Repair from UV damage in *E. coli* and *M. luteus*

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A large number of radiation sensitive mutants are known (see Table 1) of *E. coli*, which are phenotypically and genotypically well described. Little is known about the products of the different genes involved in repair. However it has been established that in the case of the cautious Rec mutants, RecB and RecC, and ATP dependent exonuclease is absent (BUTTIN & WRIGHT, 1968).

TABLE 1.

Properties of UV-sensitive mutants of « E. coli »

STRAIN TYPE	Wild type	DIR	HCR	UVR	EXR	ROR	REC	PHR
UV resistance of strain . .	++	+—	——	——	——	++	——	++
X ray resistance of strain .	++	+—	++	++	——	+—	——	++
UV resistance of phage . .	++	++	——	++	++	++	++	++
Photoreactivation	++	++	++	++	++	++	++	——
Chromosomal location of mutation		pur-E	his gal mal-B	his ilv-A	met-A	his thy	phe-A thy	gal

UVR-type mutants, which are unable to repair UV damage in cellular and in phage DNA, are known to be inhibited in the excision process ; no pyrimidine dimers are removed from bacterial DNA during post-irradiation

incubation (SETLOW & CARRIER, 1964; HOWARD-FLANDERS & BOYCE, 1964). Although the excision process itself has been thoroughly investigated the role of the three genotypes involved, *uvrA*, *uvrB* and *uvrC*, is not understood.

One approach to investigate this process is the study of excision repair *in vitro*. As described by RÖRSCH, VAN DER KAMP & ADEMA (1964) the following method can be used: double stranded DNA of phage Φ X174 (RF 1) can be isolated from infected *E. coli* cells and the biological activity of this DNA can be measured before and after irradiation using *E. coli* spheroplasts. Extracts from *M. luteus* are able to restore much of the biological activity of UV irradiated RF-DNA. These are thus believed capable of carrying out those steps in the repair process *in vitro* which are blocked in *E. coli* Hcr⁻ spheroplasts *in vivo* (see Fig. 1).

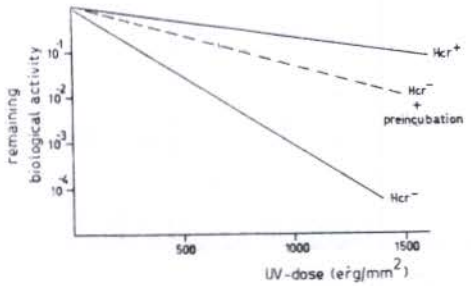

Fig. 1. — Survival curves for UV of Φ X174 RF-DNA. Hcr⁺: assayed on Hcr⁺ spheroplasts. Hcr⁻ +preincubation: DNA incubated with micrococcal extract and subsequently assayed. Hcr⁻: assayed on Hcr⁻ spheroplasts.

Several UV sensitive Hcr⁻ mutants of *M. luteus* — some as sensitive as UV sensitive mutants of *E. coli* — have been isolated (RÖRSCH *et al.*, 1966). Extracts from these mutants however were still capable of repairing UV damage of RF 1-DNA *in vitro*. This is an apparent contradiction with the Hcr⁻ property of the strains. It seems therefore that Hcr⁻ mutants of *M. luteus* are inhibited in a different step of the repair process than the step which is inhibited in Hcr⁻ mutants of *E. coli*.

The discrepancy was even more clearly demonstrated when the mutant ML9 was studied. This mutant was found to be lacking an UV-specific endonuclease. The mutant resembles the mutant isolated by OKUBO *et al.* (1967). Extracts of the mutant ML9 are unable to repair UV damage *in vitro*, but the strain itself is nearly as radiation resistant and Hcr⁺ as the wild type (see Fig. 2 and Fig. 3).

The question arises by what mechanism is UV damage repaired in strain ML9, when this mutant is lacking an UV-specific endonuclease to which a role in the excision repair process is ascribed ? Some information has been obtained by investigating bacterial DNA breakdown which accompanies

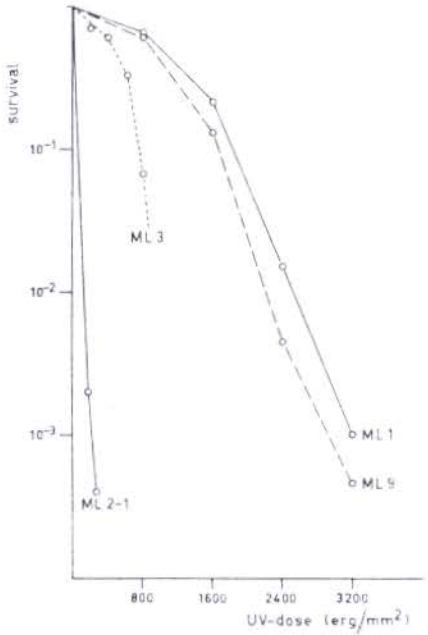

Fig. 2.

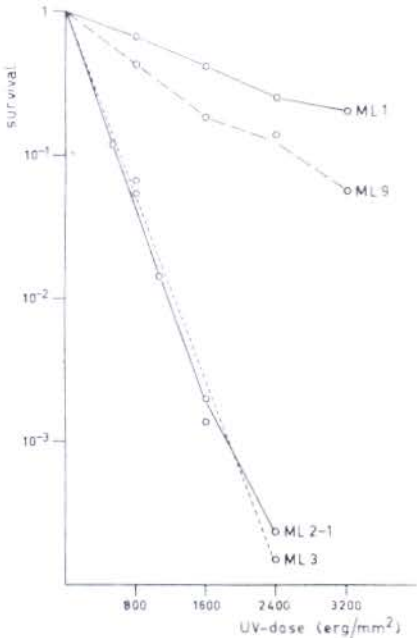

Fig. 3.

Fig. 2. — Survival curves for UV of several *Micrococcus luteus* strains. Exponential grown cells were sonicated to obtain separate cells and irradiated in 0.01 M phosphate buffer pH 7.0.

Fig. 3. — Survival curves for UV of micrococcal phage N5 on various *M. luteus* strains. The strains ML2-1 and ML3 are Her⁻.

excision after UV irradiation. As can be seen in Fig. 4 the post-irradiation degradation of DNA is as extensive in ML9 as in the wild type ML1. However in the case of ML9, degradation begins only after a delay of 60 minutes.

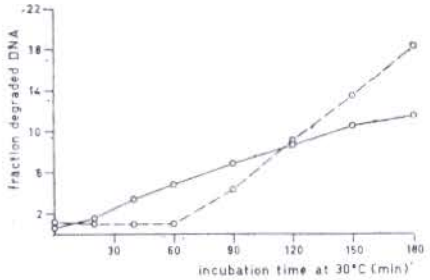

Fig. 4. — Intracellular degradation of bacterial DNA after UV irradiation. Cells were labeled overnight with [³H]-thymidine, harvested in the exponential phase and irradiated (1000 erg/mm²). The fraction degraded DNA is determined by precipitation of intact DNA by trichloroacetic acid. 0——0 ML1 wild type; 0– – –0 ML9 mutant lacking UV specific endonuclease.

The same delay was observed when the introduction of single stranded breaks was studied by alkaline sucrose gradient centrifugation in the wild type strain ML 1 and ML9 (Fig. 5 and Fig. 6). In ML9 the breaks appear later but they are repaired after prolonged incubation.

These results may indicate that the UV-specific endonuclease (incision enzyme) is induced after irradiation. However when extracts of ML9 are prepared 60 or 120 minutes after irradiation still no repair activity *in vitro* can be detected. This leads one to suggest that the repair activity in this mutant is performed by recombination and that breaks are introduced only

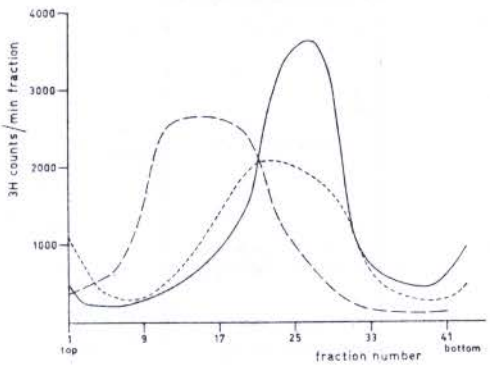

Fig. 5. — Sedimentation of DNA from strain ML1 in alkaline sucrose gradient. Cells were prepared as described under Fig. 4, converted to spheroplasts (RUPP & HOWARD-FLANDERS, 1968) and lysed in alkali. Sedimentation was for 1.5 hr at 40,000 rpm in SV50 rotor in a 5-20 % (w/w) sucrose gradient containing 10^{-3}M EDTA pH 12.1.

after one round of replication (RUPP & HOWARD-FLANDERS, 1968). To test this hypothesis more information must be obtained about DNA replication and recombination in *M. luteus*.

A disadvantage of experiments with *M. luteus* is that genetic analysis of mutations is more difficult than in *E. coli*. On the other hand, since the

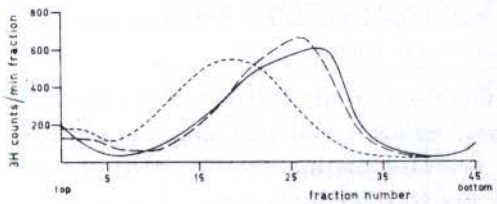

Fig. 6. — Sedimentation of DNA from strain ML9 in alkaline sucrose gradients. For details see Fig. 5.

amount of repair enzymes in *M. luteus* seems to be much greater than in *E. coli*, *M. luteus* is a more favourable organism for biochemical investigation.

Although the biochemical implications of the three mutations *uvrA*, *uvrB*, and *uvrC* in *E. coli* are known the observation that the three genes are localized at different sites on the bacterial chromosome and lead to the same phenotype is not understood. Experiments have been carried out to see if the enzymes coded by *uvrA*, *uvrB* and *uvrC* are also involved in the proliferation of the cell or in recombination.

A strain was constructed with all three *uvr* mutations. Since the UV sensitivity of a strain harbouring two *uvr* mutations cannot be distinguished from a strain with one mutation, no direct method is available to select

for a double or triple mutant. Therefore a different approach outlined in Fig. 7 was used.

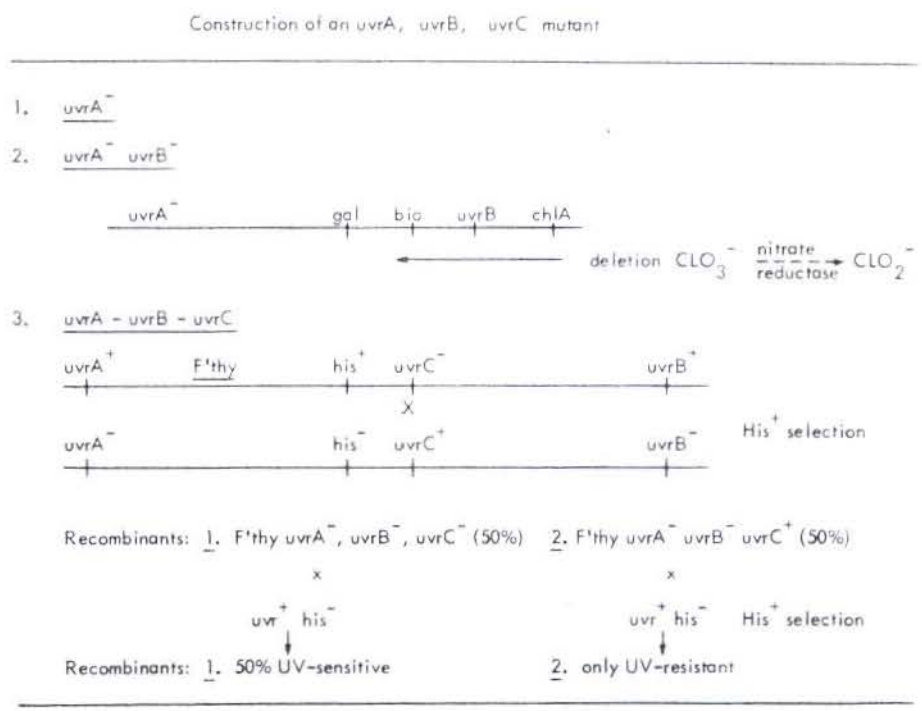

Fig. 7. — Isolation of uvrA-uvrB-uvrC triple mutant. For details see text.

First chlorate mutants were made from an *uvrA* strain. Since the *uvrB* gene lies between *chl* A and *bio* those chlorate resistant mutants obtained in a single mutagenic step which are also biotin requiring must have a *chlA-uvrB-bio* deletion. In this way a *uvrA-uvrB* double mutant was obtained.

Subsequently the double mutant, *uvrA-uvrB*, was mated with a F' *thy uvrC* strain and His$^+$ recombinants were selected. It was observed that 50 % of the recombinants had acquired the *uvrC* mutation. This was found by introduction of a F$^-$ *thy* into the recombinants and subsequently mating with a F$^-$ *uvr*$^+$ *his* strain. If the donor is *uvrC*$^-$ 50 % of the His$^+$ recombinants should be sensitive for UV, if the donor strain is *uvrC*$^+$ all recombinants are expected to be resistant.

It was proved that the *uvrA* and *uvrB* mutations were still present after the introduction of *uvrC* by preparing a Pl lysate on the triple strain followed by transduction and selecting for Met$^+$ (*uvrA*) and Gal$^+$ (*uvrB*) transductants.

The characteristics of the triple mutant were studied. It was found that the triple mutant could proliferate normally. Therefore the three *uvr* gene

products even when all three are absent are not essential for cell proliferation.

The recombination ability of the triple mutant was examined and found to be normal as illustrated in Table 2.

TABLE 2.

Chromosome mobilization by F'thy in various uvr strains

Strains	Number or tyr^+ recombinants (chromosome transfer) / Number or thy^+ recombinants (episome transfer)
uvr^+	3.5×10^{-2}
uvrA	5.0×10^{-2}
uvrB	2.8×10^{-2}
uvrC	3.7×10^{-2}
uvrA uvrB	3.5×10^{-2}
uvrA uvrB uvrC	4.0×10^{-2}
recA	$< 10^{-5}$

TABLE 3.

The effect of uvr mutations on intracodon recombination

Strains	Number of recombinants		$cys^+ trp^+$ / cys^+	Stimulation of recombination factor
	cys^+	$cys^+ trp^+$		
Codons: wild type GGA (asp); A46 GAA (glu); A23 AGA (arg)				
Wild type	2.7×10^7	127	4.7×10^{-6}	1
uvrA	1.9×10^7	328	1.7×10^{-5}	3–4
uvrB	5.7×10^6	183	3.2×10^{-5}	7
uvrC	2.0×10^7	146	7.4×10^{-6}	1–2
Codons: wild tipe GGT (gly); A78 TGT (cys); A58 GAT (asp)				
Wild type	2.3×10^7	148	5.5×10^{-6}	1
uvrA	2.5×10^7	190	7.5×10^{-6}	1–2
uvrB	3.5×10^6	192	4.2×10^{-5}	7–8
uvrC	8.1×10^6	190	2.7×10^{-5}	3–4

A more sophisticated method for the investigating of recombination is to study intracodon recombination. Two pairs of *trpA* mutants (YANOFSKY, 1965-1966) were used to investigate the effect of *uvrA*, *uvrB* and *uvrC* on intracodon recombination. The preliminary results indicate that the single mutations may have a small stimulating effect on specific types of recombination (see Table 3). The results however were obtained by Hfr-F⁻ crosses and not by transduction and have to be repeated also with isogenic strains.

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