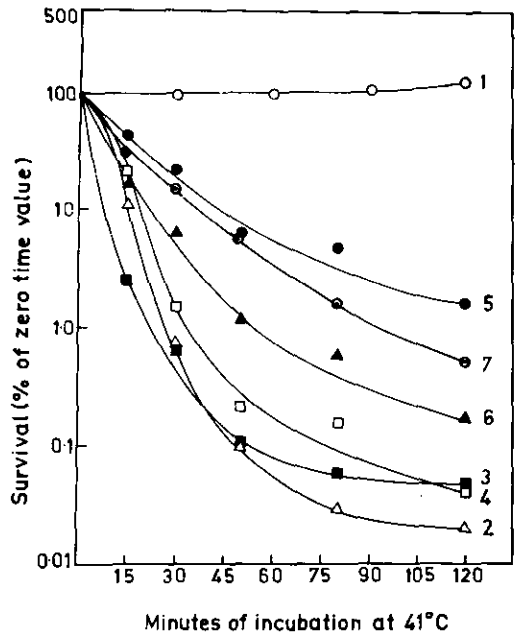

FIG. 1. Effect of growth at 41° on the survival of the lysogens of L1 phages with *ts* mutations in the immunity determinant gene. Bacteria were grown to around 2×10^8 cells/ml at 30°; these were then diluted 10^4-fold with fresh medium and incubated at 41°. At desired intervals, the viable cell count was determined at 30°. For other details, see Materials and Methods. Curves: 1, mc^26 (L1cI$^+$); 2, mc^26 (L1clts29); 3, mc^26 (L1clts391); 4, mc^26 (L1clts442); 5, mc^26 (L1clts578); 6, mc^26 (L1clts789); 7, mc^26 (L1clts339).

42° were isolated. These were classified into 28 different complementation groups which defined 28 different genes essential for L1 lytic growth. These mutants were designated *G*1-*G*28*ts*Y (*G* is the generalized symbol used for the genes and Y is the isolate number of the mutation). These genes are located serially along the L1 DNA starting from its left end (See Fig. 5). The frequency of thermostable revertants at 42° of these mutations ranged from 10^{-4} to 10^{-8}. For further studies, one mutant from each of the above 28 complementation groups was used.

Mapping of the *ts* mutations

The 28 *ts* mutations in 28 different genes essential for lytic growth were mapped by determining the frequencies of wild-type recombinants in two, three, and four factor crosses between any two such *ts* parents. The occurrence of negative interference during recombination events and/or the presence of recombinational hot spots was not considered in these analyses. First, the overall order of the genes in the L1 genome was roughly determined as follows: Different sets of 4 *ts* markers were randomly selected from the above 28, crosses were made in different combinations of two for each set, and the positions of the mutations were determined. The two terminal markers, *ts*173 (in *G*1) and *ts*895 (in *G*28), were identified by their highest recombination frequency. The results of one such cross are shown in Fig. 2. Finally, the relative positions of all the mutations were determined from the recombination data obtained in further crosses for all the successive pairs of two adjacent markers over the length of the L1 genome. These data are shown in Fig. 3. The recombination frequency for a longer interval was nearly equal to the sum of the values for the constituent short intervals within a short segment of the L1 genome (data not shown). The map distance of 18.11 units between the two terminal markers, *G*1*ts*173 and *G*28*ts*895, was given by adding together the recombination frequencies for the successive intervals for all 28 markers (See Fig. 3). Taking the whole length of L1 genome to be proportional to the above 18.11 units of map distance, the map coordinates of all 28 genes in terms of the percentage of length of L1 DNA starting from the left end were determined (Fig. 3). The genetic map of L1 was then constructed, which is shown in Fig. 5. The two terminal genes *G*1 and *G*28 have been assumed to be located at the left and right ends, respectively, of the L1 DNA. If some other genes are discovered outside this boundary, then the positions of all the genes between them may be shifted a little but their order would remain unchanged. It is seen from the map (Fig. 5) that the 28 *Gts* mutations are distributed all over the length of L1 genome, although there are two long segments from 71 to 82% and 85 to 100% coordinates within which no *Gts* mutation has been isolated, while 4 mutations have been isolated between 9 and 11% and 8 between 63 and 71% coordinates of L1 DNA.

Mapping of the *cI* gene was done as follows: The *cI* gene was first shown to lie between 10 and 20% coordinates of the L1 genome by four factor crosses using several *Gts* mutations in combination with *clts* or cI$^-$ markers, and then its position was determined from the

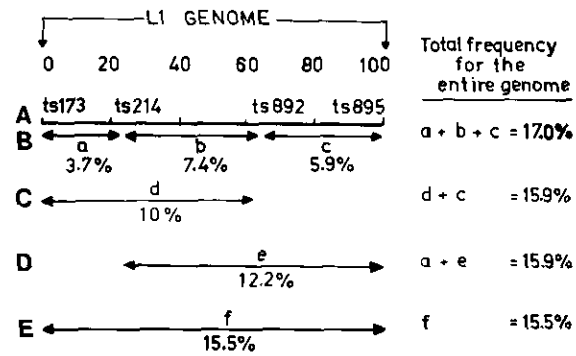

FIG. 2. Determination of the overall order of the genes of L1 defined by the growth-defective ts mutations. The crosses were done as described under Materials and Methods and in text. Line A shows the approximate positions of four mutational markers in the L1 genome, and the recombination frequencies for different intervals are shown in lines B–E. The numbers above the L1 genome show the % coordinates.

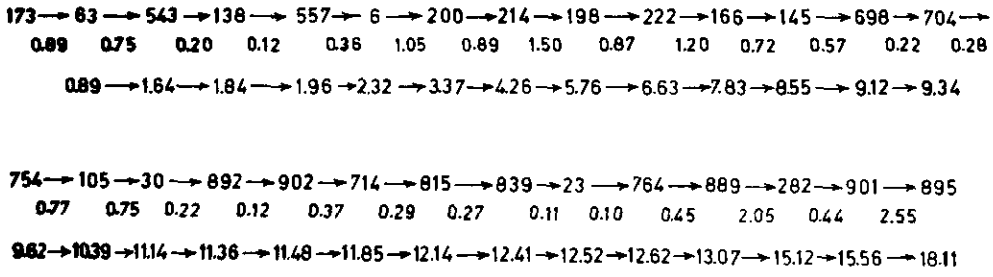

FIG. 3. Recombination frequencies of successive overlapping pairs of markers for the entire L1 genome. The results for the whole genome are shown in two blocks (upper and lower). The numbers connected by arrows in the top line in each block represent the ts mutations and those immediately below (in the second line) represent the recombination frequencies between the intervals. Numbers connected by arrows in the third line in each block represent the distance of the corresponding markers in approximate map units from the left end of the genome. For other details, see Materials and Methods and text.

recombination data in crosses of L1*G4*$^+$*G6*$^+$*cI*$^-$*G7-ts200* with L1*G4ts138cIts339G7*$^+$ and L1*G6ts6cIts-339G7*$^+$, respectively, as shown in Fig. 4. In these crosses, the relative frequencies of *G6*$^+$*cIts339G7*$^+$ (cross 2) and *G6*$^+$*cI*$^-$*G7*$^+$ (cross 3), and *G4*$^+$*cIts339G7*$^+$ (cross 1) and *G4*$^+$*cI*$^-$*G7*$^+$ (cross 3) phages, respectively, among the *G*$^+$ recombinants at 42° gave the position of cI at around 15%, between *G6ts6* and *G7ts200*. All 8 *cIts* mutations mapped very close to *cIts339* (recombination data not shown).

Effect of the *Gts* mutations on host cell lysis at 42°

The lysis genes of a phage belong to the late family and their expression are usually regulated by some of the early/delayed early genes (Dove, 1966; Calendar, 1970; Herskowitz, 1973; Geiduschek, 1991). So, all the 28 *Gts* mutations of L1 were broadly classified into two groups by studying their effects on host cell lysis at 42°. The experiment was done as described in Fig. 6. It was observed that all of these mutants lysed the host at 32° but at 42°, 14 of them failed to do so while the remaining 14 effected normal lysis. The results obtained with wild-type L1 and one mutant from each of the above two groups are shown in Fig. 6, and the distribution of all these mutations in the two groups is shown in Table 1.

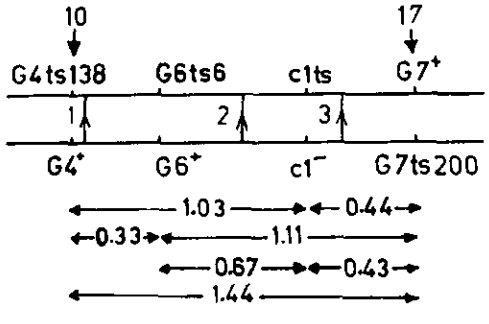

FIG. 4. Mapping of the *cI* gene of L1. Crosses were done by the procedure described under Materials and Methods. Recombination frequencies are shown below the map. The numbers 10 and 17 above the gene segment represent the % coordinates of the L1 genome. The segment of the map is drawn approximately to scale. For other details, see text.

Identification of *Gts* mutations affecting L1 DNA synthesis at 42°

During lytic development of a phage, its DNA replication often exerts a copy number-dependent control on its late gene expression (Calendar, 1970; Herskowitz, 1973; Geiduschek, 1991). It was observed that L1 DNA, on digestion with *PstI*, produced around 21 fragments of 7.5 to 0.3 kb (Fig. 7, lanes 1 and 3b). The total DNA isolated from the bacteria infected with wild-type L1 and grown at 32 and 42° also produced phage-specific *PstI* bands in the background of a light smear of chromosomal DNA (Fig. 7, lanes 5a and 5b). However, the DNAs from both uninfected mc^{2}6 and wild-type L1-infected mc^{2}6 at zero time (for input phage DNA control) did not produce an L1-specific *PstI* band pattern (Fig. 7, lanes 4b and 6, respectively). So, to identify the DNA synthesis genes of L1, the effect of the 14 lysis-defective *Gts* mutations on phage DNA synthesis at 42° was studied using the above strategy for analysis. The results in Fig. 7 show that the five mutations, *G18ts892*, *G19ts902*, *G21ts815*, *G26ts282*, and *G27ts901* caused complete inhibition (lanes 13a and 13b, 10a and 10b, 9a and 9b, 12a and 12b, and 11a and 11b, respectively) while *G20ts714* effected partial inhibition (lanes 8a and 8b) of L1 DNA synthesis at 42°. Under identical conditions, the DNA syntheses by wild-type L1, the remaining 8 mutants of the nonlysis group, and all 14 of the normal lysis group were not affected at 42°; in fact, with the latter 22 mutants and wild-type L1, phage DNA synthesis was better at 42° than at 32° [the results for only two mutants, *G4ts138* (lanes 7a and 7b) and *G9ts198* (lanes 14a and 14b), from the latter 22 and wild-type L1 (lanes 5a and 5b) are shown in Fig. 7]. These results suggest that the six genes, *G18–G21*, *G26* and *G27*, are essential for L1 DNA synthesis.

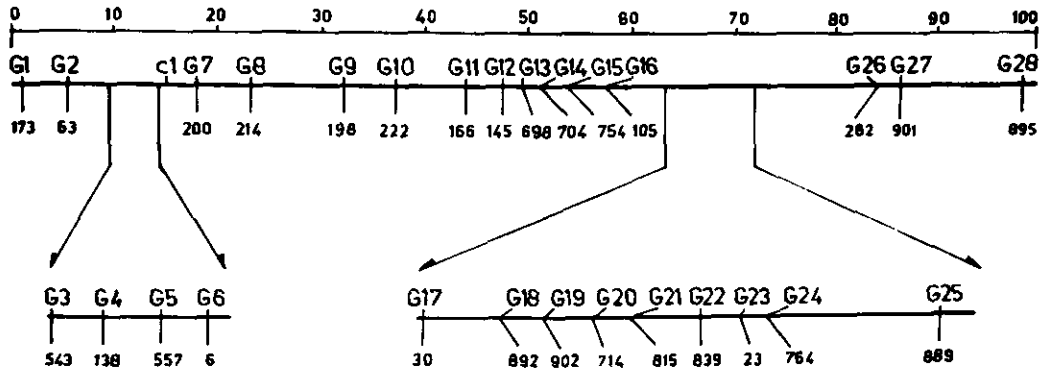

FIG. 5. Genetic map of L1. This map was constructed on the basis of the results shown in Figs. 3 and 4. Positions of the genes are shown immediately above the map. The ts mutations identifying the genes are indicated by the numbers indicated below the corresponding gene. The positions of closely located genes are shown in the expanded map below. The numbers above the map represent the % coordinates of the L1 genome. The map is drawn approximately to scale. For other details, see Materials and Methods and text.

DISCUSSION

In this paper, we have described methods by which 40 *ts* mutations in 28 genes essential for lytic growth and 8 in the repressor gene of L1 were selected with maximum scoring efficiency.

The L1 genome is around 50 kb long (Snapper *et al.*, 1988). So, after allowing room for the *cis*-specific regulatory sites and other intercistronic sequences, this phage genome is expected to code for around 50 average-sized polypeptides of which some are nonessential for the phage growth. *Ts* mutations in the latter genes could not be isolated by the selection procedure described here. Altogether, the above 29 genes constitute around 60% of the total that may be coded by the L1 genome.

It is apparent that most of the *Gts* mutations showing similar effects on host lysis at 42° are clustered within definite boundaries in the L1 genome (Table 1 and Fig. 5). Thus, all of the 10 genes (*G*18–*G*27) located between 63 and 85% coordinates are essential for host lysis, while all of the 8 (*G*10–*G*17) located between 35 and 63% coordinates are not needed for this purpose.

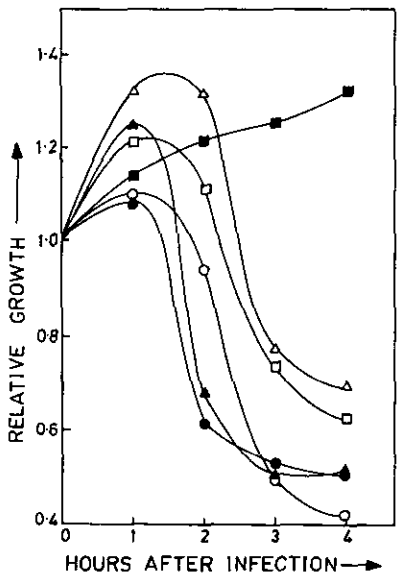

FIG. 6. Effects of *Gts* mutations in L1 on lysis of the host at 32 and 42°. A 40-ml culture of mc²6 freshly grown at 32° to around 0.25 OD was infected with the desired phage at an m.o.i. of 5 at 30° and divided into two equal parts; one was shaken at 32°, the other at 42°. The postinfection growth of the bacteria was monitored turbidimetrically at 590 nm. Lysis was maximum at 4 hr after infection in all the cases. For other details, see Materials and Methods and text. The curves are as (○) L1cI⁻ at 32°, (□) L1cI⁻*G*20ts714 at 32°, (△) L1cI⁻*G*2ts63 at 32°, (●) L1cI⁻ at 42°, (■) L1cI⁻*G*20ts714 at 42°, (▲) L1cI⁻*G*2ts63 at 42°.

TABLE 1

DISTRIBUTION OF GROWTH-DEFECTIVE *ts* MUTATIONS IN TWO DIFFERENT GROUPS SHOWING NO LYSIS AND COMPLETE LYSIS OF HOST CELLS AT 42°

Mutations[a]	Ability to lyse[b] infected bacteria at 32°	Ability to lyse[b] infected bacteria at 42°
Group I		
G3*ts*543, G6*ts*6, G8*ts*214, G9*ts*198, G18*ts*892, G19*ts*902, G20*ts*714, G21*ts*815, G22*ts*839, G23*ts*23, G24*ts*764, G25*ts*889, G26*ts*282, G27*ts*901.	Normal lysis	No lysis
Group II		
G1*ts*173, G2*ts*63, G4*ts*138, G5*ts*557, G7*ts*200, G10*ts*222, G11*ts*166, G12*ts*145, G13*ts*698, G14*ts*704, G15*ts*754, G16*ts*105, G17*ts*30, G28*ts*895	Normal lysis	Normal lysis

[a] The map positions of these mutations are given in Fig. 5. The double-underlined mutations are also defective in phage DNA synthesis at 42° (see Fig. 7).

[b] Lysis of bacteria during postinfection growth was studied as described in Fig. 6.

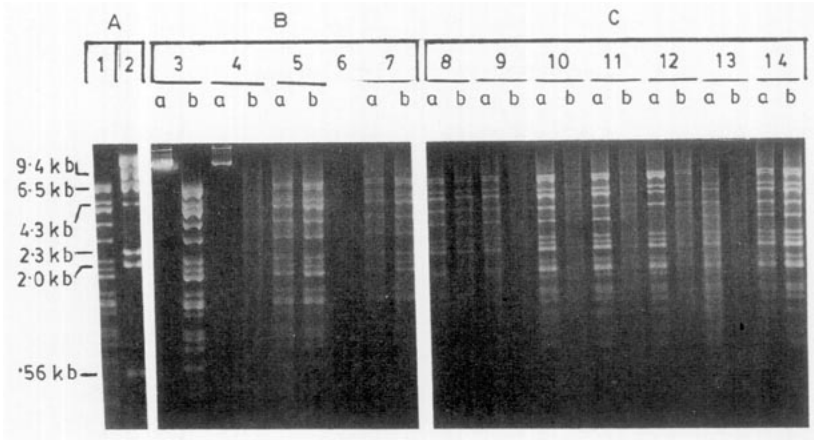

FIG. 7. Effect of *Gts* mutations on phage DNA synthesis at 42°. A 20-ml culture of mc²6 freshly grown to 1 OD was infected with mutant or wild-type L1 phage at an m.o.i. of 2 at 25°. 18-ml of enriched 7H9 broth containing 0.15 *M* glycine and 2 m*M* $CaCl_2$ was then added and mixed well. Then, one half was incubated at 32° and the other at 42°, both with shaking. After 90 min, the cells were harvested and suspended in sucrose–EDTA–Tris–HCl–pronase–lysozyme mixture, and the DNA was isolated, all by the procedures described under Materials and Methods for the isolation of mycobacterial DNA. For all the sets, the DNA obtained from 10 ml culture was finally dissolved in 400 μl TE buffer from which 6 μl DNA samples were digested with *Pst*I and electrophoresed in 1% agarose gel. The DNA bands were stained with EtBr and photographed. For other details, see Materials and Methods and text. Lanes 1, Pure L1 DNA/PstI cut; 2, pure λ DNA/*Hin*dIII cut; 3, pure L1 DNA: a, uncut and b, *Pst*I cut; 4, mc²6 DNA: a, uncut and b, *Pst*I cut; 5, wild-type L1 (L1cI⁻); 6, L1cI⁻ (zero time); 7, L1*G*4ts138; 8, L1*G*20ts714; 9, L1*G*21ts815; 10, L1*G*19ts902; 11, L1*G*27ts901; 12, L1*G*26*ts*282; 13, L1*G*18ts892; 14, L1*G*9ts198. For lanes 5 and 7–14, "a," 32° grown and "b," 42° grown DNA. Lane 6, input L1 DNA. The results in (A), (B), and (C) were obtained by electrophoretic runs at different times for different periods.

However, the 9 genes (*G*1–*G*9) located between zero and 35% coordinates have mixed distribution.

Phage-induced host lysis may be regulated by at least four types of phage gene functions: (i) genes which code for the enzymes that directly degrade or damage the cell wall and membrane to effect lysis (Mukai *et al.*, 1967; Harris *et al.*, 1967; Reader and Siminovitch, 1971), (ii) those which positively control the expression of lysis genes (Couturier *et al.*, 1973), (iii) those which increase the copy number of late genes by DNA synthesis (Calendar, 1970; Geiduschek, 1991), and (iv) genes which may positively control the expression of the genes mentioned in (ii) and (iii) above (Dove, 1966; Couturier *et al.*, 1973). The *ts* mutations in 14 genes show a lysis-defective phenotype at 42°, and 6 of them are also defective in phage DNA synthesis. So, it is likely from these results that the expression of late genes of L1 is dependent on its DNA replication.

Now, the question arises as to how many of the remaining 8 genes essential for host lysis but not for L1 DNA synthesis actually code for the lytic enzymes and how many regulate their expression. Assuming that some more genes that may be involved directly or indirectly in host lysis are yet to be identified, it seems that 8+ is quite a large number for the genes controlling the two last-mentioned functions in L1 development.

About the 6 DNA synthesis genes, the following questions may arise: (i) What are the specific functions of these genes in L1 DNA replication? Of course, any of them may regulate the expression of DNA synthesis genes [see (iii) below]. (ii) These 6 genes are present in two clusters separated by around 19% (≈9 kb) segment of DNA; four of them, *G*18–*G*21, map around 65% while the other two, *G*26 and *G*27, map at around 84% (see Fig. 5). Why are these genes in two different clusters? (iii) Of these 6 mutants, *G*20*ts*714 differs from the other five by the fact that the phage DNA synthesis by this mutant at 42° is around 50% inhibited (Fig. 7), and the cells are not lysed at all (see Fig. 6). These results indicate that in the absence of functional *G*20, the phage DNA genes are expressed to a level enough for 50% DNA synthesis yet that does not activate expression of lysis (late) genes. This suggests that possibly *G*20 is a regulatory gene having a function similar to that of the *N* gene of coliphage λ (Ogawa and Tomizawa, 1968; Friedman *et al.*, 1987; Barik *et al.*, 1987). Further work should clarify these points.

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