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The *G23* and *G25* Genes of Temperate Mycobacteriophage L1 Are Essential for The Transcription of Its Late Genes

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Two lysis-defective but DNA synthesis non-defective temperature-sensitive (*ts*) mutants of mycobacteriophage L1, L1*G23ts*23 and L1*G25ts*889 were found to be defective also in phage-specific RNA synthesis in the late period of their growth at 42°C, each to the extent of 50% of that at 32°C. The double mutant, L1*G23ts*23*G25ts*889 showed the *ts* defect in phage RNA synthesis that was nearly additive of those shown individually by the two single-mutant parents. Both *G23* and *G25* were shown to start functioning sometimes between 30 and 45 min after infection but the former gene might be dispensable after 45 min, while the latter was not. Northern analysis also shows that at 42°C, L1*G23ts23* affects RNA synthesis more strongly than L1*G25ts889* from L1 DNA segments that serve as the template for late gene transcription. Among the 21 virion and 12 non-virion late proteins synthesized by L1, L1*G23ts23* is defective in the synthesis of at least 9 virion and all of non-virion proteins at 42°C. In contrast, L1*G25ts889* is completely defective in synthesis of all the 33 late proteins. Possible roles of *G23* and *G25* in the positive regulation of transcription of different sets of late genes of L1 have been discussed.

Keywords: Bacteriophage, Late Regulatory Genes of L1, Late Transcription of L1, Mycobacteriophage L1, The *G23* and *G25* genes of L1

Introduction

The genes of a bacteriophage which are involved in host cell lysis and in phage morphogenesis are classified as the late genes (Calendar, 1970; Herskowitz, 1973; Geiduschek, 1991). Transcription of this class of genes of a phage is activated by the product(s) of late regulatory gene(s) (Roberts, 1975, 1993; Geiduschek *et al*., 1983; Geiduschek, 1991; Williams *et al*., 1994; Cho *et el*., 1995) which belong to the delayed early class that also include phage DNA replication and recombination genes. So, the transcription of phage late genes is completely dependent on the expression of its delayed early genes. Thus, a phage mutant showing defect in plaque formation but not in its DNA synthesis may not be defective in the transcription of its delayed early genes. If such a mutant phage is defective also in host cell lysis, then the mutation may be located within any of the lysis genes or in the gene(s) that regulate the transcription of late genes of the phage.

L1 is a temperate mycobacteriophage (Doke, 1960). It has a 52.1 kb long double-stranded DNA (Mandal *et al*., 2004) which remains integrated at a specific site in the host chromosome in its lysogen (Snapper *et al*., 1988). The lysogen of L1 is inducible spontaneously as well as by UV (Doke, 1960). To understand the temporal regulation of gene expression in this phage, temperature-sensitive mutations in 28 different genes of L1 essential for the lytic growth were isolated and mapped (Chaudhuri *et al*., 1993). Fourteen of those mutants showed lysis-defective phenotype at 42°C (Fig. 1). Six of those lysis-defective mutants were also defective in phage DNA synthesis at 42°C, while the remaining eight were not (Chaudhuri *et al*., 1993). Further characterization of the six DNA synthesis-defective *ts* mutants reveals that the gene *G27* positively regulates the early transcription from L1 (Datta and Mandal, 1998). Recently, other early gene regulatory elements of L1 such as an early promoter and a repressor gene have also been cloned and characterized to some extent (Chattopadhyay *et al*., 2003; Ganguly *et al*., 2004; Sau *et al*., 2004). Interestingly, structure and function of the above early promoter and repressor of L1 were found identical to those of homo-immune mycobacteriophage L5 (Donnelly-Wu *et al*., 1993; Nesbit *et al*., 1995; Brown *et al*., 1997). A comparison of combined genetic map (mutation based) and transcription map of L1 (Mandal *et al*., 2004) with the predicted genetic map of L5 derived from its DNA sequence suggest that the organization of early, delayed early and late genes of L1 and L5 possibly

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differs. Thus far gene regulatory elements that positively regulate the expression of late genes of any mycobacteriophage have not been identified and characterized though the study has potential in enriching the molecular biology of both mycobacterial and mycobacteriophage systems. In this communication, we have shown that among the above eight lysis-defective *ts* mutants, which were not defective in phage DNA synthesis at 42°C, two genes *G23* and *G25* are essential for the transcription of L1 late genes. Studies also reveal that *G23* and *G25* regulate the expression of different sets of late genes of mycobacteriophage L1.

Materials and Methods

Chemical, media and solutions. The fine chemicals were purchased from Sigma Co., USA, and [^{3}H]-uracil and [^{35}S]-methionine, from BRIT, Trombay, India. The compositions of Middlebrook 7H9 broth, enriched 7H9 broth, 7H9 hard and soft agars, phage dilution medium are described by Chaudhuri *et al.* (1993), and those of SSC, SM and TE buffers by Sambrook *et al.* (2001). The denaturing solution for RNA isolation is described by Chomczynski and Sacchi (1987).

Bacteria and bacteriophage strains. *Mycobacterium smegmatis* mc^{2}6 was obtained from Dr. B. Bloom, Albert Einstein College of Medicine, NY, USA. The mycobacteriophage strains L1*c*I$^-$, L1*c*I$^-$*G23ts*23, L1*c*I$^-$*G25ts*889 were used from our own stocks (Chaudhuri *et al*., 1993). The L1*c*I$^-$*G23ts*23*G25ts*889 double mutant was constructed by a cross between L1*c*I$^-$*G23ts*23 and L1*c*I$^-$*G25ts*889. The genetic, restriction, and transcription maps of L1 are shown in Fig. 1.

Growth of bacteria and bacteriophage L1. The mycobacterial cultures were grown on rotary shaker at 32, 37, or 42°C in enriched 7H9 broth. Growth was monitored by measuring OD_{590}. The L1 phage lysate was prepared by confluent lysis on plate, and the phage concentration determined by plaque assay on mc^{2}6 host.

Preparation of L1-infected bacteria for phage-specific RNA and Protein syntheses. *M. smegmatis* mc^{2}6 culture was grown to around 0.5 OD_{590} at 37°C in enriched 7H9 broth containing 2 mM $CaCl_2$ (no Tween 80) and mixed with desired phage at 5-10 moi (multiplicity of infection). After allowing phage adsorption at room temperature for 15 min without shaking, the culture was divided into two equal parts: one was grown at 32°C, while the other at 42°C, both with shaking. At different times, the required aliquots of these cultures were pulsed with [^{3}H]-uracil (for RNA labeling) or with [^{35}S]-methionine (for protein labeling) at desired temperatures. Uninfected culture was used as a control in each case. The labeled cells were chilled, harvested, washed and used to analyze L1-specific RNAs or proteins.

For the ^{35}S-labelling of L1 phage particles, the L1*c*I$^-$-infected mc^{2}6 culture (200 ml) was grown at 37°C for 50 min, and [^{35}S]-methionine (1,000 Ci/mmol, 4 μCi/ml) was added, and the growth was continued till complete lysis. The ^{35}S-labeled L1 particles were concentrated by precipitation with polyethylene glycol, purified by CsCl banding and used to analyze the virion proteins by SDS-PAGE.

Isolation of nucleic acid. The L1 phage DNA was prepared by the procedure described by Chaudhuri *et al.* (1993). For isolating L1-specific RNA, L1 phage infected cells suspended in 0.2 vol of denaturing solution were broken by sonication in Braunsonic Model 1510 sonicator using 100W probe with 3-4 pulses of 30 s each. Then total RNA was extracted with acid guanidinium thiocyanate-phenol-chloroform mixture (Chomczynski and Sacchi, 1987).

DNA-RNA hybridization. Preparation of nitrocellulose membrane filters containing the denatured L1 DNA (5 μg/filter) is described by Sambrook *et al*. (1989). The method of DNA-RNA hybridization on millipore filter was adapted from that described by Denhardt (1966) for DNA-DNA hybridization and using the conditions described by Sambrook *et al*. (1989). After hybridization for 16 h at 68°C, the filters were washed and dried as described (Datta and Mandal, 1998), and the radioactive counts were determined.

Northern hybridization. The desired restriction fragments of L1 DNA were labeled and used as probe to detect the late gene-specific RNAs isolated from the L1*ts* mutant-infected cells grown at 32 or 42°C exactly by the procedures described by Mandal *et al*. (2004).

Analysis of L1 phage-specific proteins. The cells labeled with [^{35}S]-methionine- or the purified L1 phage particles were lysed in a required volume of lysis buffer containing 3% SDS by keeping in a boiling water bath for 15 min. The lysates were centrifuged at 10,000 g for 15 min in cold, and the supernatants was used for analyzing the L1-specific proteins by SDS-polyacrylamide (10%) gel electrophoresis as described by Laemmli (1970). After electrophoresis, the gel was fixed and dried as described (Sambrook *et al*., 1989) and autoradiographed using Kodak XK-5 diagnostic film (Kodak, India).

Radioactivity measurement. Radioactive counts were determined in a Beckman Liquid Scintillation Counter Model LS 5000CE using aqueous or non-aqueous cocktail as required.

Results and discussion

At 42°C, the L1G23ts23 and L1G25ts889 mutants are defective in phage-specific RNA synthesis in late period but not in early and delayed early periods. A phage mutant having mutation in the gene essential for transcription of its late genes would be defective primarily in its RNA synthesis in the late period but not in any of the early and delayed early events like DNA synthesis, and early and delayed early transcriptions. Such a mutant would also show lysis-defective phenotype. So, we examined the effect of the eight lysis-defective (but phage DNA synthesis non-defective) *ts* mutations in L1 on the synthesis of phage-specific RNA at different times during post-infection growth at 42°C. In this experiment,

Table 1. Production of matured phage by the L1*G23ts*23 and L1*G25ts*889 mutants at 42°C

	Phenotype at 42°C		Average Burst at 42°C[b]	
	Host Lysis	Phage DNA Synthesis	Pfu/Cell	% of Control[a]
L1	+	+	40	100
L1*G23ts*23	-	+	4	10.00
L1*G25ts*889	-	+	4.5	11.25

[a]All the phages carry *c*I$^-$ mutation.
[b]The phage-infected mc^{2}6 (moi = 0.5) was grown at 32°C (control) and 42°C for 5 h and then treated with glycine (1 M), $CHCl_3$ (0.7%) and lysozyme (500 μg/ml) for 60 min (Chaudhuri *et al.*, 1993) at 42°C. This lysate was used to assay phage at 32°C. See text for details.

Table 2. Effect of the presence of both *G23ts*23 and *G25ts*889 mutations in the same phage on L1-specific late RNA synthesis[a]

Phage Infections	Temperature of growth	^{3}H-RNA hybridized to L1 DNA[b] (input RNA = 30 μg)	
		ng RNA hybridized[c]	% of control
L1*G23ts*23	32°C	766	100
	42°C	426	55.6
L1*G25ts*889	32°C	753	100
	42°C	462	61.4
L1*G23ts*23*G25ts*889	32°C	800	100
	42°C	133	16.6

[a]The experiment was done exactly as described in Fig. 2 except that one-time [^{3}H]-uracil pulse was given for 4 min at 66 min in each case.
[b]For each set of experiment, hybridization was done using varying concentrations of RNA. The hybridization reached saturation at 30 μg RNA/reaction under the conditions of the experiment. So, the data with 30 μg of input RNA are shown. The specific-activity of ^{3}H-RNA used varied from 2,000 to 2,500 cpm/μg RNA. For further details, see Fig. 2.

the phage-specific RNAs were quantified by their hybridization to L1 DNA on Millipore filter. In L1, the early transcription starts immediately after infection, delayed early transcription at around 8 min (Datta and Mandal, 1998), and late transcription at around 30 min. The data in Fig. 2 show that each of the two mutants, L1*G23ts*23 and L1*G25ts*889 among those eight was about 50% defective in the phage-specific RNA synthesis at around 40, 60, and 80 min but not at around 5 and 20 min. In contrast, the other six mutants, L1*G3ts*543, L1*G6ts*6, L1*G8ts*214, L1*G9ts*198, L1*G22ts*839, and L1*G24ts*764 (Chaudhuri *et al.*, 1993; Fig. 1.) were found to express nearly identical amount of RNA molecules at both 32 and 42°C during their 5-80 min post-infection growth (data not shown). So, these two mutants are defective in late but not in early or delayed early RNA synthesis.

Both the L1*G23ts*23 and L1*G25ts*889 mutants are partially defective in mature phage production at 42°C. In the temperate coliphage lambda, the mutant having mutation in the late regulatory gene Q is partially defective in the production of intracellular matured phage (Sato and Campbell, 1970). The data in Table 1 reveal that the above two L1 mutants could make intracellular matured phage particles at 42°C with a burst of 10-12% of that made at 32°C. In this respect, these two L1 mutants are comparable to the bacteriophage lambda mutant defective in the late regulatory gene Q which also produces a burst of 10% of that produced by wild-type lambda (Dambly and Couturier, 1971; Sato and Campbell, 1970). So, the results in Table 1 also support the view that G23 and G25 are involved in the positive regulation of transcription of the late genes of L1.

The *G23* gene function may be spared after 45 min of growth, while *G25* is required throughout the late period. A question arises as to whether both G23 and G25 are required throughout the period of transcription of the late genes of L1. To get an answer to this question, the effect of temperature shift up from 32 to 42°C on the L1-specific late RNA synthesis was studied. The results presented in Fig. 3 show that when the *G23ts*23 and *G25ts*889 mutant-infected cultures were shifted from 32 to 42°C at zero and 30 min (the time at which late transcription starts), the synthesis of L1-specific RNA at 38, 53, and 68 min (i.e. throughout the late period) was significantly affected by both the mutants. When shifted up at 45 or 60 min, then during the post-shift growth at 42°C, the phage could overcome the effect of the *G23ts*23 mutation on the late RNA synthesis to a large extent, while the *G25ts*889 mutant continued to show the defect though at a reduced level at least up to 68 min (the phage has a latent period of around 75 min at 37°C). These results suggest that *G23* and *G25* start functioning sometimes between 30 and 45 min and that after 45 min, the former gene is not required, while the latter is required throughout the late period.

The L1*G23ts*23 and L1*G25ts*889 mutants are defective in the transcription of different sets of late genes of L1 at 42°C**.** Partially reduced synthesis of RNA in late period by the L1*G23ts*23 and L1*G25ts*889 mutants at 42°C (Fig. 2) raises the question as to whether these two genes control the transcription of different sets of late genes of the phage. To answer this question, the effect of the presence of both *G23ts*23 and *G25ts*889 mutations in the same phage on its late RNA synthesis was examined. Data in Table 2 show that while the above two single-mutant phages could synthesize the late RNAs at 42°C respectively to the extent of 55.6 and 61.4% of those made at 32°C, the L1*G23ts*23*G25ts*889 double-mutant could make the same at 42°C only to the extent of 16.6% of those made at 32°C. Thus the *ts* defect in the late

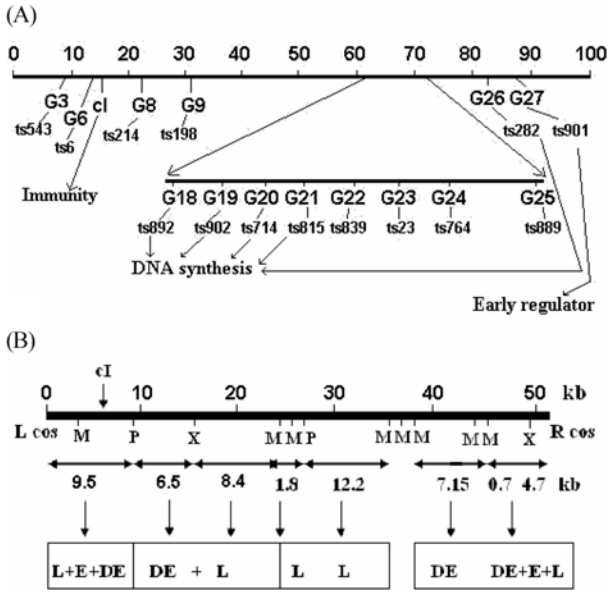

Fig. 1. Genetic, physical and transcription maps of L1. (A) In genetic map of L1, the *ts* mutations identifying the genes are indicated by the numbers shown below the gene symbols. The numbers above the map represent the coordinates of the L1 genome. 100 coordinates correspond to nearly 52 kbp. (B) L1 map has shown the kb coordinates by numbers at the top and *Mlu*I and *Pvu*II sites, respectively by M and P below. The regions of L1 DNA expressing early (E), delayed early (DE), and late (L) genes are shown (Mandal *et al*., 2004). The site of deletion located close to L1 repressor gene (*c*I) has been shown by a downward arrow.

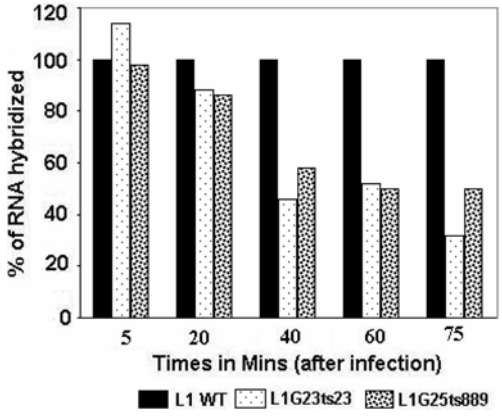

Fig. 2. Effect of *G23ts*23 and *G25ts*889 mutations on L1-specific RNA synthesis at 42°C. *M. smegmatis* mc^{2}6 culture was infected with desired phage at 5 moi and grown at 32 and 42°C. From each temperature set, 5 ml aliquots were pulsed with [^{3}H]-uracil (0.5 mCi/ml, 4.5 Ci/mmol) for 4 min at 1, 16, 36, 56, and 76 min. The total RNA was isolated and hybridized. In 32°C control, the efficiency of hybridization of ^{3}H-RNA to L1 DNA was about 3% (around 2,500 cpm) of the total input RNA at all the time periods used for labeling, except at 1-5 min when it was around 1.5% (around 1,000 cpm). Each experiment was carried out 2-3 times. One set of representative data has been shown here.

RNA synthesis by the above double-mutant phage appears to be additive [100 – {(100 – 61.4) + (100 – 55.6)} = 17 (16.6% actual)] of the same defects shown individually by its two single-mutant parents at 42°C. Further hybridization studies between a mixture carrying equal amount of each of two mutant phage-specific ^{3}H-RNAs synthesized at 42°C and L1 DNA reveal that the amount of RNA hybridized is nearly equal to the sum of those obtained from their separate hybridizations under identical conditions (data not shown). Interestingly, this additive effect was observed in both linear and plateau regions of the hybridization versus RNA concentration curves. These results suggest that *G23* and *G25* are involved in the positive regulation of transcription of different sets of late genes from different segments of L1 DNA. Now, a question may arise as to what are those 16.6% RNAs that are synthesized by L1*G23ts*23*G25ts*889 at 42°C. It may be possible that the 16% RNA are the products of a set of late genes whose transcription is regulated by a third late regulatory gene of L1 or these may be contributed by a low level transcription of late genes in the absence of functional *G23* and *G25* genes at 42°C. The fact that each of the L1*G23ts*23 and L1*G25ts*889 mutants makes matured phage particles at 42°C with a burst of about 10% of that made at 32°C (Table 2), suggests that both of them could individually synthesize the respective sets of late RNAs at 42°C to the extent of 10% of that made at 32°C, and this may contribute to the above 16% RNA synthesized by the double mutant at 42°C. This supports the above-mentioned second possibility.

It has been shown that the late gene-specific RNAs of L1 are coded by the *Pvu*II/*Mlu*I 14.9 kb and the *Mlu*I 12.2 kb DNA fragments (Mandal *et al*., 2004; Fig. 1.). To know if there is any specific template area within these regions for *G23* and *G25*-regulated late gene transcription, Northern blot experiment was done using the above two DNA fragments as probes. The results presented in Fig. 4 show that when the *Mlu*I 12.2 kb DNA was used as a probe, practically no late RNA corresponding to this region could be detected in the total RNA isolated at 60 min from L1*G23ts*23(*G25*$^+$)-infected cells grown at 42°C. However, under identical conditions, a small amount of RNA from L1*G25ts*889 (*G23*$^+$)-infected cells hybridized with the 12.2 kb DNA probe (Fig. 4). These results suggest that this 12.2 kb DNA segment codes for some late genes whose transcription is somehow dependent on the *G23* function but not on *G25*.

The *Pvu*II/*Mlu*I 14.9 kb is quite a large fragment of L1 that codes for the late genes (Mandal *et al*., 2004). So purified 14.9 kb *Pvu*II/*Mlu*I DNA fragment was further digested with *Xba*I to get *Pvu*II/*Xba*I 6.5 kb and *Xba*I/*Mlu*I 8.4 kb fragments (Fig. 1), and these were separately used as probes. Northern blot results with the 6.5 kb and 8.4 kb (Fig. 4) DNA probes show that the amounts of RNA synthesized by both *G23ts*23 and *G25ts*889 mutants were reduced at both 30 and 60 min at 42°C compared to those at 32°C, and that the effect of former

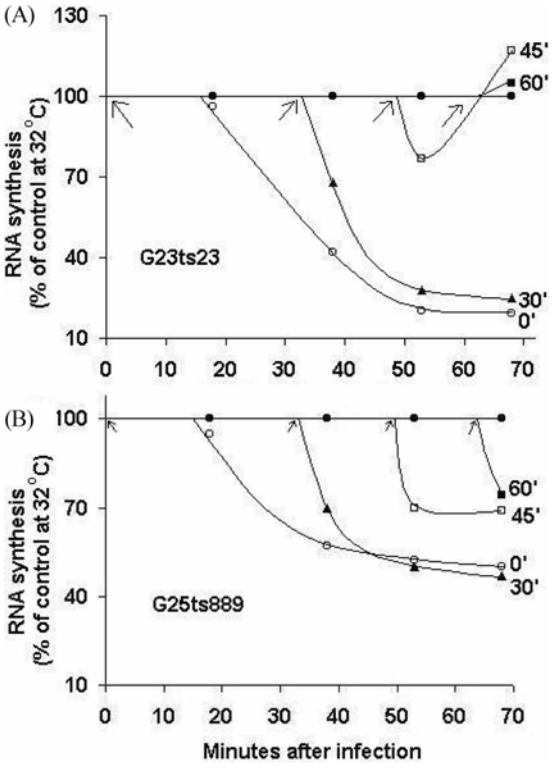

Fig. 3. Effect of temperature shift-up on L1-specific RNA synthesis by the L1*G23ts*23 and L1*G25ts*889 mutants. The phage-infected (moi = 5) $mc^2$6 culture was incubated at 32°C on a water bath shaker. At times indicated by arrows, aliquots were shifted to 42°C water bath shaker. At different times, 5 ml aliquots were taken from both 32 (control) and 42°C (shift-up) sets and pulsed with [^{3}H]uracil (0.5 μCi/ml, 4.5 Ci/mmol) for 4 min at 32 and 42°C respectively. The hybridization curves for different shift-up times are marked at the end of each curve. The efficiencies of hybridization of [^{3}H]RNA in the 32°C controls were around 3% (2,500 cpm) of the total input RNA. A, L1*G23ts*23; B, L1*G25ts*889. For other details, see Methods. Each experiment was carried out 2-3 times. One set of representative data has been presented here.

mutation is more significant than that of the later in this regard. All these results suggest that the 12.2 kb DNA codes for the *G23*-regulated genes but not the *G25*-regulated ones. It is known that the 14.9 kb DNA of L1 codes for both delayed early and late genes. So, the amounts of RNAs synthesized at 42°C in the above two mutant infected bacterial cells (that hybridized to 6.5 kb and 8.4 kb DNA segments) may be the delayed early RNAs or the transcription of late genes from both 6.5 kb and 8.4 kb DNA requires both *G23* and *G25* functions.

The *G23ts*23 and *G25ts*889 mutations are defective in L1-coded late protein synthesis at 42°C. To get further support in favor of the above conclusion that the *G23* and *G25* genes regulate the transcription of different sets of late genes, we examined the effect of the above two mutations on the synthesis of late proteins of L1 at 42°C. The Results in Fig. 5 show that under the condition of the experiment, host protein synthesis was completely stopped within the first minute of post-infection growth of L1$^+$ as well as of the L1ts mutants at both 32 and 42°C, and such inhibition remained effective till the end of the late period. Similar inhibition of host protein synthesis also occurs after infection by mycobacteriophage L5, a close relative of L1 (Hatfull and Sarkis, 1993). It is interesting to note that several early and late proteins of L1 have almost similar counterparts in L5. However, under the condition of complete inhibition of host protein synthesis after infection, the syntheses of L1-coded late proteins were thoroughly examined to see the effect of *G23* and *G25* mutations.

The virion proteins (VPs) are the late proteins, and at least 21 such proteins (V1-V21) having molecular weights ranging from 14.5 to 203 kDa were detected by SDS-PAGE analysis of the proteins from CsCl-purified ^{35}S-labeled L1 particles (Fig. 5A). The data obtained with L1$^+$-infected cells (Fig. 5A) show that the synthesis of a large number of proteins (about 33) which also include the above mentioned 21 VPs of L1

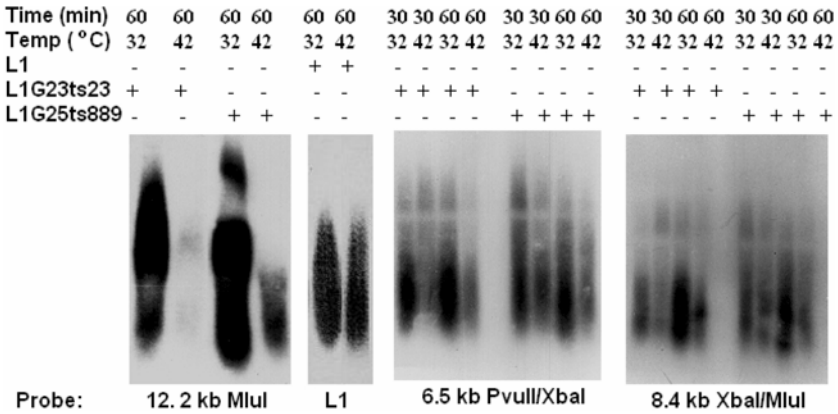

Fig. 4. Northern blots of L1-specific RNA synthesized by the wild-type L1 and its two mutants, L1*G23ts*23 and L1*G25ts*889, at 32°C and 42°C. RNAs was isolated at 30 and or 60 min from phage-infected cells according the procedure as mentioned in Materials and Methods. The probes used were L1 DNA and three restriction enzyme digested L1 DNA fragments such as 12.2 kb *Mlu*I DNA, 6.5 kb *Pvu*II/*Xba*I, and 8.4 kb *Xba*I/*Mlu*I DNA. Each of above experiments was carried out twice. One set of representative picture has been shown here.

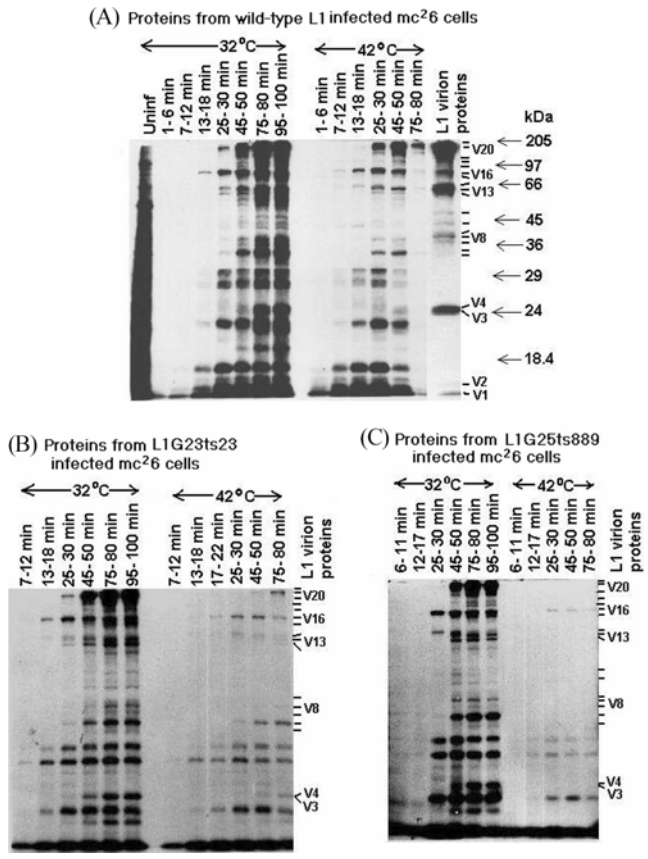

Fig. 5. Effect of the *G23ts*23 and *G25ts*889 mutations on L1-specific late protein synthesis at 42°C. The phage-infected culture was labeled by pulsing of 0.5 ml cell with [^{35}S]Met (10 μCi/ml, 1,000 Ci/mmol) for 5 min followed by 2 min chase with cold Met (100 μg/ml) at desired temperatures. Phage infections: A, wild-type L1; B, L1*G23ts*23; C, L1*G25ts*889. MOI of phage used 10. [^{35}S]-labeled virion proteins (VPs) from CsCl-purified L1 particles are shown in the right most lane in A. Positions of VPs are indicated by V1, V2, V3 etc. from bottom to top in the order of increasing molecular weights. Proteins around V1 and V2 positions in B and C were not resolved and are not marked. For further details, see Methods. Each experiment was carried out 4-5 times. One set of representative picture has been included here.

could be detected at around 35 min after infection (late transcription starts around 30 min after infection), and their synthesis continued towards the end of the latent period. So, all these proteins (21 VPs and 12 non VP proteins) are the late proteins of L1. In wild-type L1 infection, the phage-specific protein profiles at both 32 and 42°C were exactly identical except that at 42°C, the synthesis of different stage-specific proteins starts and stops much earlier and occurs at little reduced levels compared to those at 32°C. Thus, at 32°C, the synthesis of all the late proteins continues till 95 min, while at 42°C, the synthesis of these proteins practically stopped at around 75 min (Fig. 5A) but continued up to around 65 min (data not shown). In fact, the L1$^+$-infected cells did lyse much earlier at 42°C than at 32°C (our unpublished results). The data with L1*G23ts*23 (Fig. 5B) and L1*G25ts*889 (Fig. 5C) mutants show that at 42°C, the former is almost completely defective in the synthesis of at least 9 VPs namely V5, V7, V9-V11, V15, V17, V18 and V21, and nearly all of the non-virion late proteins, but not in other 12 VPs. On the other hand, the latter mutant is completely defective in all of the virion and non-virion late proteins. In this respect, L1*G25ts*889 behaves like the L1*G23ts*23*G25ts*889 double mutant (data not shown). However, this indicates that though the *G23*-regulated late RNAs are made by L1*G25ts*889 at 42°C, yet those are not translated into proteins in the absence of *G25*-regulated RNAs or their protein products. This suggests that certain gene(s) among the G25-regulated set is (are) possibly essential for the synthesis of proteins from G23-regulated late RNAs. However, the molecular mechanism of action of *G25* gene in relation to above is not yet clear.

At 42°C, the *G23ts*23 mutant transcribes the *G25*-regulated late genes but not the *G23*-regulated ones and vice versa. Also, the *G23* gene function is not needed after 45 min of postinfection growth (Fig. 3). This may be explained if it is assumed that *G23* acts through the activation/synthesis of a certain heat-stable component within 45 min, which directly activates the transcription of some of the late genes of L1. It has been observed that the *G23ts*23 mutant is also defective in L1-coded deoxyribonuclease synthesis at 42°C, while this enzyme does not code for this enzyme (P. Mandal, S. Sau, and N. C. Mandal, manuscript under preparation). So, the *G23ts*23 mutant is defective in both late RNA and the phage-coded deoxyribonuclease synthesis at 42°C. There is evidence in favor of the fact that the regulation of the expression of this phage-coded deoxyribonuclease by *G23* does not occur at the transcriptional level. Furthermore, a pseudorevertant of L1*G23ts*23 mutant capable of growing at 42°C was isolated which still retains the *ts* defect in the phage-coded deoxyribonuclease synthesis at 42°C as well as the *G23ts*23 mutation (P. Mandal, S. Sau, and N. C. Mandal, manuscript under preparation). This suggests that in the pseudorevertant of *G23ts*23 mutant, a mutation (extragenic suppressor of *G23ts*23) has occurred in a different gene, say *Gx*, that helps bypass the requirement of *G23* for late gene transcription. The above extragenic suppressor of *G23ts*23 mutation has been mapped between *G23* and *G25* in L1 genome (our unpublished results). All these imply that protein product of the wild type *Gx* gene is somehow modified by *G23* to give the functional protein that directly regulates the transcription of the particular set of late genes. It appears, therefore, that the *G23* and *G25* genes regulates the expression of late genes of L1 through a complex mechanism which can be resolved by further extensive studies involving in depth characterization of *Gx*, *G23* and *G25* genes.

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References

Brown, K. L., Sarkis, G. J., Wadsworth, C. and Hatfull, G. F. (1997) Transcriptional silencing by the mycobacteriophage L5 repressor. *EMBO J*. **16**, 5914-5921.

Calendar, R. (1970) The regulation of phage development. *Annu. Rev. Microbiol*. **24**, 241-296.

Chattopadhyay, C., Sau, S. and Mandal, N. C. (2003) Cloning and characterization of the promoters of temperate mycobacteriophage L1. *J. Biochem. Mol. Biol*. **36**, 586-592.

Chaudhuri, B., Sau, S., Datta, H. J. and Mandal, N. C. (1993) Isolation, characterization, and mapping of temperature-sensitivemutations in the genes essential for lysogenic and lytic growth of the mycobacteriophage L1. *Virology* **194**, 166-173.

Cho, N. Y., Choi, M. and Rothman-Denes, L. B. (1995) The bacteriophage N4-coded single-stranded DNA-binding protein is the transcriptional activator of *Escherichia coli* RNA polymerase at N4 late promoters. *J. Mol. Biol.* **246**, 461-471.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem*. **162**, 156-159.

Dambly, C. and Couturier, M. (1971) A minor *Q*-independent pathway for the expression of late genes in bacteriophage lambda. *Mol. Gen. Genet.* **113**, 244-250.

Datta, H. J. and Mandal, N. C. (1998) Identification of an early positive regulatory gene of mycobacteriophage L1. *J. Gen. Virol.* **79**, 205-210.

Denhardt, D. T. (1966) A membrane filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Comm*. **23**, 641-646.

Doke, S. (1960) Studies on mycobacteriophages and lysogenic mycobacteria. *Kumamoto Med. J*. **34**, 1360-1373.

Donnelly-Wu, M. K., Jacobs, W. R. Jr. and Hatfull, G. F. (1993) Superinfection immunity of mycobacteriophage L5: applications for genetic transformation of mycobacteria. *Mol. Microbiol.* **7**, 407-417.

Ganguly, T., Chattoraj, P., Das, M., Chanda, P. K., Mandal, N. C. and Sau, S. (2004) A point mutation at the C-terminal half of the repressor of temperate mycobacteriophage L1 affects its binding to the operator DNA. *J. Biochem. Mol. Biol*. **37**, 709-714.

Geiduschek, E. P. (1991) Regulation of expression of the late genes of bacteriophage T4. *Annu. Rev. Genet*. **25**, 437-460.

Geiduschek, E. P., Elliot, T. and Kassavetis, G. A. (1983) Regulation of late gene expression; in *Bacteriophage T4*, C. K. Mathews, E. M. Kutter, G. Mosig, & P. B. Berget. (eds.), pp. 189-193, American Society of Microbiology, Washington DC, USA.

Hatfull, G. F. and Sarkis, G. J. (1993) DNA sequence, structure, and gene expression of mycobacteriophage L5: a phage system for mycobacterial genetics. *Mol. Microbiol.* **7**, 395-405.

Herskowitz, I. (1973) Control of gene expression in bacteriophage lambda. *Annu. Rev. Genet*. **7**, 289-324.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**, 680-685.

Mandal, N. C., Bhattacharyya, R., Sau, S. and Chaudhuri, B. (2004) Studies on temperate mycobacteriophage L1: its physical map, site of deletion in one of its mutant, and organization of early, delayed early and late genes. *Perspective in Cytology & Genetics*, Manna, G. K. and Roy, S. C. (eds.), pp. 81-100, AICCG Publications, Kolkata, India.

Nesbit, C. E., Levin, M. E., Donnelly-Wu, M. K. and Hatfull G. F. (1995) Transcriptional regulation of repressor synthesis in mycobacteriophage L5. *Mol Microbiol*. **17**, 1045-1056.

Roberts, J. W. (1975) Transcription termination and late control in phage lambda. *Proc. Natl. Acad. Sci. USA* **72**, 3300-3304.

Roberts, J. W. (1993) RNA and protein elements of *E. coli* and transcription antitermination complexes. *Cell* **72**, 653-655.

Sambrook, J., and Russell, D. W. (2001) In *Molecular Cloning: A Laboratory Manual*. 3rd ed., Cold Spring Harbor Laboratory Press, CSH, New York, USA.

Sato, K. and Campbell, A. M. (1970) Specialized transduction of galactose by lambda phage from a deletion lysogen. *Virology* **41**, 474-487.

Sau, S., Chattoraj, P., Ganguly, T., Lee, C. Y. and Mandal, N. C. (2004). Cloning and sequencing analysis of the repressor gene of temperate mycobacteriophage L1. *J. Biochem. Mol. Biol*. **37**, 254-259.

Snapper, S. B., Lugosi, L., Jekkel, A., Melton, R. E., Kieser, T., Bloom, B. R. and Jacobs (Jr.), W. R. (1988) Lysogeny and transformation in mycobacteria: stable expression of foreign genes. *Proc. Natl. Acad. Sci. USA* **85**, 6987-6991.

Williams, K. P., Kassavetis, G. A., Herendeen, D. R. and Geiduschek, E. P. (1994) Regulation of late gene expression; in *Molecular Biology of Bacteriophage T4*, Karam, J. D. (eds.), pp. 161-175, American Society of Microbiology, Washington DC, USA.