Polish Journal of Microbiology
2008, Vol. 57, No 2, 113–119

ORIGINAL PAPER

The Delayed Early Gene *G23* of Temperate Mycobacteriophage L1 Regulates the Expression of Deoxyribonuclease, the Product of another Delayed Early Gene of the Phage

PRAJNA MANDAL, HIROCK JYOTI DATTA, SUBRATA SAU and NITAI CHANDRA MANDAL*

Department of Biochemistry, Bose Institute, Kolkata, West Bengal, India

Received 20 November 2007, revised 28 January 2008, accepted 11 February 2008

Abstract

To get clues about the genes as well as the gene regulatory circuit controlling the lytic development of temperate mycobacteriophage L1, previously we screened several conditional lethal mutants of L1 and characterized some of them to an extent. One of the mutants, L1*G23ts*23, was found defective in both growth and late gene transcription at 42°C but not at 32°C. Here we show that the above phage mutant is also defective in the expression of phage-coded deoxyribonuclease (DNase) at 42°C but not at 32°C. The *G23* gene however does not code for the above enzyme. Further analyses using the L1*G23ts23* mutant suggest that synthesis of DNase is also not regulated by G23 at transcriptional level. Expression of functional DNase in fact requires *de novo* protein synthesis. Among the 25 revertants isolated from the L1*G23ts23* mutant, which are capable of growing at 42°C (by overcoming the *ts* defect in late transcription), two, R4 and R22, have been shown to retain the *ts* defect in the expression of the above enzyme and R4, to retain also the *G23ts23* mutation. This suggests that R4 (R22 was not tested for the presence of *G23ts23* mutation) carries an extragenic suppressor of *G23ts*23 mutation in a different gene (we call this putative gene as *Gx*), which now helps bypass the requirement of G23 for late gene transcription. Possible role of G23 on the regulation of L1-coded Gx and deoxyribonuclease has been discussed at length.

Key words: bacteriophage, mycobacteriophage L1, L1-coded deoxyribonuclease, *G23* gene of L1, regulation of L1 deoxyribonuclease

Introduction

Deoxyribonucleases are essential for the DNA metabolism especially for those related to genetic recombination and repair (Clark, 1973; Kuzminov, 2001; Walker, 1985). Bacteriophages being obligate parasites, are dependent on the bacterial metabolic machineries for their own growth. Despite the synthesis of the above enzymes by bacterial hosts, most double-stranded DNA phages encode their own deoxyribonucleases for carrying out phage-specific recombination (Krüger and Schroeder, 1981; Kuzminov, 2001; Miller *et al.*, 2003; Sharples *et al.*, 1999).

Mycobacteriophage L1 is a temperate phage that infects to and grows in *Mycobacterium smegmatis* (Doke, 1960). It has a 52.1 kb long double-stranded DNA (Mandal *et al.*, 2004) and harbors at least 28 indispensable genes for carrying out its vegetative growth and one repressor gene for maintaining its lysogenic development (Chaudhuri *et al.*, 1993). Among the 28 indispensable L1 genes identified so far, the *G27* gene has been shown to positively control the transcription of the delayed early genes (Datta and Mandal, 1998). This phage codes for a deoxyribonuclease, the expression of which is also regulated by G27 at the transcriptional level (Datta and Mandal, 1998). The temperature-sensitive mutations in the two delayed early genes, *G23* and *G25* show defect in host cell lysis but not in phage DNA synthesis (Chaudhuri *et al.*, 1993). These two genes are involved in the positive regulation of the transcription of late genes of L1 (Datta *et al.*, 2007). The exact mechanism of action of none of the above 3 genes is known with certainty today. Apart from the *G23*, *G25* and *G27* genes, no other early or delayed early genes regulating the vegetative growth of mycobacteriophage has been identified so far, though genome sequences of several other mycobacteriophages are available at present in public databases (Hatfull, 2000).

From a preliminary screening experiment, it was observed that the L1*G23ts*23 mutant was defective

* Corresponding author: N. C. Mandal, Department of Biochemistry, Bose Institute, P1/12 CIT Scheme VII M, Kolkata 700 054, West Bengal, India. Phone: (91)33.23550256; **e-mail: mandalnc2003@yahoo.com** or **ncmandal@bic.boseinst.ernet.in**

also in the expression of L1-coded deoxyribonuclease (DNase) at 42°C but not at 32°C. Here we have investigated the role of the *G23* gene in the regulation of expression of DNase and reported that the synthesis of DNase is not regulated by G23 at transcriptional level, and *de novo* protein synthesis is required for the expression of functional DNase. Additional genetic study suggests that G23-mediated positive regulation of late gene transcription possibly occurs through the active involvement of a second L1-encoded protein.

Experimental

Materials and Methods

Media and solutions. 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 TE and SM buffers in Sambrook *et al*. (1989).

Bacteria and bacteriophage strains and their growth conditions. *Escherichia coli* 594 was used from the laboratory stock (Chattopadhyay and Mandal, 1982). *Mycobacterium smegmatis* mc^{2}6 was obtained from Dr. B. Bloom, Albert Einstein College of Medicine, N. Y., USA. The mycobacteriophage strains L1*c*I*ts*391, L1*c*I$^-$, L1*c*I$^-$*G3ts*543, L1*c*I$^-$*G6ts*6, L1*c*I$^-$*G8ts*214, L1*c*I$^-$*G9ts*198, L1*c*I$^-$*G23ts*23, L1*c*I$^-$*G22ts*839, L1*c*I$^-$*G24ts*764, L1*c*I$^-$*G25ts*889 were used from our laboratory stocks (Chaudhuri *et al*., 1993). The phage L1*c*I*ts*391*G23ts*23 was prepared by a cross of L1*c*I$^-$*G23ts*23 with L1*c*I*ts*391. The phages L1*c*I*ts*391*sup4* (to be called *R4sup4* or *R4* in the text) and L1*c*I*ts*391*sup22* (to be called *R22sup22* or *R22* in the text) were isolated during this work.

A portion of the genetic map showing the locations of early, delayed early and late genes is shown in Fig. 1A.

The mycobacterial cultures were grown on rotary shaker at desired temperatures in enriched 7H9 broth, and growth was monitored by measuring OD_{590}. The L1 phage lysate was prepared by confluent lysis on plate, and the pfu concentration was determined by plaque assay using *M. smegmatis* mc^{2}6 as the plating bacteria.

Preparation of [^{3}H]-labeled DNA. *E. coli* 594 was grown in TB to around 0.2 OD_{590} at 37°C on a shaker. Then [^{3}H] thymidine (2 μCi/ml, 1000 Ci/mmol) and 2-deoxyadenosine (250 μg/ml) were added, and the culture was grown further for 3 h. The cells were then harvested, and the DNA was isolated by the method of Marmur (1961). This [^{3}H]-labeled DNA was sonicated to reduce the size and extracted with phenol. The DNA was alcohol precipitated and dissolved in TE buffer.

Synthesis of L1-coded deoxyribonuclease. *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 the desired phage at 5–10 moi. The phage-infected culture was grown at 32 or 42°C with shaking. At different times, 5 ml aliquots of the culture were chilled.

In shift up and shift down experiments, the phage-infected cultures were grown at 32 or 42°C respectively, and at different times, two 5 ml aliquots were taken out. One aliquot was chilled and the other was incubated at 42 (for shift up) or 32°C (for shift down) respectively and allowed to grow for a total of 90 min followed by chilling on ice. In shift-down experiment with added antibiotic, required antibiotics were added to the shift down culture at the time of shift. The cells were harvested from all the chilled samples and used to assay deoxyribonuclease exactly by the method of Radding (1964).

Isolation of revertants of *G23ts23* mutation. The lysate of L1*c*I*ts*391*G23ts*23 was prepared starting from its plaque-purified stock. About 10^8 pfu (assayed at 32°C) of this phage was plated on *M. smegmatis* mc^{2}6 host and incubated at 42°C for about 20 h. From the plaques that appeared, 25 healthy ones were selected, and the phages were purified by single plaque procedure at 42°C. Among the 25 revertants isolated, R4 and R22 were further characterized.

Phage cross. The *M. smegmatis* mc^{2}6 culture was grown to around 0.4 OD_{590} at 32°C and infected with two parent phages in question at an moi of 5 each and grown at 32°C for about 4 h when the cells were lysed. The lysate was chilled on ice and chloroformed. The desired recombinants were selected or the recombination frequency was determined by plating this lysate at 42°C.

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

Results

The L1*G23ts23* mutant is defective in the synthesis of phage-coded deoxyribonuclease at 42°C. The results presented in Fig. 1B show that after infection of *M. smegmatis* culture with wild-type L1, the expression of a deoxyribonuclease activity started around 10 min at both 32 and 42°C (curves 2 and 3), which increased steadily with time. In contrast, there was no increase of the enzyme level in uninfected cells even up to 80 min. This suggests that the increase to nearly 10-fold excess level of this enzyme activity in wild-type L1-infected cells (over that in uninfected cells) is due to phage-dependent synthesis.

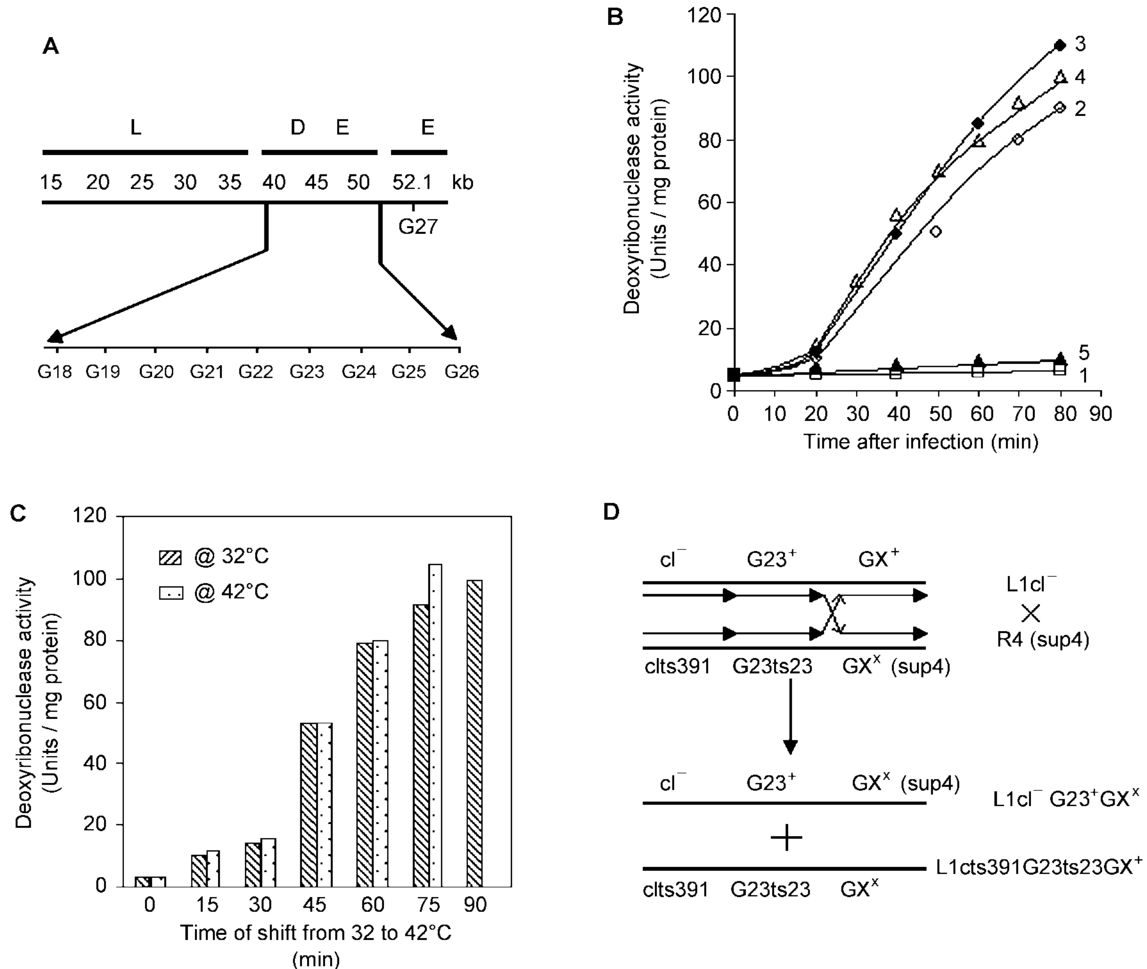

Fig. 1. Characterization of L1 *G23.*

(A) Part of the genetic map of L1. The map is shown from 15 kb to 52.1 kb. The whole length genome is equivalent to 52.1 kb. The regions of early (E), delayed early (D.E.) and late (L) genes are indicated above the map. In the expanded region are shown the approximate locations of the delayed early genes *G18* to *G26*. *G27* is an early gene. The *c*I gene encoding repressor maps at left around 5 kb co-ordinate (not shown in the map).

(B) Effect of *G23ts*23 mutation on the expression of L1-coded deoxyribonuclease. Units of Deoxyribonuclease activity was defined as the amount of enzyme which could release 1 nmol of acid-soluble nucleotide in 30 min under the condition of assay. Curves: 1, uninfected *M. smegmatis* mc^26 at 32°C; 2, wild-type L1-infected cells at 32°C; 3, wild-type L1-infected cells at 42°C; 4, L1*G23ts*23-infected cells at 32°C; 5, L1*G23ts*23-infected cells at 42°C. For other details, see Methods

(C) Effect of temperature shift up on the expression of L1-coded deoxyribonuclease by L1*G23ts*23 mutant. For the definition of the unit of the enzyme activity, see Fig. 1A. Striped bar: 32°C (at the time of shift). Pointed bar, shift up to 42°C. For other details, see Methods.

(D) Segregation of *G23ts*23 mutation from *sup4* revertant. The cross was done between L1*c*I^- and L1*c*I*ts*391*sup4* at 32°C. The phages in the lysate was assayed at 32°C, and the recombinants producing turbid plaques at 32°C but unable to grow at 42°C were selected and tested for the presence of *G23ts*23 mutation by complementation with different L1*Gts* mutants including L1*G23ts*23. The experiments reported in (B) and (C) were repeated 2–3 times, each showing reproducible results. The results of one set of experiments are presented in each case.

This enzyme was synthesized also in L1cI^-*G23ts*23 mutant-infected cells grown at 32°C (curve 4) but not in those grown at 42°C (curve 5).

The low level of L1-specific DNase activity in the L1*G23ts23*-infected bacteria grown at 42°C may be explained by one of the two possibilities: the *G23* gene of L1 may itself code for the above DNase or it may code for a protein (other than DNase) that is directly involved in the regulation of expression of the DNase. To know as to which one of these two possibilities is correct, thermal inactivation of DNase synthesized by L1+ and L1*G23ts*23 at 32°C was studied by preincubating these phage-infected cell extracts for 20 min at temperatures ranging from 37–60°C and assaying the enzyme at 37°C. It was observed that in both the cases, the enzyme was stable up to 45°C, while it was inactivated rapidly between 45 and 50°C with almost identical rates. Further, the half-life of the enzyme at 48°C in the two extracts was the same (12 min; data not shown). So, the DNase encoded by L1+ and L1*G23ts*23 phages could not be differentiated in respect of their heat stability. If the *G23* gene codes for the above DNase, then the enzyme synthesized from the *G23ts*23 mutant gene would have been temperature-sensitive and would have shown a faster thermal inactivation pattern compared to that of the enzyme

produced by the wild-type L1 (*G23*[+]) phage. This suggests that the deoxyribonuclease is not the pro duct of the *G23* gene of the phage. The above results do suggest that the *G23* gene of L1 plays a positive regulatory role in the expression of this phage-coded deoxyribonuclease.

The expression of L1-coded deoxyribonuclease is not regulated at the transcriptional level. The synthesis of L1-coded DNase has been shown to be regulated by the *G27* gene at the transcriptional level, and G27 acts as a master regulator of L1 controlling the temporal circuit of transcription of delayed early and late genes of the phage (Datta and Mandal, 1998). Now, the question arises as to how the *G23* gene is involved in the regulation of expression of the above phage-coded DNase. The results of temperature-shift up experiment presented in Fig. 1C reveal that when the L1*G23ts*23-infected culture was shifted from 32 to 42°C at 0, 15, 30, 45, and 60 min, there were no significant increase in the level of the enzyme at the end of postshift-up growth (for a total of 90 min) at 42°C over those present at the respective times of shift up. However, when shifted up at 75 min, the level of enzyme increased to a certain extent in the shifted up cells over that present at the time of shift (75 min) in 32°C-grown cells. If G23 regulates the synthesis of phage-coded DNase at the transcriptional level, then the synthesis of the mRNA encoding this enzyme would have stopped immediately following shift up, but the same mRNA already synthesized at 32°C up to the time of shift would have been translated even after the shift of the culture to 42°C to produce extra protein (enzyme) over that present at the time of shift up. But this has not happened (see Fig. 1C). So, we conclude that *G23* does not regulate the synthesis of phage-coded DNase at the transcriptional level as is done by G27.

The expression of L1-coded deoxyribonuclease is dependent on *de novo* protein synthesis during post-shift down growth of L1*G23ts23*-infected culture at 32°C. Rifampicin (Rif) inhibits RNA synthesis by inhibing DNA-dependent RNA polymerase in *E. coli* (Hartman *et al.*, 1967) as well as in mycobacteria (Harshey and Ramakrishnan, 1976; Levin and Hatfull, 1993), and chloramphenicol (Cm) inhibits bacterial protein synthesis (Renzo and Ochoa, 1962). The latter antibiotic inhibits also the growth of (Hatfull, 2000) and protein synthesis in mycobacteria (our unpublished results). In mycobacteriophage L1, the transcriptions of early and delayed early genes are totally inhibited by rifampicin (our unpublished results). The results of a temperature shift-down experiment presented in Table I show that the L1-specific DNase was synthesized to a high level by the above mutant when grown at 32°C (line 1) but to a very low level (13.7%) when grown continuously at 42°C (line 2). Among the cultures shifted down at 30 min (we selected 30 min for shift down because the late transcription starts at this time) from 42 to 32°C, the one grown in the presence of Rif could make the enzyme at 83% (line 5) of the control level (line 1) which was nearly similar to that made in the absence of any antibiotic (line 4) under identical shift down condition. In the other two shift-down cultures, one grown in the presence of Cm and the second in the presence of both Rif and Cm, the levels of the enzyme were found respectively to be at 30 (line 6) and 39% (line 7) of the control value (line 1). The level of the enzyme activity at the time of shift down (30 min) from 42°C to 32°C was around 10% (line 3) of the control value (line 1).

Table I
Effect of temperature shift down on the synthesis of phage-coded deoxyribonuclease by L1*G23ts*23 mutant

Growth of L1*G23ts*23-infeccted cells	DNase activity (Units/mg of protein)[a]
1. 70 min at 32°C (control)	80.00 (100%)
2. 70 min at 42°C	10.96 (13.7%)
3. 30 min at 42°C	8.16 (10.2%)
4. 30 min at 42°C and then shifted to 32°C and grown for 40 min	63.08 (78.85%)
5. Sample 4 + Rif at the time of shift	66.44 (83.05%)
6. Sample 4 + Cm at the time of shift	24.24 (30.30%)
7. Sample 4 + Rif + Cm at the time of shift	31.92 (39.25%)

[a] For the definition of the unit of enzyme activity, see Fig. 1B. For other details, see Text and Methods. The whole set of this experiment was repeated two times and the results in each case were reproducible. So only the results of one set of experiment are presented.

The transcription of early genes of L1 starts immediately after infection, and those of its delayed early and late genes around 8 and 30 min respectively (Datta and Mandal, 1998). So, when the *G23ts*23 mutant-infected culture was grown at 42°C for 30 min, only the early and delayed early genes of L1 (including *G23ts*23 mutant gene) were transcribed and translated but the late genes were not even transcribed. But, the mutant *G23ts*23 gene product, even if synthesized up to 30 min of growth at 42°C is not active, so the level of functional deoxyribonuclease was very low (10%) at the time of shift down. Hence, the increase of this enzyme activity in the shift-down cultures (both in the absence and presence of Rif (lines 4 and 5) from 10% at the time of shift to around 78.85 and 83% following growth at 32°C for 40 more min does suggest that such increase could occur even in the absence of new RNA synthesis during postshift-down growth. The fact that the enzyme level increased only to 30–39% (instead of to 83%) of the control level when protein synthesis was inhibited by Cm (lines 6 and 7) suggests that *de novo* protein synthesis

at the permissive temperature (32°C) is necessary for the significant increase of the enzyme level (to 83% of the control level) and such increase of the enzyme level is dependent on the functional G23 protein.

Certain revertants of L1*G23ts23* capable of growing at 42°C retain both *ts* defect in L1-coded DNase synthesis and *G23ts23* mutation. The L1*G23ts*23 mutant is defective in the phage-coded DNase synthesis (Fig. 1B) as well as in the transcription of its late genes at 42°C (Datta *et al*., 2007). A gene involved directly in the positive regulation of transcription of late genes of a phage is absolutely essential for its lytic growth, while the phage may grow even in the absence of the self-coded DNase in a wild-type host. If the *G23* gene regulates both the late gene transcription and the synthesis of DNase of L1 at the posttranslational level by a similar mechanism, then the revertants of L1*c*I*ts*391*G23ts*23 capable of growing at 42°C (by overcoming the *ts* defect in late transcription) may also include the ones which might have undergone a mutation in a putative late regulatory gene (say *Gx*), the protein product of which is the target of G23 modification (leading to conformational change). By such mutational change, the putative mutant Gx protein would acquire its functional form without G23 action, and such mutant Gx protein may be directly involved in the positive regulation of late gene transcription. To assess this proposition, around 25 revertants of *G23ts23* mutations were isolated and the property related to DNase synthesis was studied. The results presented in Table II show that among the 25 such revertants capable of forming plaques at 42°C, two (R4 and R22) retained the defect in the expression of DNase at 42°C (lines 4 and 5), while the other 23 revertants could synthesize the enzyme like wild type L1 at this non-permissive temperature (data not shown). These results suggest that in the latter revertants, possibly true reversion of the *G23ts23* mutation has occurred to produce wild-type, while in the former two (R4 and R22), the *G23ts*23 mutation has not been reverted at all, but the regain of growth capability at 42°C is possibly caused by an extragenic suppressor mutation in the putative gene *Gx,* which bypasses the requirement of *G23* function for the transcription of the late genes by Gx protein.

Table II
Deoxyribonuclease activity produced by the revertants of L1*c*I*ts*391*G23ts*23 isolated at 42°C

	DNase activity (Units/mg protein)[a]	
Phage	32°C	42°C
L1*c*I*ts*391 (*G23*$^+$)	88.86 (100)	92.00 (103%)
L1*c*I*ts*391*G23ts*23	64.00 (100)	6.70 (10.5%)
R4*sup4*	70.52 (100)	16.45 (11.6%)
R22*sup22*	85.70 (100)	24.00 (28%)

[a] For the definition of the unit of enzyme activity, see Figure 1B. For other details, see Text and Methods. The whole set of this experiment was repeated two times and the results in each case were reproducible. So only the results of one set of experiment are presented.

Since R4 and R22 retain the *ts* defect in DNase synthesis, they possibly still retain the *G23ts23* mutation. To verify this, the R4 revertant (R4 has also *c*I*ts*391 genotype) was crossed with L1*c*I$^-$ phage (Fig. 1D), and the *c*I*ts*391 containing recombinants capable of growth at 32°C forming turbid plaque but showing no growth at all at 42°C were selected and analyzed. It was observed that some of these recombinants showed *ts* defects in both phage growth and DNase synthesis at 42°C. That the *ts* defect in phage growth at 42°C of such recombinant obtained from the above cross was due to the presence of *G23ts*23 mutation was inferred by the fact that the above *ts* recombinant did not complement L1*G23ts*23 mutant for both growth and DNase synthesis at 42°C but did complement the other *ts* mutants like *G25ts*889 and *G24ts*764 of L1. This suggests that the revertant R4 retains the *G23ts*23 mutation and hence, the recovery from *ts* defect in growth is due to a second mutation (extragenic suppressor mutation *sup4*) in a different gene, which we call *G*x. From the above phage cross experiment, it appears that the *sup4* mutation (the *G*x gene) maps at the right side of *G23* gene in the L1 genetic map (Fig. 1D).

Discussion

Earlier study showed that G23 positively regulates the transcription of about 50% of the late genes of L1 and hence the L1*G23ts*23 mutant is also growth-defective at 42°C (Datta *et al*., 2007). The results presented in this paper show that L1*G23ts*23 mutant is also defective in the phage-coded deoxyribonuclease (Fig. 1B), while this enzyme synthesized at permissive temperature by L1*G23ts23* mutant showed heat-inactivation pattern similar to that shown by the enzyme synthesized by the wild type phage (data not shown). This suggests that the *G23* gene itself does not code for this enzyme. The transcription of delayed early genes starts around 8 min and that of late genes around 30 min after infection by L1 (Datta and Mandal, 1998). The expression of L1-coded DNase starts at around 10 min after infection (Fig. 1B). As the expression of functional DNase by L1 needs the functional *G23* gene (Fig. 1B), the latter gene is also expressed as early as 10 min after infection. So, both the G23 gene and the gene encoding DNase belong to the delayed early class. A comparison of genetic and transcriptional map of L1 shows that *G23* gene maps at a position which is a part of the template for

delayed early gene transcription (Mandal *et al.*, 2004; Datta *et al.*, 2007). The deoxyribonucleases coded by other double-stranded DNA phages like λ (Little, 1967), T4 (Miller *et al.*, 2003) and T7 (Krüger and Schroeder, 1981) are also the products of the delayed early genes of the respective phages. In these phages also the positive regulators of late gene transcription are the members of delayed early gene family.

From computer analysis of the DNA sequence data of L5 phage (a homoimmune sister of L1 phage), the presence of three DNase-encoding genes in this phage genome has been predicted, which appear to be located at three different base coordinates, one around 2 kb, the second around 38 kb, and the third around 43 kb from the left end of 52 kb phage DNA (Hatfull, 2000). But the expression and regulation of these genes have not been studied at all. Our data on the expression of L1-coded DNase (Fig. 1B) show that there is not much difference in the level of this enzyme in uninfected host and in L1*G23ts23*-infected host (at non-permissive temperature), while at the permissive temperature, this enzyme activity increased to about 10-fold higher level towards the end of latent period (latent period of L1 is around 110 min at 37°C; Chaudhuri *et al.*, 1993). As L1 or L5 infection immediately shuts off the protein synthesis in host *M. smegmatis* (Hatfull, 2000; Datta *et al.*, 2007), we rule out the possibility of the host-specific DNase induction following phage infection. Taken together, the data suggest that either the G23-regulated DNase of L1 is the major enzyme, if not the only one, coded by this phage or all the different DNases, if coded by L1 like L5, are possibly regulated by G23. It appears, therefore, that the delayed-early gene *G*23 of L1 phage regulates the expressions of its two other delayed early genes, one encoding DNase (present study) and the second encoding a positive regulator of transcription of late genes (Datta *et al.*, 2007). The results of temperature-shift up experiment (Fig. 1C) do suggest that the expression of L1-coded DNase is not regulated by G23 at the transcriptional level (Fig. 1C), while the *G27* gene of L1 regulates the expression of this enzyme at the transcriptional level (Datta and Mandal, 1998).

The question now arises as to how the delayed early gene *G23* regulates the expressions of two other delayed early genes, one encoding DNase and the second encoding a postive regulator of transcription of late genes of L1. Among the several revertants of L1*G23ts*23 mutant, which could grow at 42°C (by overcoming the *ts* defect in late transcription), two still retain the *ts* defect in the above enzyme synthesis at 42°C (Table II), and at least one of them (R4) has been shown to retain the *G23ts*23 mutation (the other one has not been tested by phage cross). This suggests that a mutation (extragenic suppressor of *G23ts*23) has occurred in a different gene (*Gx* which appears to map at the right side of *G23*, see Fig. 1D; however, the exact map location of this putative *Gx* gene is not known yet) that bypasses the requirement of G23 for the late gene transcription. Possibly, the protein product of wild type *Gx* gene needs to be modified by G23 protein for acquiring the functional form that actually activates late transcription, and the above extragenic suppressor mutation has possibly changed the conformation of the wild type Gx protein to one that now activates late transcription without G23 involvement. If this mechanism is the correct one then this regulation of the putative Gx by G23 will be at the posttranslational level. In a similar way, it may be speculated that the L1-coded DNase is synthesized in an inactive form, which is modified to the active form by the G23 protein. It appears therefore that the *G23* gene of L1 plays an important role in the regulation of at least a part of the genetic circuit operating in the lytic growth of this temperate phage. Cloning of the genes encoding DNase, Gx, and G23 and their extensive characterization will shed more light on the actual mechanism of regulation of the former two genes by the latter at the molecular level.

Acknowledgements

This work was supported by AD HOC Research Fellowships to P. Mandal and H. J. Datta and a Research Grant from the Council of Scientific and Industrial Research (CSIR), Govt. of India, New Delhi.

We thank Dr. B. Bloom for the mc^{2}6 strain and Mr. N.C. Datta for expert technical assistance.

Literature

Chattopadhyay D.J. and N.C. Mandal. 1982. Studies on polylysogens containing λN^-cI^- prophages. I. Control of synthesis and maintenance of a large number of integrated λ genomes. *Virology* 118: 439–448.

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

Clark A.J. 1973. Recombination-deficient mutants of *E. coli* and other bacteria. *Annu. Rev. Genet.* **7**: 67–86.

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

Datta H.J., P. Mandal, R. Bhattacharyya, N. Das, S. Sau and N.C. Mandal. 2007. The *G23* and *G25* genes of temperate mycobacteriophage L1 are essential for the transcription of its late genes. *J. Biochem. Mol. Biol.* 40: 156–162.

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

Harshey R.M. and T. Ramakrishnan. 1976. Purification and properties of DNA-dependent RNA polymerase from *Mycobacterium tuberculosis H37RV*. *Biochim. Biophys. Acta* 432: 49–59.

Hartmann G., K.O. Honikel, F. Knusel and J. Nuesch. 1967. The specific inhibition of the DNA-directed RNA synthesis by rifamycin. *Biochim. Biophys. Acta* 145: 843–844.

Hatfull G.F. 2000. Molecular genetics of mycobacteriophages. In: *Molecular Genetics of Mycobacteria*. Ed by Hatfull, G. F. and Jacobs, W. R.,Jr. ASM Press (Washington D.C.).

Kuzminov A. 2001. DNA replication meets genetic exchange: chromosomal damage and its repair by homologous recombination. *Proc. Natl. Acad. Sci.* USA 98: 8461–8.

Krüger D.H. and C. Schroeder. 1981. Bacteriophage T3 and bacteriophage T7 virus-host cell interactions. *Microbiol. Rev.* 45: 9–51.

Levin N.C. and G.F. Hatfull. 1993. *Mycobacterium smegmatis* RNA polymerase: RNA supercoiling, action of rifampicin and mechanism of rifampicin resistance. *Mol. Microbiol*. 8: 277–285.

Little J.W. 1967. An exonuclease induced by bacteriophage lambda. II. Nature of the enzymatic reaction. *J. Biol. Chem.* 242: 679–686.

Mandal N.C., R. Bhattacharyya, S. Sau and B. Chaudhuri. 2004. Studies on temperate mycobacteriophage L1: Its physical map, site of deletion in one of its mutants, and organization of early, delayed early and late genes. In *Perspectives in Cytology and Genetics* (Eds: Manna, G.K. and Roy, S. C.) Vol. 11: 81–100.

Marmur J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* 3: 208–218.

Miller E.S., E. Kutter, G. Mosig, F. Arisaka, T. Kunisawa and W. Ruger. 2003. Bacteriophage T4 genome. *Microbiol. Mol. Biol. Rev.* 67: 86–156.

Radding C.M. 1964**.** Nuclease activity in defective lysogens of phage lambda. *Biochem. Biophys. Res. Commun*. 15: 8–12.

Renzo R. and S. Ochoa. 1962. Effect of chloramphenicol on protein synthesis in cell-free preparations of *Escherichia coli. Cell* 78: 317–324.

Sambrook J., E.F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sharples G.J., L.M. Corbett and P. McGlynn. 1999. DNA structure specificity of Rap endonuclease. *Nucl. Acids Res.* 27: 4121–4127.

Walker G.C. 1985. Inducible DNA repair systems. *Annu. Rev. Biochem.* 54: 425–457.