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Indirect Ultraviolet-Reactivation of Phage λ

(mutagenesis/repair of DNA/bacterial conjugation/*Escherichia coli*)

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ABSTRACT When an F⁻ recipient *Escherichia coli* K12 bacterium receives Hfr or F-*lac*⁺ DNA from an ultraviolet-irradiated donor, its capacity to promote DNA repair and mutagenesis of ultraviolet-damaged phage λ is substantially increased.

We call this phenomenon indirect ultraviolet-reactivation, since its features are essentially the same as those of ultraviolet-reactivation; this repair process occurs in pyrimidine dimer excision-deficient strains and produces clear plaque mutations of the restored phage. Moreover, this process is similar to indirect ultraviolet-induction of prophage λ, since it is promoted by conjugation. However, contrarily to indirect induction, it is produced by Hfr donors and occurs in recipients restricting the incoming ultraviolet-damaged donor DNA.

The occurrence of indirect ultraviolet-reactivation provides evidence for the existence in *E. coli* of an inducible error-prone mechanism for the repair of DNA.

The survival of ultraviolet (UV)-irradiated phage λ is increased when the host cell has been exposed to a low UV dose before infection. This phenomenon, known as UV-reactivation (1), is accompanied by a high rate of mutation (UV-mutagenesis) of the reactivated phage (2).

UV-reactivation as well as UV-mutagenesis of phage λ require for their occurrence the bacterial *recA* and *lex* gene functions (3, 4). This requirement is also shared by UV-induction of prophage λ (5–8) as well as by UV-mutagenesis of *Escherichia coli* K12 (9, 10). This has led Defais *et al.* (4) to suggest that common pathways might be involved in the above-mentioned phenomena.

Since lysogenic induction in a lysogenic recipient cell can be obtained "indirectly" by conjugation with a UV-irradiated F⁺ (11, 12) or F′ donor (13), if the above hypothesis is correct, one should be able to produce UV-reactivation of phage λ as well as its mutagenesis in a recipient host cell mated with a UV-damaged F′ donor. This paper shows that this is indeed the case. Therefore, we call the repair process described here indirect UV-reactivation. Its conditions of occurrence have been compared to those of indirect UV-induction.

The occurrence of indirect UV-reactivation provides evidence for the existence in *E. coli* of an inducible error-prone DNA repair mechanism.

MATERIALS AND METHODS

Bacteria and Bacteriophages. Bacteria used are described in Table 1. Symbols conform to the recommendations of Demerec *et al.* (14). All F⁻ strains were derived from GY 158: F⁻ *thr-4 leu-8 thi-1 pyrF49 dra-34 thyA34 lacY1 tonA101 supE*, except AB 2480. F_2-*lac*⁺ was that from Jacob and Adelberg. Bacteria were rendered resistant to streptomycin (Str^R) or spectinomycin (Spc^R) by transduction with phage P1 grown on GY 2049 *str* or on GY 2339 *spc*. Bacteria resistant to phage λ (Lam^R) were selected against λvir as white colonies on eosin–methylene blue–maltose plates. Bacteria resistant to phage P1 (Pon^R) were obtained as resistant to phage 62c (this paper). Phage λpapa was used throughout for UV-reactivation experiments. In restricting crosses (see Table 2), it was modified by previous growth on P1CM (15) lysogens.

Indirect UV-Reactivation Experiments were performed as follows: logarithmic phase cultures of donor and recipient bacteria at 2×10^8 cells per ml were centrifuged at 4°, and the pellets were resuspended in cold 0.01 M $MgSO_4$ solution. Aliquots of the suspensions were exposed to UV light; when only one UV dose was given to the donor it was 1600 ergs/mm². Irradiated and nonirradiated samples were centrifuged and the pellets resuspended in broth, donor bacteria being concentrated 5-fold. The survival of donor and recipient bacteria was then measured. Bacterial conjugation was carried out by mixing equal volumes of donor and recipient cultures, which were immediately distributed into small tubes and incubated in the dark for 25 min at 37° with gentle agitation. Then, dilutions of UV-irradiated (or not) phage λ were added at multiplicities of infection of less than 10^{-2} to the small tubes in which phage adsorption took place for 25 min at 37°. The suspensions were then plated with AB 2480 indicator bacteria. In the mating mixture, the strain that was not host for phage λ was always Lam^R. Plaques were counted after overnight incubation at 37°. The efficiency of conjugation was determined at 25 min, before the addition of phage to the mixture, by measuring the number of recipients having acquired the *lac*⁺ gene from the nonirradiated donor.

Direct UV-Reactivation Experiments were performed under conditions similar to those described above, except that donors were not added, since their addition gave identical results in control experiments. Host bacteria were exposed to 800 or 50 ergs/mm², according to whether or not they were able to excise pyrimidine dimers (Uvr⁺ or Uvr⁻).

UV-Reactivation Factor. UV-reactivation of λ was always determined at about 10^{-2} survival of the phage plated either on Uvr⁺ or Uvr⁻ bacteria; exposure doses of the phage to UV light were 1500 and 450 ergs/mm², respectively. The reactivation factor was estimated as the ratio of λ survival on a host carrying UV-damaged bacterial DNA over that on a host without UV-damage to the bacterial DNA. For the sake of

Abbreviation: UV, ultraviolet.

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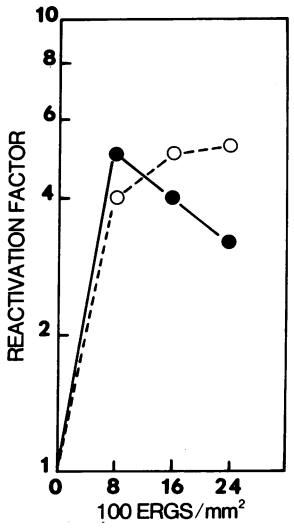

FIG. 1. Indirect UV-reactivation was determined on GY 688 mated with UV-irradiated Hfr GY 1151 (○- - -○) and with F-*lac*$^+$ GY 854 (●——●). The titer of phage λ used was 3 × 10^6/ml; its survival was precisely 10^{-2} in this experiment. The percentages of recipients having acquired Lac$^+$ were 59% (cross GY 854 × GY 688); 17% (cross GY 1151 × GY 688). The reactivation factor is plotted on the ordinate against the UV dose given to the donor, on the abscissa.

brevity, in the tables, phage and bacteria that were exposed to UV light have been labeled with an asterisk.

Miscellaneous. Media and experiments on indirect induction of prophage λ were as published previously (12). UV doses were measured with a Latarjet dosimeter.

RESULTS

Indirect UV-Reactivation of Phage λ. The number of plaques formed by UV-irradiated phage λ on an F$^-$ recipient host cell crossed with an F-*lac*$^+$ or Hfr donor increases when the donor has been exposed to UV light before mating (Fig. 1). However, the reactivation factor under these conditions is 3–5, lower than that found (about 10) on directly UV-irradiated host cells (*direct UV-reactivation*). Such a difference can almost completely be accounted for by the variable efficiency of DNA transfer to the recipient.

The phage reactivation process described here is not an enhanced *host-cell reactivation*, since it is not abolished in an F$^-$ recipient λ host deficient in pyrimidine dimer excision repair (Table 2, Exp. A_1 and A_2). This finding parallels what

TABLE 1. *Bacterial strains*

Strains	Sex	Chromosomal markers used	Origin
GY 688	F$^-$	*lacY1 strA2049*	GY 158
GY 695	HfrH	*uvrB5*	Transductant no. 10 of KML 516 (ref. 16)
GY 701	F$^-$	*lacY1 strA2049 lam-701* (λref)$^+$	GY 688
GY 743	F$^-$	*lacY1 strA2049* (P1CM)$^+$	GY 688
GY 744	F$^-$	*lacY1 strA2049 uvrB5*	GY 695 × GY 688
GY 745	F$^-$	*lacY1 strA2049 uvrB5 pon*	GY 744
GY 746	HfrH	*uvrB5 lam-1151 pon*	GY 1151
GY 854	F$_2$-*lac*$^+$	*uvrB501 lam-854*	(ref 13)
GY 1151	HfrH	*uvrB5 lam-1151*	GY 695
GY 4006		*lacY1 lam-4005 spc-2339*	GY 158
GY 4007	F$_2$-*lac*$^+$	*strA2049*	GY 688 × F$_2$-*lac*$^+$
GY 4010	F$_2$-*lac*$^+$	*lacY1 lam-4005 spc-2339*	GY 4006 × F$_2$-*lac*$^+$
AB 2480	F$^-$	*uvrA recA13 str-1157*	(ref. 30)

TABLE 2. *Phage reactivation experiments*

Exp. no.	Donor	λ Host	Thousand PFU/ml λ	Thousand PFU/ml λ*	λ Survival (%)	Reactivation factor
A_1	GY 854 F-*lac*$^+$	GY 688	1000	3.9	0.4	11.7
	GY 854 F-*lac*$^+$*	GY 688	1000	47	4.7	
	None	GY 688	1000	2.6	0.26	18.4
	None	GY 688*	1000	48	4.8	
A_2	GY 854 F-*lac*$^+$	GY 744 *uvrB*	1000	1.4	0.14	3.8
	GY 854 F-*lac*$^+$*	GY 744 *uvrB*	1000	5.3	0.53	
	None	GY 744 *uvrB*	1000	0.8	0.08	3.1
	None	GY 744 *uvrB**	1000	2.5	0.25	
B_1	GY 4010 F-*lac*$^+$	GY 688	900	10	1.1	3.3
	GY 4010 F-*lac*$^+$*	GY 688	800	30	3.7	
	None	GY 688	800	5	0.6	8.5
	None	GY 688*	700	36	5.1	
B_2	GY 4006 F$^-$	GY 4007 F-*lac*$^+$	800	6	0.75	1
	GY 4006 F$^-$*	GY 4007 F-*lac*$^+$	800	6	0.75	
	None	GY 4007 F-*lac*$^+$	800	3.5	0.4	16
	None	GY 4007 F-*lac*$^+$*	5000	32	6.4	
C_1	GY 746 Hfr	GY 688	5200	87	1.7	4.4
	GY 746 Hfr*	GY 688	6500	490	7.5	
	None	GY 688	8000	80	1	2.6
	None	GY 688*	6500	170	2.6	
C_2	GY 746 Hfr	GY 743 (P1CM)$^+$	4000	92	2.3	2.3
	GY 746 Hfr*	GY 743 (P1CM)$^+$	7000	380	5.4	
	None	GY 743 (P1CM)$^+$	7900	94	1.2	5
	None	GY 743 (P1CM)$^+$*	5900	350	5.9	

* The sign * in this and subsequent tables indicates that bacteria or phage have been UV-irradiated. The percentages of recipients having acquired Lac$^+$ were 72% in A_1, 82% in A_2; 80% in B_1, and 100% in B_2; 27% in C_1 and less than 10^{-4} in the restrictive cross C_2. To avoid its restriction in GY 743, λ.P1CM (λ grown on a P1CM lysogen) was used in experiments C_1 and C_2.

TABLE 3. *Mutagenesis during reactivation*

Donor	λ Host	Thousand PFU/ml λ	Thousand PFU/ml λ*	λ Survival (%)	Reactivation factor	$10^4 \times$ Frequency of mutants
GY 1151 Hfr	GY 688	11,000	110	1	2.9	3.6
GY 1151 Hfr*	GY 688	12,000	350	2.9		13.5
None	GY 688	14,000	105	0.75	9.2	1.7
None	GY 688*	9,000	620	6.9		18

Mutagenesis was scored by the number of clear plaque mutants arising from about 10^5 plaques of λ survivors. Lac$^+$ transfer was 10%.

is found in direct UV-reactivation (4, 16). Yet, bacteria carrying *uvrB5*, used in this series of experiments, appear to be less proficient in UV-reactivation of λ than some other *uvrA*, *uvrB*, or *uvrC* bacteria (16).

Furthermore, a high rate of mutation of λ to clear plaque mutants is observed when UV-irradiated phage λ is reactivated in a recipient crossed with a UV-damaged HfrH donor (Table 3).

The rate of mutation is of the same magnitude as that found in direct UV-reactivation.

Since UV-reactivation can be characterized as (*i*) a repair process that is highly mutagenic for the recovered phage, and (*ii*) taking place in *uvr*$^-$ hosts, we propose to call this repair process *indirect UV-reactivation* by analogy to *indirect UV-induction* of prophage λ (12).

Indirect UV-Reactivation Is Induced by Transferred UV-Damaged DNA. To prove this point and eliminate the possibility that indirect UV-reactivation could be either due to some component released into the medium by the irradiated donor or promoted solely by the cell wall contact, we sought to demonstrate that indirect UV-reactivation is determined by the direction of conjugal transfer. Indeed, only in those λ hosts that were recipients of the UV-damaged transferred DNA, did UV-reactivation of λ take place (Table 2, B_1 and B_2).

It has been demonstrated that conjugal transfer of UV-damaged episomal DNA (13, 17), as well as Hfr DNA (J. George, unpublished), is not diminished after UV-irradiation of the donor bacteria, at least in the range of UV doses used here. This, taken together with the control experiments described just above, suggests that indirect UV-reactivation is induced in a nonirradiated host cell by the introduction of UV-damaged bacterial DNA.

Indirect UV-Reactivation Versus Indirect UV-Induction. It was previously established (12, 13, 18) that indirect UV-induction of prophage λ results from the transfer of an entire UV-damaged *replicon*. Restriction in the recipient lysogen of the incoming DNA abolishes prophage induction (19). In contrast to indirect prophage induction, indirect UV-reactivation can be promoted with equal efficiency by either F′ or Hfr donors as shown in Fig. 1, Table 2, and Table 4.

Moreover, restriction of the incoming UV-damaged donor DNA in the recipient by a factor of 10^4 only slightly decreases the efficiency of indirect UV-reactivation of unrestricted phage λ infecting the same recipient (Table 2, C_1 and C_2). If degradation of the transferred DNA takes place, a very likely occurrence, this does not prevent the appearance of the phenomenon.

A repeated decrease of direct UV-reactivation of λ. P1CM in GY 688 not lysogenic for P1CM was found (Exp. C_1, lines 3 and 4). This observation remains unexplained, although it could be that modification of the phage DNA competes with DNA repair.

DISCUSSION

This paper demonstrates that the introduction of UV-damaged DNA into a host cell provides a means of promoting UV-reactivation of UV-irradiated phage λ. The fact that the efficiency of indirect UV-reactivation under our experimental conditions is less than that produced by direct UV-exposure of the host cell is not at all surprising if one takes into account limiting factors such as the efficiencies of mating and of DNA transmission.

This new kind of phage reactivation has the same essential

TABLE 4. *Promotion of indirect UV-reactivation and indirect prophage induction by F′ and Hfr donors*

	Indirect UV-reactivation in GY 688 F$^-$				Indirect prophage induction in GY 701 F$^-$(λ)$^+$ Lam^R	
Donor	Infective centers (thousand PFU/ml) λ	Infective centers (thousand PFU/ml) λ*	λ Survival (%)	Reactivation factor	Free phages (thousand PFU/ml)	Efficiency of indirect induction
GY 854 F-*lac*	800	3.5	0.4	5	4,300 (A)	B/A = 30
GY 854 F-*lac**	800	16	2		130,000 (B)	
GY 1151 Hfr	800	4.1	0.5	3.4	3,200 (a)	b/a = 2.5
GY 1151 Hfr*	700	12	1.7		8000 (b)	

The host for indirect UV-reactivation of phage λ was GY 688 and the recipient lysogen used for indirect UV induction was GY 701. Indirect UV-reactivation was estimated as described in *Material and Methods*. The technique for obtaining and estimating indirect UV-induction has been fully described in ref. 12. The percentages of recipients which had received Lac$^+$ at 20 min were 55% (cross GY 854 × GY 688); 28% (cross GY 854 × GY 701); 19% (cross GY 1151 × GY 688); 4.6% (cross GY 1151 × GY 701).

features as direct UV-reactivation: it is associated with the mutagenesis of the restored phage and it can be produced in a pyrimidine dimer excision-deficient strain. The question of the involvement of pyrimidine dimer excision in UV-reactivation has been a controversial matter in the past (20–22). Yet, it has been shown that UV-reactivation is not the result of an enhanced dimer excision in Uvr^+ cells (23), and that it takes place in Uvr^- bacteria in which there is no pyrimidine dimer excision (4, 16). We are unable, as yet, to account for the lower efficiency of UV-reactivation (direct or indirect) in bacteria carrying the *uvrB5* mutation. This is in contrast with what was found in other *uvrA*, *uvrB*, and *uvrC* strains (4, 16). An explanation for this difference in behavior must await the elucidation of what the *uvrB* function is.

Mutagenesis of the restored phage is an intrinsic feature of the UV-reactivation process (1). The mutagenic effect was found to be the same whether the F^- recipient had been directly exposed to UV or had received by conjugation a relatively small piece of DNA exposed to an almost equivalent dose. This suggests that introduction by conjugation of UV-damaged DNA has a catalytic effect in inducing an "error-prone" (9) repair process. This is in line with the fact that mutagenesis and repair of the UV-damaged phage can also be produced as a consequence of a thermal shift of *tif* bacteria (24), under which condition no obvious DNA lesions are revealed (25, 24).

Treatments that in lysogens promote lysogenic induction, such as UV-irradiation, thymineless death, or thermal shift of *tif* bacteria, also promote UV-reactivation of phage λ in the non-lysogenic host (26, 24). However, conditions that promote indirect UV-reactivation are not sufficient to promote indirect lysogenic induction of prophage λ. Both phenomena are triggered by pyrimidine dimers carried by the donor DNA transferred to the recipient, but lysogenic induction, in order to be produced, requires their presence on a transmitted replicon (12, 13, 18).

Repair processes such as pyrimidine dimer excision or recombination between phage and host DNAs cannot account for the mechanism of UV-reactivation (4, 16, 27).

We think that the introduction into a host cell of bacterial DNA carrying UV lesions *induces* a repair process less accurate than excision and post-replicative recombination repair (28); we postulate that such a phenomenon implies the existence of a third mechanism of repair of UV-damage. This idea is supported by other studies (29, 31).

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