CHAPTER IV: SURVIVAL OF BACTERIOPHAGE LAMBDA EXPOSED TO NON PHYSIOLOGICAL IONIC STRENGTH
Introduction

*Escherichia coli* responds to DNA damage with the expression of a set of functions usually termed as SOS response. This includes the induction of a transitory mutagenic DNA repair system, the activation of inducible prophage and of several other functions involved in cell division and DNA metabolism (Radman, 1974; Witkin, 1976). Induction of 'SOS' functions occurs on treatment with the hazardous agents such as UV radiations and γ-rays (Witkin, 1976; Bresler et al., 1978).

With regard to the damaging effect of non physiological conditions, Ahmad and Srivastava (1980) reported that repair to mildly heated (52°C) intracellular phage lambda requires the same radiation repair machinery which is involved in the radiation damage. Moreover, it is already a well known fact that exposure to non physiological pH, ionic environment and temperature significantly affect the stability of viruses (Gard and Maaloe, 1959; Pollard and Solosko, 1971). The *in vitro* studies on lambda DNA revealed the increase in the pitch of DNA duplex with increasing ionic strength (Bode, 1968). However, no serious attempt has been made to identify the damages induced in DNA and their repair as a result of non physiological ionic strength.
The work included in this chapter was initiated with a view to understand the nature of the lesions induced in DNA as a result of high ionic strength treatment. Our contention was that bacteriophage lambda might serve as a convenient model for this purpose because even a slight change in the DNA could be reflected in its plaque forming capacity.

**Materials and Methods**

The strains of lambda phage used in this study were $\lambda c^+$, $\lambda ci857$, $\lambda red$, $\lambda bia1$ and $\lambda vir$. Repair proficient strain of *E. coli* K-12 (AB1157) was lysogenised with $\lambda ci857$ in our laboratory. The relevant genetic markers associated with each *E. coli* and lambda strain are given in chapter II.

Bacterial cultures were raised in nutrient broth obtained from Hi-Media (India). Treatment buffers given in the chapter II were employed for the present work, while 0.01M Tris Mg solution at pH 8.0 was used as dilution buffer and to run the untreated control. Except the experiments for the survival of bacteriophages, all the studies were carried out under the high ionic strength treatment conditions.

**Extracellular non physiological ionic strength treatment:** Phage lambda stock was purified according to the method of Ahmad et al.
(1977). Purified phage lambda (10^8 PFU/ml) was incubated at 37°C in the treatment buffers of 2M, 10^{-2}M as well as 10^{-5}M Mg^{++} + 5% NaCl buffer and 10^{-5}M MgSO_4. Aliquots of 0.1 ml were withdrawn at regular intervals, suitably diluted in normal buffer of pH 8.0 and allowed to adsorb on radiation sensitive and wild-type host strains at 37°C. The infective centres were plated on nutrient agar by the double layer method. Plaques were counted after overnight incubation of the plates at 37°C except for λci857 which was incubated at 42°C.

**Intracellular non physiological ionic strength treatment:**
Bacteria were harvested from exponential culture and suspended in MgSO_4 dilution buffer. Infective centres were prepared by adsorbing lambda particles at high multiplicity of infection (5:1) to bacteria (2x10^8 cells/ml). The complexes were then treated with the buffers of various ionic strengths (chapter II) at 37°C. Aliquots were withdrawn at regular intervals, suitably diluted and plated with 0.3 ml of C600 cells along with the soft agar by double layer method. Plaque forming units (PFU) were counted after 0/N incubation of plates at 37°C.

**Liquid holding recovery:** Phage λc^+ treated intracellularly, was subjected to liquid holding recovery. The treated
complexes were diluted ten fold in the recovery medium (chapter II) and incubated at 37°C for 2 h. Samples were taken out at regular intervals to assay PFU.

HIS induced c mutations of λc⁺: λc⁺ was treated with the high ionic strength as free particles and intracellularly for studying HIS mutagenesis in wild-type, lexA, recA and uvrA strains. The c mutants were scored according to the methods of Defais et al. (1971).

**Weigle mutagenesis:** Exponentially grown AB1157 and λc⁺ were separately treated with HIS for 6 h at 37°C and the infective centres were prepared at low multiplicity of infection. Unadsorbed phage particles were removed by centrifugation. The untreated controls were also run simultaneously. c mutants were scored on nutrient agar plates after 24 h incubation.

**Prophage induction:** Exponentially grown lysogen λcl857/AB1157 (1-4 x 10⁸ cells/ml) was centrifuged, suspended in the HIS buffer and incubated at 32°C for 3 h. The cells were again centrifuged and resuspended in nutrient broth with or without chloramphenicol (100 µg ml⁻¹) and incubated
for 3 h at 32°C. Aliquots were taken out at regular intervals, suitably diluted and plated with C600 cells. The plaques were scored after O/N incubation of plates at 42°C. Untreated controls were also run simultaneously.

Results

Survival of lambda on extracellular non physiological ionic strength treatment: Extracellular exposure of λc⁺ to both high and low ionic strength treatments had no effect on plaque forming ability. Similar results were also obtained with λvir, λbio1 and λred. Because there was no effect of non physiological ionic strength on lambda, the data are not shown.

Survival of on intracellular ionic strength treatment:

1. 1M MgSO₄ treatment. Intracellular treatment to lambda phage under this condition resulted in the loss of PFU (Fig.1). There was more decline in PFU in λc⁺ complexes with uvrArecA, recB, rer, recA, uvrA and lexA mutants compared with the wild-
type strain. Moreover, the complexes prepared with λ\text{red} and λ\text{bio1} were found to be relatively more sensitive as compared to those with the λc\textsuperscript{+} strain (Fig. 5a). λ\text{bio1} complexes with w.t. strain were considerably more sensitive as compared with λc\textsuperscript{+} - recA complexes (Figs. 1, 5a) but the effect of red mutation in lambda was quantitatively equal to that of rec mutation in E. coli. λc\textsuperscript{+} - polA\textsuperscript{-} complexes also exhibited damaging effect due to this treatment as compared to their isogenic counterparts (Fig. 1).

2. 1M MgSO\textsubscript{4} + 5% NaCl (HIS) treatment. The survival of λc\textsuperscript{+} phage exhibited a marked decline under this condition of treatment. The loss in PFU was more pronounced with λc\textsuperscript{+} - uvrArecA complexes followed by the complexes with recA, uvrA, rer, lexA and recB in order of decreasing sensitivity (Fig. 2). The relative sensitivity of λc\textsuperscript{+}, λ\text{red} and λ\text{bio1} complexes with wild-type E. coli strain is shown in Fig. 5b. Both the mutants were more HIS sensitive as compared to the wild-type λc\textsuperscript{+} strain. Furthermore, the survival of λc\textsuperscript{+} - polA\textsuperscript{-} complexes was also found to be significantly low as compared to that of λc\textsuperscript{+} - polA\textsuperscript{+} complexes (Fig. 2).

3. 10^{-2} M MgSO\textsubscript{4} + 5% NaCl treatment. The survival of phage lambda in various radiation sensitive mutants is given in Fig. 3. Plaque forming ability of λc\textsuperscript{+} - uvrArecA complexes was found to be
reduced significantly as compared to the complexes obtained with recB, rer, recA, uvrA and lexA strains. The λred-w.t. and λbio1-w.t. complexes exhibited a high degree of sensitivity towards the HIS treatment as compared to the complexes prepared with the λc+ strain (Fig. 5c). λc+-polA+ was again found to be exhibiting significantly high tolerance towards the said treatment compared to complexes with the polA− strain (Fig. 3).

4. 10−5 M MgSO4 + 5% NaCl treatment. A significant reduction in the PFU was found under this particular condition of treatment (Fig. 4). λc+-uvrArecA complexes again exhibited a remarkable decline in PFU followed by recA, recB, uvrA, rer and lexA lambda complexes. The complexes of λc+ with polA− were significantly sensitive to the treatment as compared to λc+-polA+ (Fig. 4). The λred and λbio1 complexes exhibited the same pattern of survival as found with other treatments (Fig. 5d).

The complexes of λc+ with recF and uvrD mutants did not show any remarkable change in survival as compared to those with the isogenic wild-type counterparts (data not shown).

Liquid holding recovery: The lambda complexes with w.t., recA, uvrA, lexA strains were exposed to HIS condition for 4 h
for this purpose. Treated complexes did not exhibit any increase in the PFU on liquid holding in the recovery medium upto 60 min (data not shown).

**HIS induced mutagenesis:** The results on the c mutation in lambda are incorporated in Table 1. HIS treatment for 6 h to the phage λc⁺ did not result in the induction of clear plaque mutation. However, the mutation frequency for this test marker was found to be slightly increased when the untreated λc⁺ was propagated in the HIS treated host bacterium. The mutagenic effect of HIS treatment was even more pronounced when the lambda as well as *E. coli* host were separately exposed to the HIS condition (Table 1). Mutagenic potential of HIS treatment was also investigated in various complexes. Intra-cellular treatment of λc⁺ in the w.t., recA, lexA and uvrA strains resulted in the varying degree of c mutation (Table 2). The recA and lexA mutations in the host apparently abolished the mutational capacity of the complexes towards the HIS treatment. However, the λc⁺-uvrA complexes exhibited a significant level of HIS induced mutagenesis. The w.t. and uvrA hosts apparently provide the same degree of mutagenic potential for the said marker in the *E. coli* complexes following the HIS treatment.
Prophage induction: Table 3 shows the induction of prophage as a result of HIS treatment to lysogen during the post treatment liquid holding in the nutrient broth. A fraction of the lysogenic population exhibited induction of lytic cycle during the liquid holding at 32°C. In the presence of chloramphenicol, however, the prophage induction was not observed.

Discussion

Lethal effects of high and low ionic strength of the growth medium are well documented (McCarthy, 1962; Costilow, 1981; Roth et al., 1985a). However, a little effort seems to have been devoted for the in vivo DNA damage. We have selected lambda/E.coli system as a model to investigate the HIS induced lesions in DNA. The plaque forming ability of the complexes was used as an index for HIS induced damage and its repair. Under the extracellular treatment conditions, both at high and low ionic strength, the survival of lambda was unaffected. In case of foot and mouth disease virus (FMDV), a remarkable decline in the infective potential has been reported (Fellows, 1964). However, it seems likely that under the non physiological ionic strength conditions, lambda proteins as well as its DNA are not damaged to a large extent. Since the treated phage DNA was allowed to infect on its host, it is also possible that micro-
lesions in DNA, if any, might have been repaired inside the normal host. On the other hand, intracellular treatment to phage DNA with non physiological ionic strength resulted in the loss of plaque forming units (PFU) indicating thereby, a major damage in the metabolic machinery of host bacterium which in turn, affected the vegetative multiplication of the phage (Figs. 1-5).

Regarding the involvement of DNA repair genes, defects in the recA, polA, rer, uvrA, recB and lexA loci render the bacterial cells more sensitive to the exposure to non physiological ionic strength environment.

The close examination of the survival patterns under the different ionic strength treatment conditions revealed that 1M MgSO₄ + 5% NaCl treatment condition was equally damaging as 10⁻⁵M MgSO₄ + 5% NaCl and the rest of the two conditions which were less damaging seem to have quantitatively similar contribution on the injury to E.coli cells. This finding is consistent with the treatment of bacteria alone (chapter III). But there were three distinct differences in case of λ-E.coli complexes: (1) All the complexes were more resistant towards the HIS treatment as compared to the corresponding bacterial strains treated alone, (2) The uvrA gene seems to have profound effect on the recovery of PFU in the case of treated λ-E.coli complexes contrary to the bacterial systems, and (3) The con-
tribution of \textit{lexA} gene in the case of complexes was less significant as observed with the bacteria alone.

The low injury found in this system could be explained on the basis of different number of affected targets in the two systems. The $\lambda$-\textit{E.coli} complex system is a direct index of the damages induced in DNA only, whereas the treatment to bacteria involves a multitarget system owing to the less defined parameter such as viability. Similar results were also obtained with mild heat and alkali treatments (Ahmad and Srivastava, 1980; Musarrat, 1987). The anomalous behaviour of \textit{uvrA} in the two systems was also demonstrated earlier (Ahmad and Srivastava, 1980). Figs. 1-4 also directed us towards the additive effect of \textit{recA} and \textit{uvrA} genes in our system.

Moreover, the $\lambda$\textit{red} and $\lambda$\textit{bio1} mutants were also more sensitive as compared to the wild-type strain suggesting the role of \textit{red}$^+$ and \textit{gam}$^+$ genes in the repair of the lesions induced by non physiological ionic strength (Figs. 5a,b,c,d). The $\lambda$\textit{bio1} mutant was invariably more sensitive to all the treatment conditions indicating thereby, the active role of \textit{gam} gene in this type of repair process. The involvement of \textit{red} and \textit{gam} genes in the repair of radiation induced damage is a well established fact (Srivastava, 1973; Trgovcevic and Rupp, 1975). Ahmad \textit{et al.} (1978) and Ahmad and Srivastava (1980) have also reported the involvement of \textit{recA, lexA} and \textit{polA} genes of \textit{E.coli} and \textit{red} gene of lambda in
the repair of heat induced lesions. The contribution of \textit{recA} and \textit{red} genes was almost identical regardless of the treatment conditions. The \textit{red} gene of lambda and \textit{recA} gene of \textit{E. coli} seem to be exerting complementary effect on the ionic strength induced lesions. Complementarity of the two genes has also been reported for the radiation, heat and pH repair processes (Srivastava, 1973; Ahmad and Srivastava, 1980; Husarrat, 1987). In view of the involvement of \textit{recA} and \textit{lexA} genes, we suggest the induction of 'SOS' response at least during the HIS treatment. The idea also gains an appreciable support by the induction of prophage in the lysogen and induction of \( \xi \) mutation as a result of HIS treatment (Tables 1-3). The induction of prophage also required \textit{de novo} protein biosynthesis. The role of \textit{rer} gene for the initiation of 'SOS' response was also suggested by Srivastava (1978). Prophage induction by UV and X-ray is well established and is considered to be one among the many 'SOS' responses (Lwoff \textit{et al.}, 1950; Witkin, 1976). This is also used as a simple parameter for testing the potentially mutagenic agents (Moreau \textit{et al.}, 1976). When the HIS treated bacteria were allowed to infect by the untreated phage, the frequency of \( \xi \) mutation was found to be enhanced to an appreciable extent. The enhancement was much more pronounced with treated phage. This finding clearly suggested that the diminished proof reading activity of DNA polymerase(s) was the major cause of mutagenesis. Villani \textit{et al.} (1978) also suggested that treatment of DNA damaging agents induced.
an inhibitor which inhibits or reduces the proof reading, $3' \rightarrow 5'$ exonucleolytic activity of constitutive DNA polymerase. Recently, Lu et al. (1986) have provided evidence that reca protein binds to \( \subunit \) of DNA polymerase III and thus reduces the proof reading activity of the enzyme.

As compared to \( E. coli \), liquid holding recovery of PFU of lambda was not observed when HIS treated $\lambda_c^+$-host complexes were held in the recovery medium for 1 h at $37^\circ$C. The lack of recovery may be due to the inability of infected bacteria to recover in the recovery medium since the repair of DNA and the multiplication of lambda depend upon the state of host. In the case of intracellular treatment, where the host has also been injured, the recovery involving de novo synthesis probably did not take place due to the infection of phage. It is well known that DNA, RNA and protein syntheses of host are inhibited following infection with lambda (Cohen and Chang, 1970).

In view of the present work, it seems beyond doubt that after the external ionic strength exceeds to the tolerable limit, the internal ionic strength is also affected resulting in the damage to the metabolic machinery of the bacterial cells. Moreover, the chromosomal as well as other DNA molecules also get injured to an appreciable extent. To cope with the hazardous effects of non physiological ionic strength of the environment,
the cell protects itself utilizing its constitutive as well as inducible repair machinery. Interestingly, the well documented 'SOS' response seems to play an active role to deal with this offensive non physiological stress. Schuldiner et al. (1986) and Musarrat (1987) independently reached to the same conclusion in the case of non physiological pH.
Fig. 1  Survival of $\lambda c^+$ on intracellular exposure to 1M MgSO$_4$ treatment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Survival Pattern</th>
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<tbody>
<tr>
<td>w.t.</td>
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</tr>
<tr>
<td>recA</td>
<td>Δ—Δ</td>
</tr>
<tr>
<td>recB</td>
<td>□—□</td>
</tr>
<tr>
<td>rer</td>
<td>▲—▲</td>
</tr>
<tr>
<td>lexA</td>
<td>●—●</td>
</tr>
<tr>
<td>uvrA</td>
<td>x—x</td>
</tr>
<tr>
<td>uvrArecA</td>
<td>□—□</td>
</tr>
<tr>
<td>polA$^+$</td>
<td>0—0</td>
</tr>
<tr>
<td>polA$^-$</td>
<td>□—□</td>
</tr>
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</table>
Fig. 2 Survival of $\lambda_c^+$ on intracellular exposure to $1M \text{MgSO}_4 + 5\% \text{NaCl}$ treatment

<table>
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<tr>
<th>Gene</th>
<th>Symbols</th>
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<tr>
<td>w.t.</td>
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</tr>
<tr>
<td>recA</td>
<td>△——△</td>
</tr>
<tr>
<td>recB</td>
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</tr>
<tr>
<td>rer</td>
<td>△——△</td>
</tr>
<tr>
<td>lexA</td>
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<tr>
<td>uvrA</td>
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</tr>
<tr>
<td>uvrRecA</td>
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<tr>
<td>polA$^+$</td>
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</tr>
<tr>
<td>polA$^-$</td>
<td>□——□</td>
</tr>
</tbody>
</table>
Fig. 3  Survival of $\lambda_c^+$ on intracellular exposure to $10^{-2}\text{M MgSO}_4 + 5\% \text{ NaCl}$ treatment

| w.t.           | □□□□□□ □□□□□□       |
| recA           | △△△△△△ △△△△△△       |
| recB           | □□□□□□ □□□□□□       |
| rer            | △△△△△△ △△△△△△       |
| lexA           | ●●●●●● ●●●●●●       |
| uvrA           | ×××××× ××××××       |
| uvrArecA       | ■■■■■■ ■■■■■■       |
| polA$^+$       | □□□□□□ □□□□□□       |
| polA$^-$       | □□□□□□ □□□□□□       |


Fig. 4  Survival of $\lambda_c^+$ on intracellular exposure to $10^{-5} \text{M} \ Mg\text{SO}_4 + 5\% \ NaCl$ treatment

- w.t. : 0---0
- recA : △---△
- recB : ▽---▽
- rer : ▲---▲
- lexA : ●---●
- uvrA : ×---×
- uvrArecA : ■---■
- polA$^+$ : 0----0
- polA$^-$ : ◻---◻
Fig. 5 Survival of phage λ on intracellular exposure to (a) 1M MgSO$_4$, (b) 1M MgSO$_4$ + 5% NaCl, (c) $10^{-2}$M MgSO$_4$ + 5% NaCl and (d) $10^{-5}$M MgSO$_4$ + 5% NaCl treatments

$\lambda c^+$ w.t. : 0--0

$\lambda red$ w.t. : X--X

$\lambda bio1$ w.t. : •--•
Table 1. HIS induced mutagenesis in phage $\lambda c^+$

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<tr>
<th>Time of incubation (h)</th>
<th>Mutation frequency per $10^3$ PFU</th>
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<td></td>
<td>Extracellularly treated phage</td>
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<tr>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>2.9</td>
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<tr>
<td>Strains</td>
<td>Spontaneous mutation frequency</td>
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<td>---------</td>
<td>-------------------------------</td>
</tr>
<tr>
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</tr>
<tr>
<td>recA</td>
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<tr>
<td>lexA</td>
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<td>uvrA</td>
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Table 3. Induction of prophage on HIS treatment

<table>
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<tr>
<td></td>
<td>Untreated control</td>
<td>After 3 h HIS treatment</td>
</tr>
<tr>
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<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
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</tr>
<tr>
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<td>3</td>
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