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UV resistance of *E. coli* K-12 deficient in cAMP/CRP regulation

Marie-France Puyo, Patrick Calsou and Bernard Salles

Laboratoire de Pharmacologie et Toxicologie Fondamentales CNRS, Toulouse, France

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Summary

Deletion of genes for adenylate cyclase (*Δcya*) or cAMP receptor protein (*Δcrp*) in *E. coli* K-12 confers a phenotype that includes resistance to UV radiation (254 nm). Such mutations lead to UV resistance of *uvr*$^+$, *uvrA*, *lexA* and *recA* strains which could partly be abolished by the addition of cAMP to *Δcya* but not to *Δcrp* strain culture medium. This effect was not related to either inducibility of major DNA repair genes or growth rate of the bacteria. Enhanced survival was also observed for UV-irradiated λ bacteriophage indicating that a repair mechanism of UV lesions was involved in this phenomenon.

In *Escherichia coli*, cyclic AMP receptor protein (CRP, also known as CAP) forms a complex with cyclic AMP (cAMP) synthesized by adenylate cyclase; this complex binds next to numerous promoter sites, stimulating or inhibiting transcription initiation (De Combrugghe et al., 1984). Basically, cAMP exerts its action on catabolic operons and has been shown to reverse the negative effect of glucose on the synthesis of metabolic enzymes (Pastan and Adhya, 1976; Ullmann and Danchin, 1983). However, mutants in genes for either adenylate cyclase (*cya*$^-$) or CRP protein (*crp*$^-$) have a highly pleiotropic phenotype in addition to their inability to catabolize some carbon sources. This phenotype includes non-exhaustively: (i) a decrease in the frequency of lysogeny of λ bacteriophage (Grodzicker et al., 1972); (ii) a negative effect of the induction of the plasmidic *cea-kil* operon which also belongs to the SOS regulon (Nakazawa and Tamada, 1972; Salles et al., 1987); (iii) in *E. coli* B/r strains, a lack of respiration shut-off after DNA-damaging treatment, an SOS function triggered by UV irradiation (Swenson et al., 1978; Swenson and Norton, 1984). In addition, this phenotype is partly reverted to wild type by addition of cAMP to the culture medium, though this is not the case with *crp*$^-$ mutants.

SOS response is induced either directly or indirectly by DNA-damaging treatments such as UV irradiation that lead to the formation of DNA lesions that interfere with the replication fork (Walker, 1987). RecA protein, the central regulator, is then activated and allows the cleavage of LexA protein, the common repressor of SOS genes. Subsequently, more than 20 SOS genes, including excision repair genes (*uvrA, B,*

Correspondence: Dr. B. Salles, Laboratoire de Pharmacologie et Toxicologie Fondamentales CNRS, 205 route de Narbonne, F-31400 Toulouse, France.

TABLE 1

BACTERIAL STRAINS

Strain	Genotype	Source or reference
MC4100	F⁻ *araD139 Δ(argF-lac) U169 rpsL150 relA1 flbB5301 ptsF25 deoC1(rbsR)*	Salles and Weinstock, 1989
GE1068	As MC4100 but *Δ(cya)854*	Salles and Weinstock, 1989
GE1050	As MC4100 but *Δ(crp)* cam^R	Salles and Weinstock, 1989
TP2139	F⁻ *xyl ilvA argH1 lacΔX74 crpΔ39*	Roy et al., 1983
BE87	As MC4100 but *uvrA6 malE57*::Tn*5*	This study
BE88	As GE1068 but *uvrA6 malE57*::Tn*5*	This study
BE134	As MC4100 but *Δ(recA-srl)306 srlR301*::Tn*10*	This study
BE327	As GE1068 but *Δ(recA-srl)306 srlR301*::Tn*10*	This study
BE326	As GE1050 but *Δ(recA-srl)306 srlR301*::Tn*10*	This study
RH4539	*trpA540 strA lacZ*(ICR36) (Mu c^+)	P. Caillet-Fauquet
RH4681	As RH4539 but *dinA1*::Mud(Ap^R, *lac*)	P. Caillet-Fauquet
GW1060	As GW1000 but *uvrA*::Mud(Ap^R, *lac*) (Mu *c ts*)	Kenyon and Walker, 1980
BE132	As RH4539 but *Δ(cya)854*	This study
BE149	As BE132 but *uvrA*::Mud(Ap^R, *lac*)	This study
BE307	As RH4681 but *Δ(cya)854*	This study
GE94	As MC4100 but *Δ(gal-G*[I]*b2*(I)::(*recA*::*lacZ*)	Weisemann et al., 1984
BE291	As GE94 but *Δ(cya)*854	This study

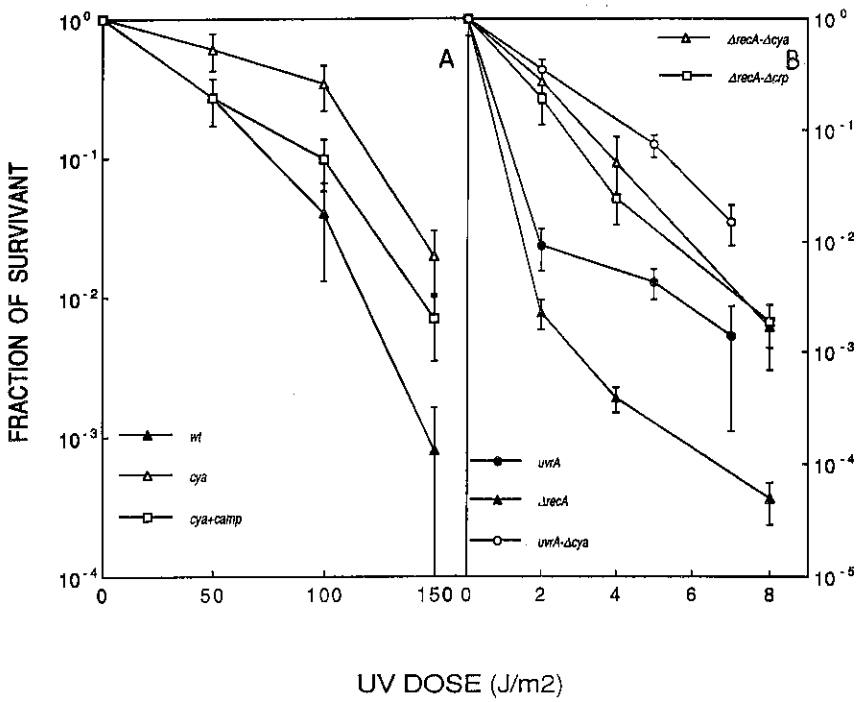

Fig. 1. Survival of UV-irradiated bacteria. (A) UV survival curves of (△) MC4100, (▲) GE1068 and (□) GE1068+3 mM cAMP. (B) UV survival curves of *uvrA* mutants (○) BE87, (●) BE88 and *ΔrecA* mutants (▲) BE134, (△) BE327 and (□) BE326. Mean of 3 independent experiments with SD.

D) are coordinately expressed at a high level. Superimposed on this regulatory circuitry, the fine-tuning of some SOS repair functions relies on additional controls (Neidhardt, 1987; Van Bogelen et al., 1987).

The possible role of cAMP in the modulation of repair gene expression as determined by bacterial and phage survival after UV irradiation is discussed. The UV resistance property of mutants lacking either adenylate cyclase or CRP protein is reported. This effect is considered in the light of known mechanisms of DNA repair processes.

Material and methods

E. coli K12 strains are listed in Table 1. P1 transductions and λ phage lysates were performed as previously described (Silhavy et al., 1984) as well as the construction of isogenic *Δcya* and *Δcrp* derivatives (D'Ari et al., 1988). *Δcya-834* mutation (Brickman et al., 1973) has recently been sequenced and corresponds to a 200-base pair deletion (Glaser et al., 1989). *Δcrp* derivative was either *crp::Tncam* in MC4100 genetic background (Salles and Weinstock, 1989) or *Δcrp39* (Roy et al., 1983) constructed by the two-step procedure in AB1157 genetic background (D'Ari et al., 1988). Similar results were obtained both with MC4100 and AB1157 derivatives and the two *crp* and *cya* deletions used. Bacteria were grown at 37°C in the mineral salts medium M63 (Silhavy et al., 1984) supplemented with 0.5% casamino acid (Difco), 1 μg/ml thiamine, 10 mM $MgSO_4$, 20 μg/ml tryptophan when needed and 0.5% glucose (medium hereafter termed MMC). When necessary, 3 mM cAMP was added to the MMC medium. Bacterial and phage survival experiments after UV irradiation were performed as already reported (Defais et al., 1971). β-Galactosidase activity was determined as described (Silhavy et al., 1984) and RecA protein was quantified by a two-site radioimmunological assay (Paoletti et al., 1982).

Results

Survival curves of bacteria after UV irradiation (Fig. 1A) showed that *Δcya* (GE1068) and *Δcrp* (GE1050) mutants were equally UV-resistant. This phenotype was partly reverted by an exogenous supply of cAMP in the case of *Δcya* but not *Δcrp* (Fig. 1A and data not shown) indicating that the CRP/cAMP complex was somehow involved in this UV resistance. The same deletions transduced in a different genetic background (AB1157) gave identical results (data not shown). Deletion of *cya* does not lead to additional mutation since reversion of *Δcya* mutation by P1 transduction also reverted the resistance phenotype (data not shown).

TABLE 2

EXPRESSION OF *lacZ* GENETIC FUSIONS WITH SOS GENES IN *cya*$^+$ AND *Δcya* STRAINS

Fusion	UV dose [a] (J/m^2)	Relevant genotype		
		wild type	*Δcya*	*Δcya* + cAMP
dinA	0	11 [b]	9	9
	15	48 [c]	40	31
uvrA	0	64	82	83
	3	319	385	408
recA	0	289	414	351
	50	1530	3805	2174

[a] The UV dose corresponds to the maximal SOS induction as determined from dose–response curves.

[b] β-Galactosidase activity (Miller units per OD578) was determined on duplicate samples with at least 3 determinations during bacterial exponential growth. Each value represents the mean of at least 3 independent experiments with SD < 30%.

[c] Induced level of fusion expression was checked 90 min after UV irradiation. Each value represents the mean of 2 independent experiments.

This enhanced survival could result in a higher level of expression and/or inducibility of some SOS genes by comparison with wild-type bacteria. To test this hypothesis the expression of *din* (*d*amage *in*ducible genes) (Kenyon and Walker, 1980), *recA* and *uvrA* gene fusions with *lacZ* was quantified (Table 2). Both *cya*$^-$ and *cya*$^+$ strains displayed a similar basal level of expression of *dinA* and *uvrA* operon fusions. The same result was observed after UV irradiation leading to complete derepression of SOS fusions. Similar results were found with *dinB*, *D* and *F* operon fusions (data not shown). In contrast, basal and induced expression of *recA::lacZ* protein fusion seemed slightly and negatively regulated by

cAMP. In order to confirm this result RecA protein was quantified by an immunological radiometric assay in uninduced and UV-induced conditions. However, no significant variation of RecA protein concentration in *cya*$^-$ versus *cya*$^+$ strains was found (data not shown).

In parallel to the study of the putative modulation of SOS genes by cAMP, we chose a genetic approach and constructed derivatives deficient in the excision repair pathway (*uvrA*) or the SOS regulatory network (non-inducible SOS functions with *ΔrecA* and *lexA3* mutations). Neither *uvrA6*, nor *ΔrecA* and *lexA3* mutations abolished the UV resistance of *Δcya* or *Δcrp* strains (Fig. 1B and data not shown). Therefore, it appears that DNA repair involving *uvrA, recA* or *lexA* genes was not responsible for the UV resistance displayed by *cya* or *crp* mutants. The increase in UV survival provoked by *cya* or *crp* mutations might be related to their slow growth rate. Survival after UV irradiation was tested in *uvrA* derivatives growing in medium supplemented with 2 mM adenosine, which has been shown to stimulate the growth of *crp* and *cya* mutants (Hammer-Jesperson and Nygaard, 1976). In spite of an approximately similar doubling time of about 40 min for *Δcya*, *Δcrp* or *cya*$^+$*crp*$^+$ strains, mutants remained more resistant to UV damage (data not shown).

UV resistance could be related to differences in quality or quantity of UV-induced DNA lesions in wild-type versus *cya* or *crp* mutants. This possibility was tested by determining the survival of a UV-damaged *λ(h80)* bacteriophage after infection. Phage survival was higher in *Δcya* and *Δcrp* mutants, in both *uvrA*$^+$ and *uvrA*$^-$ derivatives (Table 3). The increase in survival was abolished by addition of cAMP to the culture medium in the case of *Δcya* mutant.

Discussion

The bacterial response to various stresses such as DNA damage, free radicals, temperature and osmotic change is dependent upon the corresponding regulons. However, there are connections between these regulons at different steps of their regulatory circuitry, which permit the fine control of the physiological response (Neidhardt, 1987; Van Bogelen et al., 1987). Deletion mutants in *cya* and *crp* genes were constructed and exhibited a phenotype resistant to UV irradiation.

This resistance was independent from *recA* and *uvrA* genes and was also effective for the survival of UV-irradiated *λ* phage, which indicates that either a repair or a tolerance mechanism of UV damage was somehow regulated by the CRP/cAMP complex. Alternatively, the global effect of cAMP and CRP may well be involved in processes not directly related to UV resistance, though they actually affect this resistance. For instance, the change in the intracellular levels of nucleotides following UV irradiation (Barbé et al., 1986) could affect UV mutagenesis and may also be involved in UV survival. Other nucleotides such as 3,5′ phosphoguanosine (ppGpp) which is produced during stress responses (Van Bogelen et al., 1987) were not involved in the resistance since (i) MC4100 was *relA1*, (ii) and AB1157 *Δcya relA*$^+$ derivative was still resistant when the *relA1* derivative was constructed (data not shown).

cAMP does not modulate the basal and induced expression of the SOS gene operon fusions

TABLE 3

EFFECT OF *cya* AND *crp* MUTATIONS ON SURVIVAL OF UV-IRRAIDATED *λ(h80)* PHAGE

Excision repair genotype of recipient bacteria	UV dose to phage (J/m^2)	Relevant catabolite repression genotype			
		cya$^+$ *crp*$^+$	*Δcya*	*Δcya* +cAMP	*Δcrp*
uvrA$^+$	300	5.2×10^{-3}	2.3×10^{-2}	7.6×10^{-3}	1.2×10^{-2}
uvrA$^-$	100	4.0×10^{-3}	3.9×10^{-2}	4.4×10^{-3}	N.T.

Infection of damaged *λ(h80)* was determined on *uvrA*$^+$ strains MC4100, GE1068 and GE1050 or on *uvrA*$^-$ strains BE87 and BE88. Each value represents the mean of at least 2 independent experiments.

tested. The slight variation in *recA::lacZ* protein fusion expression could be due to post-transcriptional regulation of cAMP on the *recA-lacZ* RNA messenger (Simon and Apirion, 1972; Ullmann and Danchin, 1983), when this regulation was not present with *recA*. On the other hand, it is interesting to note that expression of *dinA*, which encodes the mutagenic DNA pol II (Chen et al., 1989), is not regulated by the CRP/cAMP complex. Induced mutation yield has been reported to be depressed by glucose when compared to that of bacteria growing in glycerol-supplemented medium (MacPhee, 1985). However, reports on the possible effect of catabolite repression on mutagenesis were contradictory (Pons and Müller, 1989; Thomas and MacPhee, 1987). The glucose effect was reported to enhance mismatch repair which consequently affected the mutant yield. In addition, by numbering λ phage mutants (clear plaques) when it infected *cya* mutants, no significant difference in the frequency of mutants was found (data not shown).

Interestingly, disruption of adenylate cyclase gene in *Saccharomyces cerevisiae* generates a multistress resistance phenotype including UV light, ethanol and heat shock stresses by an unknown mechanism (Iida, 1988). Preliminary results indicate that *E. coli Δcya* and *Δcrp* mutants also display a phenotype resistant towards heat and hydrogen peroxide (unpublished results). This suggests that there is a conserved overlapping regulation between metabolic and damage repair pathways in both prokaryotic and eukaryotic cells that might be essential for cell survival to environmental stresses.

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