

A Point Mutation at the C-Terminal Half of the Repressor of Temperate Mycobacteriophage L1 Affects Its Binding to the Operator DNA

Tridib Ganguly, Partho Chattoraj, Malabika Das, Palas K. Chanda,
Nitai. C. Mandal, Chia Y. Lee[†] and Subrata Sau*

Department of Biochemistry, Bose Institute, P1/12-CIT Scheme VII M, Calcutta 700 054, India

[†]Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center,
3901 Rainbow Blvd., Kansas City, Kansas 66160, USA

Received 31 March 2004, Accepted 6 July 2004

The wild-type repressor CI of temperate mycobacteriophage L1 and the temperature-sensitive (ts) repressor CI_{ts391} of a mutant L1 phage, L1c_{Its391}, have been separately overexpressed in *E. coli*. Both these repressors were observed to specifically bind with the same cognate operator DNA. The operator-binding activity of CI_{ts391} was shown to differ significantly than that of the CI at 32 to 42°C. While 40-95% operator-binding activity was shown to be retained at 35 to 42°C in CI, more than 75% operator-binding activity was lost in CI_{ts391} at 35 to 38°C, although the latter showed only 10% less binding compared to that of the former at 32°C. The CI_{ts391} showed almost no binding at 42°C. An *in vivo* study showed that the CI repressor inhibited the growth of a clear plaque former mutant of the L1 phage more strongly than that of the CI_{ts391} repressor at both 32 and 42°C. The half-life of the CI_{ts391}-operator complex was found to be about 8 times less than that of the CI-operator complex at 32°C. Interestingly, the repressor-operator complexes preformed at 0°C have shown varying degrees of resistance to dissociation at the temperatures which inhibit the formation of these complexes are inhibited. The CI repressor, but not that of CI_{ts391}, regains most of the DNA-binding activity on cooling to 32°C after preincubation at 42 to 52°C. All these data suggest that the 131st proline residue at the C-terminal half of CI, which changed to leucine in the CI_{ts391}, plays a crucial role in binding the L1 repressor to the cognate operator DNA, although the helix-turn-helix DNA-binding motif of the L1 repressor is located at its N-terminal end.

Keywords: Gel shift assay, Mycobacteriophage L1, Temperature-sensitive mutant CI_{ts391} repressor, Wild-type CI repressor

Introduction

Mycobacteriophage L1 is a temperate phage, whose natural host is an unspiciated *Mycobacterium* ATCC27199 (Doke, 1960). It has a 50 kb double-stranded DNA genome, which remains integrated at a specific site in the chromosome of *M. smegmatis* (Snapper *et al.*, 1988). Genetic studies have shown that the *cI* gene of L1 is essential for both the establishment and maintenance of lysogeny, and the lysogen of L1 carrying a temperature-sensitive mutation in its *cI* gene is induced at non-permissive temperatures (Chaudhuri *et al.*, 1993). This suggests that the *cI* gene of L1 codes for its repressor. The *cI* gene is located at the left-hand side in around 15% of the coordinates of its genome (Chaudhuri *et al.*, 1993). The wild-type *cI* and mutant *cI_{ts391}* genes of L1 were cloned and sequenced (Sau *et al.*, 2004). Sequence analysis showed that the *cI* gene encodes a 183 amino acid residue protein, and in the CI_{ts391} repressor, the proline residue at the 131st position is replaced by leucine. Incidentally, the amino acid sequence of the CI repressor of L1 (Sau *et al.*, 2004) was found to be 100% identical to that of the repressor gp71 of the homoimmune phage L5 (Hatfull, 2000). L5 gp71 negatively regulates the transcription from the early promoter, *P_{left}* and was shown to bind at a specific operator region encompassing the -35 element of the promoter, *P_{left}* (Hatfull, 2000). The promoter, having 100% sequence similarity with the *P_{left}* of L5, was also recently cloned from phage L1 (Chattopadhyay *et al.*, 2003). Interestingly, the operator present in *P_{left}* was also present in 29 other locations within the L5 genome (Hatfull, 2000).

The temperature-sensitive repressors of lambda (Mandal and Leib, 1976; Jana *et al.*, 1999), P1 (Heinrich *et al.*, 1989) and Mu (Vogel *et al.*, 1991) have been used as model systems to study the structure-function relationship of these negative regulatory proteins and to construct several genetic tools (Sambrook *et al.*, 1989). Several mutants carrying the

*To whom correspondence should be addressed.
Tel: 91-33-2337-9416; Fax: 91-33-2334-3886
E-mail: sau@bic.boseinst.ernet.in

temperature-sensitive mutations in the repressor gene were also isolated from both mycobacteriophages L1 (Chaudhuri *et al.*, 1993) and L5 (Donnelly-wu *et al.*, 1993), and the mutations mapped (Donnelly-wu *et al.*, 1993; Sau *et al.*, 2004). However, such mutant repressors remain to be exploited at length in the elucidation of the structure-function relationship of any mycobacteriophage-specific repressor. In the present communication, in order to decipher the role of the 131st proline residue on the structure and function of the L1 repressor, both the wild-type and the CI_{ts391} repressors were overexpressed and their operator DNA-binding activities compared at different temperatures. Our data suggests that the point mutation resulting in the P131L change in the CI_{ts391} repressor significantly affects its binding to the operator at 32°C and above.

Materials and Methods

Bacterial and phage strains, plasmids and growth conditions

M. smegmatis mc²155 and LR222, were obtained from Dr. Anil Tyagi (University of Delhi, South Campus, New Delhi, India), and routinely grown in Middlebrook 7H9 medium (Chaudhuri *et al.*, 1993). The wild-type mycobacteriophage L1, its mutants L1cI and L1cI_{ts391}, and their assay procedures have been described previously (Chaudhuri *et al.*, 1993). The *E. coli*-*M. smegmatis* shuttle vector pSD5S30, containing a mycobacteria-specific promoter S30, was obtained from Dr. Anil Tyagi (University of Delhi, South Campus, India). The vector pM_{PMK4} (Mayer, 1995) was a generous gift from Dr. S. Yasuda (Japan).

DNA isolations and manipulations Genomic DNAs were isolated from both the wild-type L1 and mutant L1cI_{ts391} phages, according to the standard procedure (Sau *et al.*, 2004). The cI and cI_{ts391} genes were amplified by Taq polymerase using the above genomic DNAs as templates and the same pair of primers; LCP2 (5'-AAGCTTCCTTTCGTTGCGCGGC) and LCP3 (5'-GAATTCATGAGCGGCAAAAATC). The resulting fragments were cloned separately into a TA cloning vector, according to the manufacturer's protocol (Invitrogen Inc., Carlsbad, USA). The recombinant TA vectors carrying the cI and cI_{ts391} genes were designated pSAU1047 and pSAU1076, respectively. Sequences of the repressor genes were confirmed by the sequencing of the inserts of both pSAU1047 and pSAU1076 using an ABI automated sequencer, according to the manufacturer's protocol. All other molecular biological techniques, including plasmid DNA isolation, digestion of DNA by restriction enzymes, ligation by T4 DNA ligase, transformation, etc. were carried out according to the standard procedures (Sambrook and Russell, 2001). All the restriction & modifying enzymes, PCR kits and oligonucleotides were purchased from Bangalore Genei (India).

Overexpression of the wild-type CI and CI_{ts391} repressors To overexpress the wild-type CI and CI_{ts391} repressors, the cI and cI_{ts391} genes, generated from pSAU1047 and pSAU1076, respectively, were separately subcloned downstream of the L-arabinose inducible promoter, pBAD, in an *E. coli*-specific

expression vector, pM_{PMK4} (Mayer, 1995). The resulting pM_{PMK4} derivatives carrying the cI and cI_{ts391} genes were named pSAU1049 and pSAU1085, respectively.

E. coli DH5α cells carrying pSAU1049 were grown for 16 h at 32°C in the presence of 0.1 (w/v) L-arabinose and then harvested by centrifugation. The cell pellet was resuspended in 1/10 volume of TEN buffer (10 mM Tris-Cl, pH 7.9, 1 mM EDTA and 50 mM NaCl) and sonicated with 5-30 sec pulses at 100 W using a sonicator (Microsonics XL2000). The extract was centrifuged at 37,000 rpm at 4°C, and both the supernatant and pellet fractions were stored on ice till used. The proteins in these cell extracts were estimated by a standard procedure (Bradford, 1976) and those in the different fractions analyzed by SDS-PAGE (Ausubel *et al.*, 1998). A protein band having a size (~21 kDa) similar to that of the L1 repressor was found in the extracts made from the induced *E. coli* cells carrying pSAU1049 only (data not shown).

The cell extracts were prepared from *E. coli* DH5α (pSAU1085) cells by the same procedure described above. As shown in the case of the wild-type L1 repressor above, a 21 kDa protein was also detected in the extracts prepared from the induced *E. coli* cells carrying pSAU1085 only (data not shown).

Gel shift assay For the gel shift assay, a 97 bp *Pst*I-*Bam*HI fragment of L1 DNA, equivalent to 51740-51644 bp co-ordinate of L5 genome carrying one repressor binding site at the immediate upstream of the promoter, *P*_{left} (Brown *et al.*, 1997) was cloned in a separate study and used here as an operator DNA in the gel shift assay. It was end-labeled with [³²P-γ] ATP, according to the standard procedure (Sambrook and Russell, 2001). The gel shift assay was performed by a modification to the method of Brown *et al.*, (1997). Briefly, an aliquot of the soluble extract, containing around 5 μg protein, was mixed with 2 ηg of ³²P end- labeled operator DNA in a 20 μl reaction mixture (containing 2 μg of calf thymus DNA, 100 μg/ml of BSA, 50 mM NaCl, 10 mM Tris pH 7.9, 5% glycerol and 1 mM EDTA) and incubated on ice for 15 min. The reaction was stopped by the addition of 3 μl of loading dye (containing 5% glycerol and Bromophenol blue) and the samples loaded onto a pre-run native polyacrylamide gel (6 % acrylamide, 1 X TBE pH 8.3) in 0.25 X TBE buffer (Ouyang *et al.*, 1999). After electrophoresis for 4 h at 50 V, the gel was dried and autoradiographed. The autoradiographs were scanned either by a densitometer (Molecular Dynamics, USA) or by a Gel Doc 1000 system (BioRad, Hercules, USA). Each lane was scanned 3-5 times and the average value of each lane was used to determine the amount of both the bound (by L1 repressor) and free operator DNA that had been separated by the above gel electrophoresis. As demonstrated for many DNA-binding proteins before (Ausubel *et al.*, 1998), the amount of operator DNA shifted (bound) by the L1 repressor was used as a direct measure of the operator DNA-binding activity of L1 repressor. For the incubation of the L1 repressor or L1 repressor-operator complexes at other temperatures, such as 32, 35, 38, 42, 45, 48 and 52°C, different temperature-regulated water baths were used.

To see the effect of temperatures on the DNA binding activities of the L1 repressor, equal amounts (5 μg) of *E. coli* cell extracts carrying either the wild-type or CI_{ts391} repressors were incubated at 32, 35, 38 and 42°C for 30 min. Then, 2 μg of ³²P end- labeled operator DNA was added to each extract and the incubation continued for another 20 min. The rest of the steps of gel shift assay

were the same as those described above.

To study the refolding of the L1 repressor, equal amounts (5 μ g) of *E. coli* cell extracts carrying either the wild-type or CIIts391 repressors were incubated at 42, 45, 48 and 52°C for 30 min, and then cooled very slowly to 32°C. An equal amount of 32 P end-labeled operator DNA was added to each extract and the reactions continued for 20 min at 32°C. The rest of the steps of gel shift assay were performed according to the procedure described above.

To study the stability of the complexes formed between the wild type CI (or CIIts391) and the operator at different temperatures, equal (5 μ g) amounts of *E. coli* extract were mixed with 2 μ g of 32 P end-labeled operator DNA at 0°C and incubated for about 1 min. Then, the reaction mixtures were transferred to 32, 42, 45, 48 and 52°C incubators for 20 min. Next, all the mixtures were resolved, autoradiographed and quantified by the procedures described above.

To study the rate of dissociation of the repressor-operator complexes at 32°C, equal (5 μ g) amounts of *E. coli* cells extracts (carrying either the wild-type or L1CIIts391 repressor) were mixed with 2 ng of 32 P end-labeled operator DNA and incubated at 32°C. After 20 min, a 400-fold excess of unlabeled operator DNA was added, and 25 μ l aliquots taken at 0, 5, 15, 30, 45, 60 and 90 min. Each time the reaction was stopped by the addition of an equal volume of the sample loading buffer. These reaction mixtures were subjected to electrophoresis, autoradiographed and scanned by the procedures described above. The half-life of the repressor-operator complex was determined as the time point where 50% of the repressor-operator complex of the zero time point was present.

Results and Discussion

The wild-type CI and its mutant derivative, CIIts391 bind to the same cognate operator specifically Ascertain that the 21 kDa protein present in the extracts of the L-arabinose induced *E. coli* cells carrying pSAU1049 is indeed an L1-specific repressor, a gel shift assay was performed using the 32 P end-labeled L1 operator DNA and different *E. coli* cell extracts. The results shown in Fig. 1 reveal that the operator DNA-binding activity (assayed at 0°C) was present in the cell extract prepared from induced *E. coli* carrying pSAU1049 plasmid (lane 3) only, but in neither the same cells grown in the absence of the inducer (lane 2) nor the *E. coli* cells harboring no plasmid but grown in presence of L-arabinose (data not shown). The binding to the 32 P-labeled operator DNA was competed with cold operator DNA (lane 4), but not with non-specific DNA (lane 5). It was concluded that this DNA-binding activity was specific for the operator DNA of L1. Identical DNA-binding results were also obtained with the extract prepared from the L-arabinose induced *E. coli* cells carrying pSAU1085 (data not shown). Therefore, the above 21 kDa protein was truly the *cI* gene-coded repressor of L1. The operator DNA-binding results also suggest that the L1 repressor expressed in the heterologous system of *E. coli* had attained biologically active conformation.

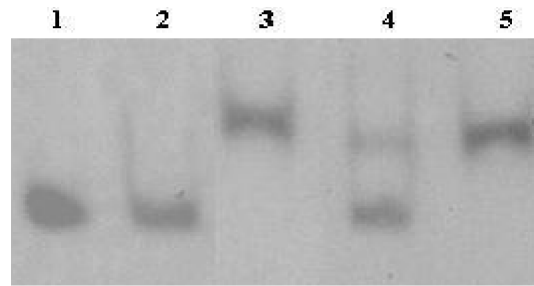


Fig. 1. Operator DNA-binding specificity of the L1 repressor. To determine the operator DNA-binding specificity of the L1 repressor, the gel shift assay was carried out according to the procedure described in Materials and Methods. Lanes: 1, end-labeled operator; 2, as of lane 1 plus uninduced *E. coli* (pSAU1049) cell extract; 3, as of lane 1 plus induced *E. coli* (pSAU1049) extract; 4, as of lane 3 plus 100 fold excess of cold operator DNA; 5, as of lane 3 plus 100 fold excess of a DNA fragment carrying a truncated *E. coli* β -galactosidase gene. See text for details.

Comparison of the operator DNA binding activities between the wild-type CI and CIIts391 repressors at different temperatures

The temperate mycobacteriophage L1 forms turbid plaques on the lawn of *M. smegmatis* at both 32 and 42°C, whereas, its mutant L1cIts391, forms turbid plaques at 32°C, but clear plaques at 42°C (Chaudhuri *et al.*, 1993). It was suggested that the point mutation present in CIIts391 makes it more heat-labile compared to the wild-type repressor above 32°C (Sau *et al.*, 2004). To determine whether the repressor CIIts391 is really more thermosensitive than that of its wild-type counterpart, their operator DNA-binding activities were compared by gel shift assays at temperatures ranging from 32 to 42°C. It was reasoned that a repressor, if sensitive to a particular temperature, would fail to bind its cognate operator DNA at that temperature, and as a result, the repressor-operator complex will not form. As shown in Fig. 2, the operator-binding activity of the wild-type repressor was affected nearly 5-10% at 35 to 38°C compared with that obtained at 32°C (lanes 2 versus 4 & 6). However, about 40% binding was found at 42°C (Fig. 2, lanes 2 versus 8). The binding activity of CI was dramatically reduced, to about 95-98%, at temperatures from 45 to 52°C (data not shown). On the contrary, the operator DNA-binding activity of the CIIts391 repressor was estimated to be about 10% less than that of the wild-type CI at 32°C (Fig. 2, lanes 2 versus 3). Most notably, around 75% of the CIIts391-specific activity was lost at 35 and 38°C (Fig. 2, lanes 3 versus 5 & 7), and at 42°C there was barely any CIIts391 activity left (Fig. 2, lane 9). The data indeed indicates that the DNA-binding activity in the CIIts391 repressor was more thermolabile than that in the wild-type repressor at 35°C and above. Such temperature-dependent behavior of CIIts391 has not been previously reported for any other temperature-sensitive mycobacteriophage-specific repressor.

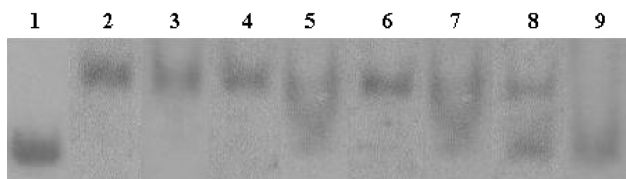


Fig. 2. The effect of preincubation at different temperatures on the DNA-binding activities in L1 repressors. The gel shift assay picture showing the amount of complexes formed between the wild-type CI (or CIIts391) repressor and operator DNA at different temperatures. See Materials and Methods for details. Lanes 2 & 3, 4 & 5, 6 & 7 and 8 & 9 contain reaction mixtures at 32, 35, 38 and 42°C, respectively. Lane 1 contains labeled operator only. The reaction mixtures containing the wild-type repressor were loaded into lanes 2, 4, 6 and 8, whereas, the mixtures carrying the CIIts391 repressor were loaded into the remaining lanes.

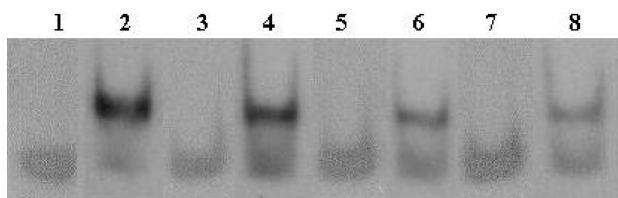


Fig. 3. The effect of cooling of the pre-heated L1 repressor on the operator DNA-binding activity. The autoradiograph showing the extent of refolding of both the CI and CIIts391 repressors preheated at different temperatures. See Materials and Methods for details. Lanes 2, 4, 6 and 8, contained the assay mixture carrying the wild-type repressors preincubated at 42, 45, 48 and 52°C, respectively. Lane 1 contained ^{32}P end labeled operator DNA only. In the rest of the lanes, the CIIts391 repressors, preincubated at 42, 45 and 48°C, were loaded after their reactions.

Heat-treated wild-type repressor CI regains its DNA-binding activity after gradual cooling The loss of DNA-binding activity in the above two repressors suggests that the active conformations of the repressor proteins are changed to inactive forms at higher temperatures. To test if such changes of the conformations of the repressor proteins were reversible or irreversible, the DNA-binding activities of the heat-treated repressors were determined after their slow cooling. As shown in Fig. 3 (lane 2), the wild-type repressor was estimated to shift by around 80% that of the input operator when cooled to 32°C after heating at 42°C. This indicates that the wild-type repressor substantially regained its DNA-binding activity as it showed only about 40% DNA-binding activity at 42°C (Fig. 2, lane 8). In addition, around 45-60% DNA-binding activity was shown when cooled slowly to 32°C after preincubation at temperatures from 45 to 52°C (Fig. 3, lanes 4, 6 and 8). This is highly surprising as the wild-type CI was found not to bind the operator DNA at a significant level when the binding assays were carried out directly between 45 and 52°C (data not shown). Contrary to the above, the CIIts391 repressor was

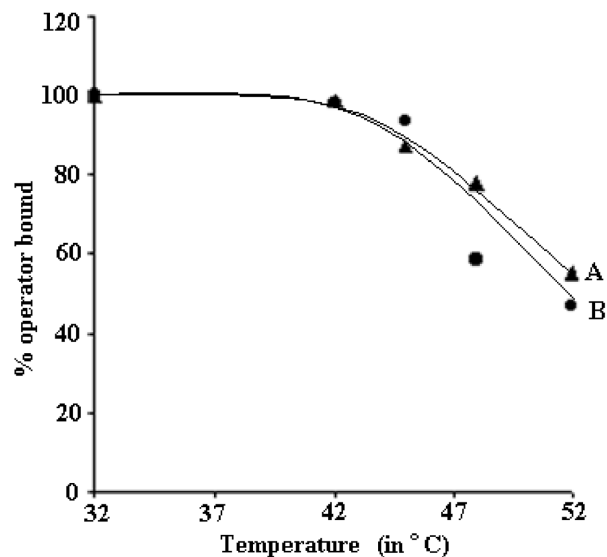


Fig. 4. The difference in stability between the wild type CI and CIIts391 repressor operator complexes at different temperatures. The plot of % operator bound versus temperature show the varying degrees of resistance of the CI-(curve A) or CIIts391-(curve B) operator complexes to dissociation at 32-52°C. As described in Materials and Methods, the amount of repressor-bound operator, not undergoing dissociation on shifting the reaction mixtures from 0 to 32, 42, 45, 48 and 52°C, respectively, were determined from a gel shift assay picture, and then plotted. The amount of repressor-bound operator at 32°C was defined as 100%.

observed not to regain its DNA-binding activity to an appreciable level, even when cooled slowly to 32°C after 30 min preincubation at 42°C (Fig. 3, lane 3). These results differ from the observation with some of the temperature-sensitive mutant repressors of phage λ , which regained substantial amounts of DNA binding activities under similar treatments (Mandal and Leib, 1976). However, our results suggest that the 131st proline residue of the L1 repressor plays crucial roles in maintaining its active form and in the binding of the repressor to the cognate operator.

Stability of repressor-operator complex at different temperatures It was shown that a mutant repressor of bacteriophage P1 could not bind with its operator DNA in the temperature ranging 40 to 45°C, but the repressor-operator complex formed at room temperature was somewhat resistant to the above temperatures (Heinrich *et al.*, 1989). To see whether the CIIts391 repressor of L1 behaves in a similar way, the stability of the CIIts391-operator DNA complexes preformed at 0°C was checked by the gel shift assay after 20 min incubation at 32 to 52°C. As shown in Fig. 4, the complexes had varying degrees of resistance to dissociation during incubation at the above temperatures (curve B). The preformed CI-operator DNA complexes were also found to be heat-tolerant within the above temperature range (Fig. 4,

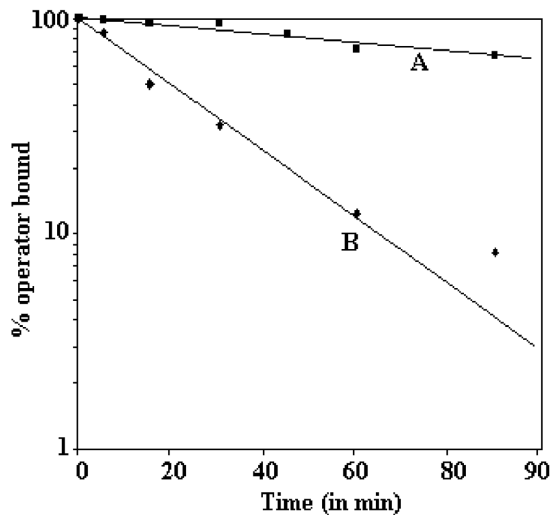


Fig. 5. Dissociation of the repressor-operator complexes at 32°C. The amount of repressor-bound operator, remaining undisturbed for 0-90 min after the addition of excess cold operator DNA, were determined from gel shift assay pictures, and then plotted against time. For details, see Materials and Methods. The amount of operator bound in the shifted complex of the zero time aliquot was defined as 100%. Curves: A, wild-type repressor and B, CIts391 repressor, respectively.

Curve A). The only difference between the two types of complex is that the CI-operator complexes are slightly more stable than those of the CIts391-operator at temperatures above 42°C (curves A versus B). In general, about 45-98% of either type of complex was found to be unperturbed between 42 and 52°C compared to that at 32°C. Such results, however, were unexpected compared to the observation mentioned above (Fig. 2). Also, this can not properly explain how the L1CIts391 lysogen in *M. smegmatis* is induced and lysed at 42°C (Chaudhuri *et al.*, 1993), when the CIts391 repressor-operator complex is resistant to a certain extent at this non-permissive temperature. This indicates that some other regulators may also be involved in the induction of the above lysogen at non-permissive temperatures.

Plating efficiency of L1cI phage on *M. smegmatis* strains carrying the wild-type cI and CIts391 repressor genes

Both the wild-type L1 and its mutant L1CIts391 phages form

turbid plaques on the lawn of *M. smegmatis* at 32°C (Chaudhuri *et al.*, 1993). Our *in vitro* data have also shown (above) that both the repressors bind well with the same operator DNA at 32°C. To test whether the two repressors behave similarly *in vivo*, the efficiency of the plating of L1cI (Chaudhuri *et al.*, 1993) on *M. smegmatis* mc²155 carrying either the wild-type or the CIts391 repressor genes was measured. To this end, the plasmids pCP1 and pCP2 (Sau *et al.*, 2004) were constructed by cloning the wild-type cI and CIts391 genes into pSD5S30 (Jain *et al.*, 1997). Next, L1cI phages were plated on *M. smegmatis* mc²155 carrying either pCP1 or pCP2, and the efficiencies of the plating of L1cI as well as its plaque size were determined at both 32 and 42°C, and the data are presented in Table 1. At either temperature, both the efficiency of the plating (e.o.p) of L1cI phages and the plaque size determined on mc²155 (pCP2) were increased significantly compared to those on mc²155 (pCP1), but reduced to an extent comparable with those on mc²155 (pSD5S30). The reduction of both the determinants, especially at 42°C, most possibly occurred due to assaying the L1cI phage at room temperature. However, these results suggest that the wild-type repressor gene of pCP1 is more active in repressing the growth of L1cI compared with that of the CIts391 repressor gene at both 32 and 42°C. It was interesting to note that the L1cI phages were unable to form plaques on either the wild-type L1 lysogen or L1CIts391 lysogens in mc²155 at 32°C (data not shown). This may have been that the levels of expression of the repressors by pCP1 and pCP2 were less than those of the lysogens.

Half-lives of the repressor-operator complexes The reduced level of repression of L1cI phage growth by CIts391, as found above, may have been due to the weaker binding of CIts391 to the operator DNA than that of the wild-type CI at 32°C. To test this hypothesis, the rate of dissociation of the CIts391-operator complex was compared with that of the wild-type CI-operator complex at 32°C. As shown in Fig. 5, the rate of dissociation of the CIts391-operator complex was much higher than that of the CI-operator complex at 32°C. While the half-life of the latter complex was estimated to be 160 min, that of the former was only 20 min. On the contrary, at 0°C, the half-lives of the two complexes were 90 and 130 min, respectively (data not shown). These results suggest that the operator binding activity of CIts391 is weaker than that of

Table 1. Plating of L1cI phages on *M. smegmatis* mc²155 carrying different plasmids

<i>M. smegmatis</i> Strains	Repressor gene present	Percent e.o.p.* of L1cI		Diameter of plaque (mm)	
		32°C	42°C	32°C	42°C
mc ² 155 (pSD5S30)	nil	100	100	2.5	3.0
mc ² 155 (pCP1)	cI	9.5	20	0.5	1.8
mc ² 155 (pCP2)	CIts391	22	63	1.0	2.5

*The efficiency of plating (e.o.p) was determined by dividing the plaque-forming units on *M. smegmatis* mc²155 carrying either pCP1 or pCP2 by those formed on *M. smegmatis* mc²155 harboring pSD5S30. See text for details.

the wild-type repressor at 32°C.

Acknowledgments We thank Drs. B. Bloom, S. Yasuda and Anil Tyagi for the several strains, such as mycobacteriophage L1, *M. smegmatis*, and plasmids, like pMPPMK4 and pSD5S30. The technical assistance of Mr. N. C. Datta is thankfully acknowledged. This research work was supported by the Junior Research fellowships to Mr. Tridib Ganguly (from the CSIR, Govt. of India, New Delhi) Mr. Partho Chatteraj and Mr. Palas Chanda (both from UGC, Govt. of India, New Delhi).

References

- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (ed.) (1998) Current Protocols in Molecular Biology, Vol. 2, pp. 12.2.1-12.2.11 copyright by John Wiley & Sons, Inc., USA.
- Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
- 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.
- 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-sensitive mutations in the genes essential for lysogenic and lytic growth of the mycobacteriophage L1. *Virology.* **194**, 166-172.
- 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: application for genetic transformation of mycobacteria. *Mol. Microbiol.* **7**, 407-417.
- Hatfull, G. F. (2000) Molecular Genetics of Mycobacteriophages; *In Molecular Genetics of Mycobacteria*, Hatfull, G. F. and Jacob, W. R., Jr. (eds.), pp. 37-54, ASM Press, Washington D.C., USA.
- Heinrich, J., Riedel, H-D., Baumstark, B. R., Kimura, M. and Schuster, H. (1989) The cI repressor of bacteriophage P1 operator-repressor interaction of wild-type and mutant repressor proteins. *Nucl. Acids Res.* **17**, 7681-7692.
- Jain, S., Kaushal, D., DasGupta, S. K. and Tyagi, A. K. (1997) Construction of shuttle vectors for genetic manipulation and molecular analysis of Mycobacteria. *Gene* **190**, 37-44.
- Jana, N. K., Roy, S., Bhattacharyya, B. and Mandal, N. C. (1999) Amino acid changes in the repressor of bacteriophage lambda due to temperature-sensitive mutations in its cI gene and the structure of a high temperature-sensitive mutant repressor. *Protein Eng.* **12**, 225-233.
- Mandal, N. C. and Leib, M. (1976) Heat-sensitive DNA-binding activity of the cI product of bacteriophage Lambda. *Molec. Gen. Genet.* **146**, 299-302.
- Mayer, M. P. (1995) A new set of useful cloning and expression vectors derived from pBlueScript. *Gene.* **163**, 41-46.
- Ouyang, S., Sau, S. and Lee, C. Y. (1999) Promoter analysis of the *cap8* operon, involved in type 8 capsular polysaccharide production in *Staphylococcus aureus*. *J. Bacteriol.* **181**, 2492-2500.
- Sau, S., Chatteraj, P., Ganguly, T., Lee, C. Y. and Mandal, N. C. (2004) Cloning and sequencing 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, Br. and Jacobs, W. R. Jr. (1988) Lysogeny and transformation in mycobacteria: stable expression of foreign genes. *Proc Natl Acad Sci. USA.* **85**, 6987-6991.
- Sambrook, J. and Russell, D. W. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.
- Vogel, J. L., Li, Z. J., Howe, M. M., Toussaint, A. and Higgins, N. P. (1991) Temperature-sensitive mutations in the bacteriophage Mu c repressor locate a 63-amino acid DNA-binding domain. *J. Bacteriol.* **173**, 6568-6577.