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The genetics of the Luria–Latarjet effect in bacteriophage T4: evidence for the involvement of multiple DNA repair pathways

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Summary

The Luria–Latarjet effect is an increase in resistance of a virus to DNA damage during infection of a host. It has often been assumed to involve recombinational repair, but this has never been demonstrated experimentally. Using nine bacteriophage (phage) T4 mutants, I present evidence indicating that, for phage T4, the Luria–Latarjet effect is due to three repair pathways – excision repair, post-replication-recombinational-repair (PRRR) and multiplicity reactivation (MR) (a second form of recombinational repair). The results also show that the Luria–Latarjet effect develops in two stages. The first stage starts soon after infection. Damage which occurs during the first stage can be repaired by excision repair or PRRR. The second stage appears to start after the first round of DNA replication is complete. DNA damage which occurs during this stage can apparently be repaired by MR as well as the other two repair pathways. The results of this study support the hypothesis that recombinational repair has been selected to ensure that the progeny phage genomes which are packaged have minimum DNA damage. Since other viruses which infect bacterial, animal and plant cells show a Luria–Latarjet effect similar to that in phage T4, the conclusions from this study may have wide applicability.

1. Introduction

DNA repair mechanisms are ubiquitous among living organisms. Viruses either encode their own DNA repair enzymes or use those made by their hosts. Luria & Latarjet (1947) carried out one of the earliest studies of DNA repair, although the result they described, later designated the Luria–Latarjet (LL) effect, was not interpreted in terms of DNA repair at the time of the study.

Luria and Latarjet observed that during the course of infection, the resistance of bacteriophage (phage) T2 to ultraviolet light irradiation (UV) increased throughout the first part of the infection and then decreased during the latter part. This was attributed to changes in the phage structure during infection, specifically to the build-up and subsequent dissipation of a UV-absorbing material. Later the same effect was found with phage T4 (Symonds & McCloy, 1958) and phage T5 (Luria & Steiner, 1954). The effect could also be obtained with X-rays (Latarjet, 1948) or disintegration of incorporated ^{32}P (Stent, 1955) as the damaging agent. Further, the decrease in resistance during the latter portion of the infection was demonstrated to be a reflection of the packaging of phage DNA and the onset of cell lysis (Symonds, 1957). At this time in the infection the bulk of the DNA that will contribute to the progeny pool is already packaged into virion particles. The DNA in these particles is metabolically inactive just as that of the free phage is. The remaining resistance of infective centres to UV irradiation at this time depends on being able to: (i) repair damage in unpackaged DNA; (ii) package the repaired DNA; and (iii) complete virion assembly before the cell lyses (Symonds, 1957).

A number of other features of the LL effect have been described: (i) The increase in resistance begins immediately after infection (Benzer, 1952). (ii) Resistance develops in two stages (Symonds & McCloy, 1958). (iii) In multiple infections, the resistance and subsequent decline occur sooner than in single infections (Luria & Latarjet, 1947). These results have been explained by a number of mechanisms including DNA repair (Harm, 1980).

The third feature has been interpreted as indicating a role for multiplicity reactivation (MR) in the LL effect (Symonds & McCloy, 1958; Ritchie & Symonds, 1970). MR is a recombinational repair mechanism which can occur when more than one phage T4 genome is present in a cell (Bernstein & Wallace,

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Table 1. *Bacterial and bacteriophage strains*

Strain	Relevant genotype	Relevant phenotype and gene product function
Bacteria		
S/6/5	wild-type (Epstein *et al.* 1963)	
CR63	*supD* (Epstein *et al.* 1963)	Partially suppresses amber mutations, approximately 28% suppression (Garen 1968)
Bacteriophage		
T4D	wild-type (Epstein *et al.* 1963)	
*am*A453	*32* (Berger, Warren & Fry, 1969)	DNA replication, recombination and recombinational repair deficient; gene product is a single strand DNA-binding protein Bernstein & Wallace, 1983; Kodadek, 1990)
*am*N91	*37* (Epstein *et al.* 1963)	Gene encodes distal portion of the tail fibre (Mosig & Eiserling, 1988)
*am*N116	*39* (Mufti & Bernstein, 1974)	DNA replication deficient (*E. coli* gyrase can substitute), deficient in MR but not in PRRR; gene encodes one of three subunits of DNA topoisomerase (McCarthy, 1979; Miskimins *et al.* 1982)
*am*NO5	*41* (unpublished data)	DNA replication and recombinational repair deficient; gene product is one of two subunits of the RNA primase/helicase (Mosig & Eiserling, 1988; Bernstein & Wallace, 1983)
*am*NG37	*46* (unpublished data)	DNA replication, recombination and recombinational repair deficient; with gene *47*, encodes a DNA endonuclease (Prashad & Hosoda, 1972; Warner & Snustad, 1983)
*am*NO11	*47* (Minner & Bernstein, 1976)	Same as *am*NG37 (gene *46*)
v	*denV* (Harm, 1963)	Excision repair deficient; gene product has glycosylase and AP endonuclease activities which excise pyrimidine dimers from DNA (Friedberg, 1985)
w_m	*uvsW* (Hamlett & Berger, 1975)	Recombination and recombinational repair deficient; gene product function is unknown but it may regulate initiation of DNA replication (Derr & Kreuzer, 1990)
x (*px*)	*uvsX* (Harm, 1963)	Recombination and recombinational repair deficient; gene product catalyses homologous pairing and strand exchange (Yonesaki & Minagawa, 1985)
y_m	*uvsY* (Hamlett & Berger, 1975)	Recombination and recombinational repair deficient; gene product acts as a *uvsX* accessory protein stabilizing pre-synaptic complexes (Minagawa *et al.* 1988; Kodadek *et al.* 1989)

1983). Phage T4 mutants which have reduced levels of MR also have decreased levels of LL resistance (Symonds, Heindl & White, 1973). However, MR cannot be the sole mechanism behind the LL effect since a significant amount of LL resistance to damage develops during the early portion of the infection before any DNA synthesis has begun and only one genome is present (Pratt, Stent & Harriman, 1961; Ritchie & Symonds, 1970).

While it is clear that DNA repair must be involved in the LL effect, the specific DNA repair mechanism(s) employed have not been identified. A role for some type of recombinational repair is indicated (Symonds *et al.* 1973). Only recently, however, have the specific functions of many phage DNA repair genes been identified (see Mosig & Eiserling, 1988, for a review) allowing investigation of individual repair mechanisms.

In this paper, I study the effect of mutations in nine DNA repair genes on the LL effect with phage T4. These include genes involved in excision repair, post-replication-recombinational-repair (PRRR) and multiplicity reactivation (MR).

2. Materials and methods

(i) *Strains*

Table 1 lists the bacterial and phage strains used in this study. All strains were from the Bernstein stock collection and have been previously characterized by this laboratory or others (see Table 1 for references). High titre stocks were grown using the bottle lysate method (Adams, 1959). *E. coli* S/6/5 was used as the host in LL experiments with wild-type T4D and the phage mutants *am*N116 (gene 39), *v*, w_m, *x* and y_m. Stocks of these phage were also grown on *E. coli* S/6/5 except for *am*N116 which was grown on *E. coli* CR63. All other phage mutants were grown on *E. coli* CR63 in LL experiments and to produce stocks. Since the amber mutants are defective in essential phage functions it was not possible to test their influence on the LL effect under completely restrictive conditions (*i.e.* in a su^- host).

(ii) *Production of infected complexes and survival curves*

E. coli cells were grown to a concentration of approximately 1×10^8 ml^{-1} in 200 ml Hershey broth

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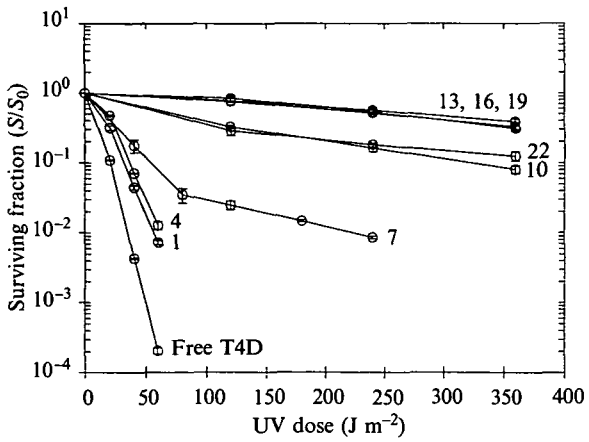

Fig. 1. Development of LL resistance by phage T4D infecting *E. coli* S/6/5. Each curve represents the survival of phage/cell complexes sampled at different times after infection was begun by the addition of 2× Hershey broth. The number next to the curve indicates the time after infection in minutes when the sample was taken for UV irradiation. Surviving fraction is calculated as the viable count at a given dose of UV divided by the viable count at 0 J m^{-2} UV. Each point represents the mean of three experiments and error bars were calculated as the standard error of the mean. This is true for all subsequent figures showing survival curves.

(Steinberg & Edgar, 1962) at 37 °C. The cells were then recovered by centrifugation and washed twice with M9 salts solution (6 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4Cl per litre ddH_2O, pH 6·8) (modified from Adams, 1959). The cells were resuspended in a final volume of 25 ml M9 salts solution. Phage were added to an aliquot of these concentrated log phase cells at a multiplicity of infection of 5×10^{-3} and the phage/cell mixtures were allowed to incubate for a sufficient time (10–15 min) at 37 °C for 95% of the phage to absorb. Under these conditions phage can infect a cell but, because the cells are starved, the phage cannot begin the infectious cycle (Benzer, 1952). At the end of the adsorption period, an equal volume of 2× Hershey broth was added, starting the infectious cycle. Thus the infection of all cells was synchronized. At various times aliquots of the culture were diluted 100-fold into ice-cold M9 salts solution. The diluted, cooled infected complexes are stable for several hours (Benzer, 1952).

To prevent photoreactivation, the remainder of the procedure was done in a darkened room under dim yellow light. Aliquots of the phage/cell complexes were titred using the soft agar overlay method (Adams, 1959). The remaining phage/cell complexes were then UV irradiated at a rate of 1 J m^{-2} s^{-1} using a General Electric G8T5 germicidal lamp (maximum output at 260–300 nm). At various times during irradiation samples were taken and plated to measure survivors. The plates were incubated at 37 °C overnight.

Plaques were counted the next day. Surviving fractions were calculated as the titre of the survivors at a given UV dose divided by the titre of the same free phage or phage/cell complexes at the zero dose. All experiments were performed in triplicate and the results obtained at each dose of UV were averaged. Best fit curves were determined using the GRAPH computer program (MicroMath Scientific Software, Salt Lake City, Utah). Confidence limits were calculated using the method of Miller & Freund (1985).

3. Results

(i) *Development of LL resistance by wild-type phage T4*

In Fig.1 the surviving fraction of infective centres (phage infected cells) is plotted against UV dose for samples taken at various times after infection. Thus this figure shows the development of LL resistance during the course of infection by phage T4D. Some increase in UV resistance can be seen to occur after only 1 min of infection. The level of resistance is about the same 4 min after infection. At 7 min the survival curve has both decreased in slope and become biphasic indicating a mixed population of infective centres with higher levels of resistance. One sub-population is relatively more sensitive to UV as indicated by the initially steeper slope of the survival curve. The other sub-population is much more resistant to UV; its slope is similar to those seen later in the infection.

At 10 min after infection about 70–80% of the population of infective centres (as indicated by extrapolating the portion of the curve with the smallest slope back to the ordinate) have achieved maximal resistance. By 13 min, UV resistance has reached its maximum value for the entire population. UV resistance remains unchanged at 16 and 19 min. At 22 min, about 40–50% of the population continues to show maximum resistance while the remainder of the population is less resistant.

This figure shows, in agreement with the results of others (Latarjet, 1948; Pratt, Stent & Harriman, 1961), that there are three stages to the LL effect. The first stage develops immediately after infection and represents a modest increase in resistance over that of the free phage (approximately 2-fold based on the slope of the inactivation curves). By 7 min after infection about 7% of the infective centres have entered the second stage of resistance. From 13–19 min the maximum level of resistance is achieved by the entire population. The decrease in resistance observed at 22 min signals the start of the third stage of LL resistance which, as described earlier, results from the packaging of phage DNA.

In order to identify the mechanisms involved in the first two stages of LL resistance, phage T4 mutants defective in genes involved in excision repair, PRRR and MR were tested for their ability to develop LL resistance.

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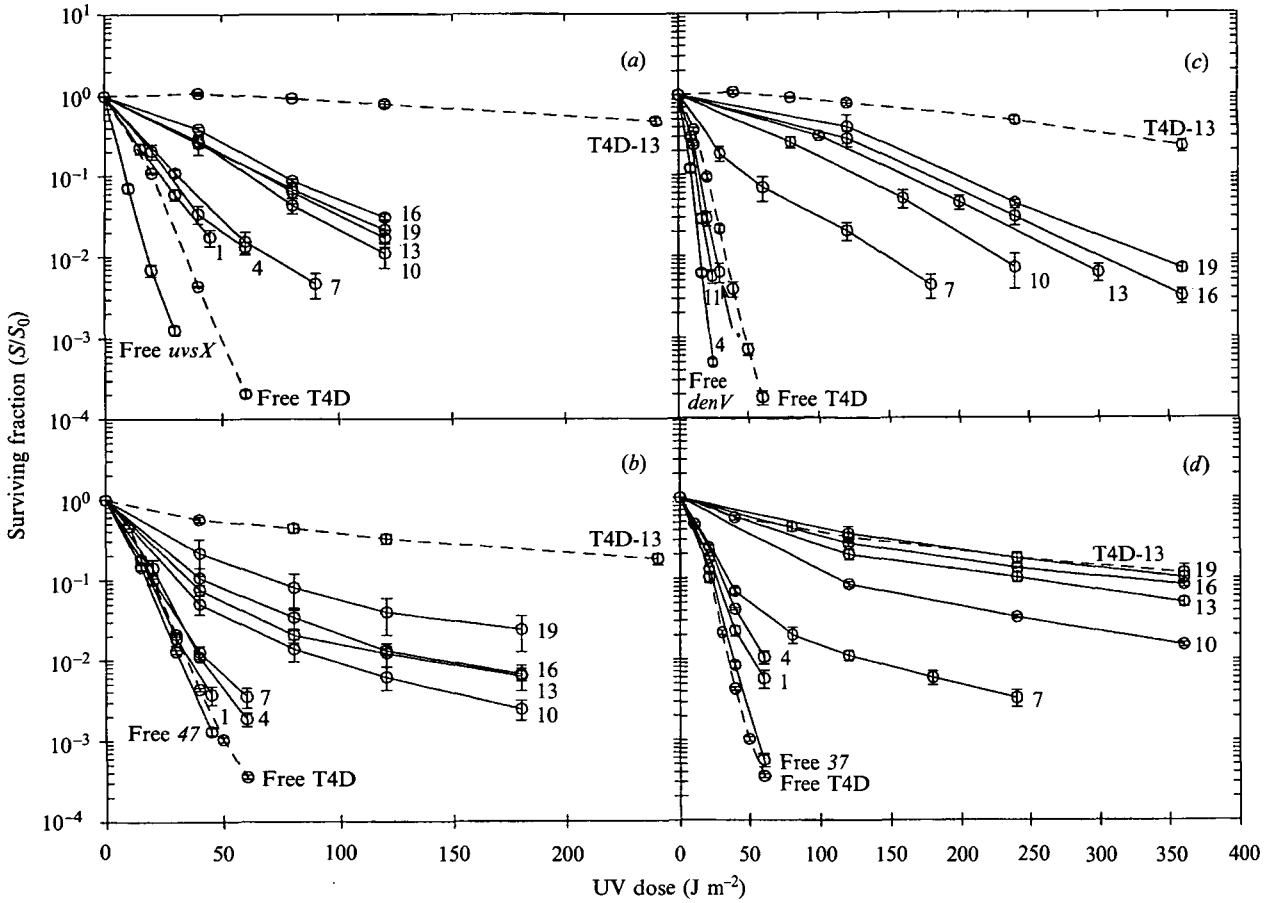

Fig. 2. Development of LL resistance by various phage T4 mutant strains. (*a*). *uvsX* mutant survival. (*b*) Gene *47* mutant survival. (*c*) *denV* mutant survival. (*d*) Gene *37* mutant survival. UV dose scales are chosen to show the maximum detail for each set of curves. Times when samples were taken for subsequent irradiation are indicated as in Fig. 1. Wild-type T4D curves (broken lines) are taken from Fig. 3*a* for (*a*) and (*c*). For (*b*) and (*d*) they are taken from the analogous survival curves of T4D infecting *E. coli* CR63 (data not shown).

(ii) *Development of LL resistance by mutant phage T4*

The development of LL resistance by a phage T4 *uvsX* mutant is shown in Fig. 2*a*. LL resistance appears at 1 min after infection just as in wild-type phage T4D infections. As the *uvsX*$^-$ infection continues, resistance develops in a patten similar to that of wild-type infections. However, the maximum resistance reached is much lower than that of wild-type (approximately 27%). This suggests that repair of damage to the maximally resistant complexes depends to a large extent on gp uvsX-mediated recombinational repair.

Fig. 2*b* shows the development of LL resistance by a phage T4 amber mutant defective in gene *47* when infecting *E. coli* CR63 which carries an amber suppressor that allows partial expression of gene *47* activity. [Development of LL resistance by wild-type phage T4D infecting *E. coli* CR63 was also determined and is nearly identical to that seen in Fig. 1 where *E. coli* S/6/5 is the host except for a delay in the decrease in resistance until 31 min after infection (data not shown).] Unlike the phage *uvsX*$^-$ mutant or T4D, the *47*$^-$ mutant does not develop much resistance during the initial portion of the infection.

A portion of the infective complexes develops resistance at 10 min and the resistance of this portion increases at 13 min. Although not as clearly defined as with T4D at 7 min after infection, the *47*$^-$ mutant's survival curves at 10, 13, 16 and 19 min are also biphasic. As described above, this indicates a mixed population, with differing levels of resistance. The portion of infective centres in the most resistant sub-population increases during the course of the infection. This increase is probably due to increasing levels of suppression of the amber mutation (see also Fig. 3*d* below). At no point however does the resistance ever become as great as that of phage T4D at 13 min.

Development of LL resistance by a phage T4 *denV*$^-$ mutant is shown in Fig. 2*c*. There is almost no development of LL resistance at 1 or 4 min after infection. By 7 min of infection there is a dramatic increase in resistance. Although it is not as well defined in these curves as for phage T4D, there appears to be a biphasic curve at 7 min indicating a mixed population of resistant and sensitive infective centres. The LL resistance continues to increase at 10, 13, 16 and 19 min but never achieves the levels seen in the wild-type infection.

A phage amber mutant defective in gene *37* was

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tested as a negative control for these LL experiments. This gene has no role in phage T4 DNA metabolism or repair. Fig. 2*d* shows the development of LL resistance by a gene *37* mutant infecting *E. coli* CR63. The mutant develops LL resistance in a manner very similar to wild-type phage T4D infecting *E. coli* CR63. There is a slight delay in reaching the maximum survival level attained with wild-type T4, but this delay is within the range of variation with this system. This indicates that the changes seen with the other mutants are due to the specific nature of the mutations, rather than to a general effect that any mutation might have.

(iii) *Effect of various mutations in phage T4 on LL resistance*

The data in Fig. 2 suggest that the LL effect is a reflection of both excision repair and recombinational repair. Furthermore, the change from the first stage (a small increase in resistance to UV) to the second stage (a larger increase in resistance to UV) occurs consistently between 4 and 10 min after infection regardless of which DNA repair pathways are impaired. Based on the time–dose studies presented above it should be possible to determine a gene's involvement in the LL effect by measuring the resistance at selected times of each stage rather than throughout the infection. Fig. 3 shows the results of these experiments.

Fig. 3(*a*, *b*) shows the survival curves for the 1-min complexes of mutant phage (as well as T4D) infecting *E. coli* S/6/5 and CR63, respectively, while Fig. 3(*c*, *d*) shows the corresponding survival curves for the 13-min complexes. The rate of inactivation (defined by the slope of a best fit line) of the wild-type 1-min infected complexes is half that of the wild-type free phage. For the wild-type 13-min complexes the inactivation rate is 22-fold less than that of the wild-type free phage, *i.e.* the resistance is 22 times greater. Thus, while the first stage of the LL effect endows the infected complex with a significant increase in resistance, the second stage is much more robust.

Fig. 3*a* compares LL resistance between one minute complexes of phage T4D and phage with mutations in genes *39*, *uvsW*, *uvsX*, *uvsY* and *denV*. The gene *39* mutant has an initial decrease in resistance, although the overall slope of the survival curve is not significantly different from that of T4D. This implies that the phage-encoded topoisomerase is not required for the first stage of LL resistance.

The products of genes uvsW, uvsX and uvsY are all required for efficient recombination. Fig. 3*a* shows that all three of these mutants have a decrease in first stage LL resistance, suggesting that recombinational repair is involved in repairing damage which occurs at this time. [It is important to keep in mind that the repair does not occur at this time. Both forms of recombinational repair (PRRR and MR) cannot function until after DNA replication begins and there are two copies of the DNA segment to be repaired. In fact the enzymes needed for excision repair are not present this early in the infection either (see below). However the decrease in the resistance seen at this time indicates that the mutated gene causes a decrease in the repair pathway(s) that will repair damage that is formed at this time.]

Finally, Fig. 3*a* shows the survival curve for the *denV* mutant 1-min complexes. It confirms the results shown in Fig. 2*c*. Since the *denV* gene product is expressed early in infection, these results suggest a role for this gene (and excision repair) in the first stage of the LL effect.

Fig. 3*b* presents the comparison of LL resistance between 1-min complexes of T4D and phage with mutations in genes *32*, *41*, *46* and *47*. It shows that there may be a slight decrease in the resistance of the 1-min complexes of the 32^- mutant relative to T4D. There is a larger decrease in first-stage LL resistance for the gene *41* mutant indicating a role for this gene in this stage of LL resistance.

Fig. 3*b* shows that the gene *46* mutant does not have decreased first-stage LL resistance while the gene *47* mutant does have a significant decrease in first-stage resistance. Since *am* mutants defective in gene *46* ordinarily act similarly to gene *47 am* mutants in other assays of function this result is somewhat unusual. The difference may be due to unequal levels of amber suppression by the *E. coli* CR63 host.

Fig. 3*c* compares LL resistance between 13-min complexes of phage T4D and phage with mutations in genes *39*, *uvsW*, *uvsX*, *uvsY* and *denV*. The gene 39^- mutant, which had no overall effect on the LL resistance of the 1-min complexes, does exhibit a decrease in the resistance of the 13-min complexes. As the phage topoisomerase is required for MR but not for PRRR (Bernstein & Wallace, 1983) this would imply that the recombinational repair utilized in the first stage of the LL effect is PRRR while MR is only involved in the second stage (see Discussion).

Phage harbouring mutations in genes *uvsW*, *uvsX* and *uvsY* all show a severe decrease in the LL resistance of their 13-min complexes. This indicates a strong role for recombinational repair in the second stage of LL resistance. The $denV^-$ mutant phage, which had the greatest decrease in resistance of the 1-min complexes, has the least decrease in resistance for the 13-min complexes. This difference implies that while excision repair has a role in both the first and second stages of LL resistance, its relative contribution is greater in the first stage than in the second stage (see Discussion).

Fig. 3*d* shows the comparison of LL resistance between 13-min complexes of phage T4D and phage with mutations in genes *32*, *41*, *46* and *47*. The resistance of the 13-min complexes of the 32^- is decreased approximately two-fold relative to T4D

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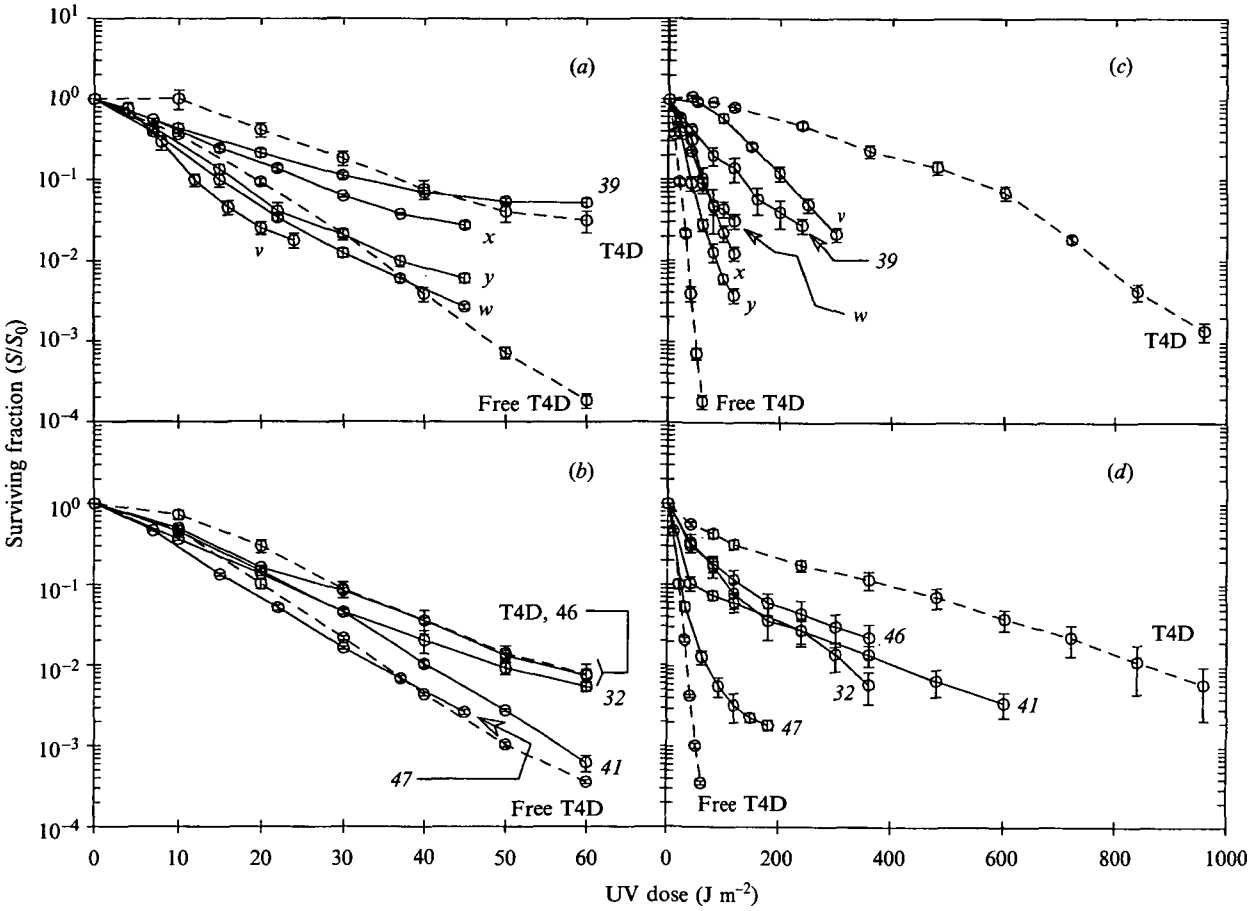

Fig. 3. Comparison of phage T4 gene products in Luria–Latarjet resistance to UV irradiation. (*a*) Comparison of LL resistance of 1-min complexes with *E. coli* S/6/5 as the host. (*b*) Comparison of LL resistance of 1-min complexes with *E. coli* CR63 as the host. (*c*) Comparison of LL resistance of 13-min complexes with *E. coli* S/6/5 as the host. (*d*) Comparison of LL resistance of 13-min complexes with *E. coli* CR63 as the host.

indicating that the gene 32 protein is involved in repair in this later stage of the LL effect.

Interestingly, the remaining three mutant phage have 13-min complex survival curves which are somewhat biphasic. In the *41*$^-$ phage survival curve there are two distinct populations as was seen with T4D at 7 min after infection (Fig. 1). As mentioned earlier, this type of biphasic curve is indicative of a mixed population of infected complexes with two levels of resistance. Extrapolating the higher resistance portion of curve to the ordinate indicates that about 10% of the gene *41*$^-$ mutant population is maximally resistant at this time. For the gene *46* and *47* mutant phage about 17 and 1·5%, respectively, of the population is maximally resistant in the 13-min complexes. In contrast to the gene *41* mutant phage, the gene *46* and *47* mutant phage 13-min survival curves do not show the sharp transition indicating two distinct populations. Instead there is a gradual change in the slope of the survival curves indicating a mixed population of infected complexes with a range of different resistance levels.

Just as was seen with the 1-min complexes (Fig. 3*b*) the overall LL resistance of the *47*$^-$ mutant phage 13-min complexes were more strongly decreased than that of the *46*$^-$ mutant phage complexes. However, the reduction in second-stage resistance in both strains is consistent with the idea that recombinational repair is largely responsible for this resistance.

One final observation is that with one exception (*denV* 13-min complexes) all of the mutants were more sensitive to the lowest doses of UV than T4D. At low doses of UV, T4D survival curves have a shoulder indicating that the repair mechanisms can tolerate some amount of damage before becoming saturated (Bernstein, 1981). While it is common for the shoulder to be eliminated in the survival curves of phage with mutations in DNA repair genes involved in MR, it is unclear why this shoulder should be lost in cases (such as the gene *39* mutant's 1-min complexes) where the overall rate of resistance does not differ from that of T4D.

4. Discussion

(i) *Summary of genes involved in the LL effect*

LL resistance develops in two stages. Based on the timing of the transition from the first to the second

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Table 2. *Summary of phage T4 gene involvement in the LL effect*

Gene	Function	Repair pathway*	Pre-replication	Post-replication
denV	AP endonuclease	Excision, PRRR, MR	+ +	+
uvsW	Unknown	PRRR, MR	+ +	+ +
uvsX	Strand pairing, exchange	PRRR, MR	+	+ +
uvsY	*uvsX* accessory protein	PRRR, MR	+ +	+ +
32	Single strand DNA binding	Excision, PRRR, MR	±	+
39	Topoisomerase	MR	−	+
41	Primase	PRRR, MR	+ +	+(I)
46	Exonuclease	PRRR, MR	−	+(I)
47	Exonuclease	PRRR, MR	+ +	+ +(I)
37	Tail fibre	None	−	−

' + + ' signifies that a gene is required for the indicated stage of LL resistance. ' + ' indicates that a gene is partially involved. ' ± ' indicates a gene that is slightly involved. ' − ' indicates that a gene is not involved. 'I' indicates a gene is required to initiate this stage.

* This column lists which of the three major repair pathways are deficient in mutants defective in the indicated genes.

stage it appears that the completion of the first round of replication could be the key event responsible for this transition. For wild-type phage, DNA replication begins about 5 min after infection (Mosig & Eiserling, 1988). Two minutes is more than sufficient for some of the phage to complete the first round of replication (McCarthy *et al.* 1976). Thus the biphasic survival curve seen at 7 min after infection by wild-type phage T4 (Fig. 1) probably represents a mix of infective centres which have completed the first round of replication (second stage, more resistant) and others which have not (first stage, less resistant). The results of the experiments with the gene *41* (primase) mutant also support this conclusion. The survival curve of the 13-min complexes of the gene *41* mutant (Fig. 3*d*) is biphasic as is the survival curve of the 7-min complexes of wild-type phage T4 (Fig. 1). It is likely that for the gene *41* mutant, the reduced levels of primase (which is required for DNA replication) could prevent many of the infected complexes from completing the first round of replication at 13 min after infection. I will therefore be describing these two stages as the pre-replicative (first stage) and post-replicative (second stage) stages.

Table 2 contains a summary of the results of the mutant studies. It shows that the products of the denV, uvsW, uvsY, 41 and 47 genes are strongly required for resistance to DNA damages introduced during the pre-replicative stage and that the uvsX and (perhaps) 32 gene products are also involved. The gene 39 and 46 proteins may have no role in this resistance. [Alternatively, it may be that the products of these two mutant genes are sufficiently active due to suppression by the host to allow normal function.] For resistance to DNA damages introduced during the post-replicative stage, the products of genes uvsW, uvsX, uvsY and 47 are strongly required; the denV, 32, 39, 41 and 46 proteins are also involved.

(ii) *Repair pathways contributing to the LL effect*

Phage T4 is thought to have five DNA repair pathways which are able to overcome DNA damage – photoreactivation, error-prone repair, excision repair, post-replication-recombinational-repair (PRRR) and multiplicity reactivation (MR) (Bernstein & Wallace, 1983). [Two other repair pathways – replication repair and methylation repair have also been described. They were identified on the basis of particular mutations in genes *32* and *41* which reduced resistance to DNA damage and were epistatic to both an excision repair mutant (*denV*) and a recombinational repair mutant (*uvsX*). However, as noted by Wachsman & Drake (1987), these epistasis tests indicate that genes act in separate pathways only if the individual mutations completely block their particular pathway. Subsequently, a lethal mutation in the *uvsX* gene has been noted (Selick *et al.* 1987). This means that all previous known mutations of *uvsX* including those used in the identification of replication repair and methylation repair were somewhat leaky. Therefore the mutations assigned to these pathways most likely cause a reduction in recombinational repair as other mutations in genes *32* and *41* do.]

Photoreactivation, which is host-encoded rather than phage-encoded, was prevented in these experiments by keeping the infected complexes in dim light. Thus photoreactivation is not relevant to this study. Error prone repair is likely a mutation-inducing damage bypass mechanism rather than a true repair mechanism (Friedberg, 1985). Strains deficient in error-prone repair do not have an increased sensitivity

to UV irradiation (Bernstein & Wallace, 1983) indicating that it repairs little if any DNA damage. Thus it is unlikely that error prone repair would significantly contribute to LL resistance.

The three remaining repair pathways – excision repair, PRRR and MR – represent substantially all the repair capacity available to phage T4 under the conditions in this study in which the LL effect develops. The results shown in Fig. 3 implicate all three in LL resistance.

In this study, excision repair and PRRR can operate on DNA damage formed throughout the infection while MR can only act on damage which occurs after there is more than one copy of the phage genome present, *i.e.* after the first round of replication is completed. This suggests that most of the increase in LL resistance in the post-replicative stage is due to MR. [Strictly speaking, this repair is not MR since MR is defined as the recombinational repair observed in multiply infected complexes. Given the results summarized in Table 2, it seems likely that MR is mechanistically identical to this recombinational repair.]

5. Conclusions

Phage T4 has a life-cycle which is dependent on recombination for both DNA replication and, as shown in this study, much of its DNA repair capacity. Thus the LL effect can be seen as a consequence of the phage life-cycle rather than as an independent phenomenon. During the latter half of the infectious cycle large amounts of DNA are being made. About 15 min after infection packaging of newly synthesized DNA begins. Throughout infection in the colon, phage DNA is exposed to damaging agents including bile acids and fecapentaenes which are abundant in the colon (Cheah, 1990) and possibly oxygen from the capillary-rich intestinal lining (Savage, 1977). It would be advantageous for a phage to produce progeny that are as damage free as possible. Thus it is reasonable that phage T4 would have a robust repair capacity just before progeny are packaged – in effect clearing as much damage as possible from its 'germ line'.

LL resistance has been demonstrated in other coliphage (T2 and T5) (Luria & Latarjet, 1947; Luria & Steiner, 1954) as well as the mammalian herpes simplex virus (Lytle & Hester, 1976) and the cyanophage AS-1 which infects the blue-green alga *Anacystis nidulans* (Amla, 1979). Furthermore, if MR is considered to be indicative of LL recombinational repair then many other examples can be identified. Bernstein, Hopf & Michod (1987) describe 14 viruses which undergo MR. These include the *E. coli* viruses T1, T2, T4, T5, T6, λ and ϕX174; the *S. typhimurium* phage Vi; and the mammalian viruses herpes simplex, adenovirus, SV40, reovirus, influenza virus and poxvirus (the last two having an RNA genome). These viruses ordinarily go through a period during their infectious cycle when multiple copies of the genome are present (Fenner *et al.* 1974; Kornberg, 1980). This period of potential interaction and the ability to undergo MR satisfies the conditions needed for LL recombinational repair. The ability of viruses whose hosts are bacteria, animals and plants, with both DNA and RNA genomes, to utilize the LL effect suggests that the LL effect may represent a general strategy used by a wide variety of viruses to deal with genome damage.

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