MUTAGENESIS IN VITRO *AND USE OF DNA RECOMBINANT GENOME FOR EVALUATION OF DNA DAMAGE AND REPAIR*

Ann. Ist. Super. Sanità
Vol. 25, N. 1 (1989), pp. 145-148

THE pR PLASMID: A TOOL FOR STUDYING DNA REPAIR AND MUTAGENESIS IN PROKARYOTIC AND EUKARYOTIC CELLS

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Summary. - *The pR plasmid, a derivative of R46 plasmid, offers the possibility to have an experimental approach to three important problems related to UV repair and mutagenesis. By using this plasmid we were able to show: a) the pR* mucAB *genes need the cooperation of uvpl gene product to carry out their UV repair function; b) the expression of* mucAB *genes is regulated not only by* lexA *gene, but by a gene localized in the rep region of pR itself. This gene acts as an anti-repressor of* lexA; *c) mammalian cells show an enhanced resistance to UV light when transformed by pR plasmid carrying the* mucAB *genes.*

Riassunto (Il plasmide pR: un nuovo efficiente mezzo per studiare la riparazione del DNA e la mutagenesi sia nei procarioti che negli eucarioti). - *Il plasmide pR, un derivato del plasmide naturale R46, offre la possibilità di affrontare sperimentalmente tre importanti problemi connessi con l'UV repair e la mutagenesi. Grazie all'uso di questo plasmide abbiamo rilevato infatti che: a) i geni* mucAB *hanno bisogno della cooperazione del gene uvpl per svolgere la loro funzione riparatrice ai raggi ultravioletti; b) l'espressione dei geni* mucAB *è regolata non soltanto dal gene* lexA, *ma da un altro gene, presente nella regione rep del plamside pR, che svolge la funzione di antirepressore di* lexA; *c) le cellule di mammifero sono trasformate dal plasmide pR e presentano resistenza ai raggi UV quando il pR porta i geni* mucAB.

Introduction

The R46 plasmid (TP120) [1], its derivatives pKM101 [2] and pR [3] provide an efficient way of studying the function and regulation of the mutagenic component of the *Escherichia coli (E. coli)* SOS system, since their *mucAB* genes encode a function that is homologous to the one encoded by the *umuCD* genes. Both the repair capacity and the mutation rate of a cell that contains these plasmids are increased, and mutations that affect *mucA* or *mucB* decrease both repair and mutation rates to wild-type levels [4].The expression of the *mucAB* operon [5] is repressed by the product of the *lexA* gene. These properties and the facility of use make the pR plasmid very suitable to investigate on topics such as: are other pR genes, in addition to *mucAB* genes, involved in UV-repair? How are the *mucAB* genes regulated? Do *mucAB* genes products work in Eukaryotes?

The UV-phenotype and the UV-induced mutagenesis in pR uvp1-plasmid

We have shown previously [6] that the pR plasmid possesses, in addition to *mucAB* genes, the uvp1 region involved in UV resistance in *E. coli*. The insertion of the Tn5 transposon in the uvp1 region, as well as in the *mucAB* genes, impairs the ability of the pR plasmid to enhance the UV survival in *E. coli* cells.

In order to obtain a smaller (uvp1$^-$ muc$^+$) plasmid that could be handled and analyzed more easily, we constructed a new plasmid (pL11.5) which carries the *mucAB* genes and a deletion in the uvp1 region; to test its ability to enhance the UV resistance of *E. coli* cells, *E. coli* C600 strain was transformed with the plasmid and the transformants were assayed for their sensitivity to UV light. As Fig. 1 shows, the UV survival curve of C600 cells harboring the pL11.5 plasmid is superimposable on that of C600 cells used as the control. In order to ascertain whether the uvp1 region resembles the *mucAB* genes in increasing UV-induced mutagenesis [4], *E. coli* AB1157 strain was transformed with either the pR or the pL11.5 plasmid and the cells were assayed for their ability to increase the mutation rate, following UV-irradiation. This was accomplished by scoring the number of rifampicin-resistant colonies on indicator L-agar plates.

As shown in Table 1 both the pR and the pL11.5 plasmids elicit a significant increase in the mutation rate of *E. coli* cells.

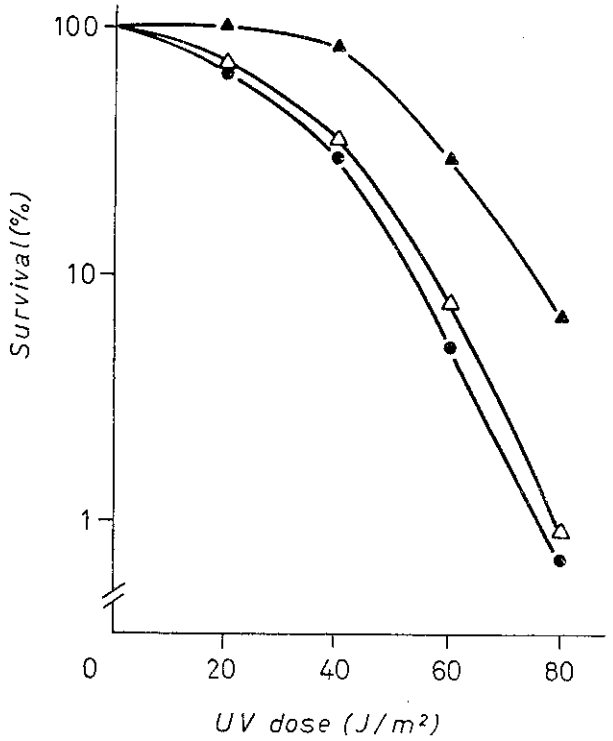

Fig. 1. - UV survival curves of *E. coli* C600 transformed with pR or with pL11.5. Cells were irradiated at a dose rate of 5 J/m²/s *Symbols*: ● = C600; ▲ = C600/pR; Δ = C600/pL11.5.

Table 1. - *UV-induced mutagenesis in* E. coli *AB1157 strain transformed by pR or by pL11.5 plasmids*

Strains	UVdose (J/m²/s)	Rifr/10⁸ survivors
pR (uvp1⁺ muc⁺)	5	202
pL11.5 (uvp1⁻ muc⁺)	5	219
E. coli AB1157	5	20

These results indicate that the uvp1 region cooperates with the *muc* genes in repairing UV damage, without being involved in UV-induced mutagenesis. Recently, we identified a protein of 20 kDa molecular weight, coded by uvp1 region, responsible for UV resistance in bacteria.

Induction by λpR of *mucAB* and *sfiA* genes

The rate of mutation in strains containing R46 and its derivatives is not limited to those mutations that are induced by chemical or physical agents. In fact, Mortelmans and Stocker [7] showed that the presence of this plasmid in bacterial cells increases the level of spontaneous mutations by a factor of 10. This phenomenon could not be explained if the *muc* genes were costantly repressed by the *lexA* gene product. On the other hand, if the *muc* genes have even a low level of constitutive expression they should still exhibit a gene-dosage effect when cloned in a multicopy plasmid. This effect, however, was not observed by Elledge and Walker [5]. Alternatively, the *muc* genes might be subject to positive control.

To test whether the pR plasmid encoded a function that regulates the expression of SOS pathways (*i.e.*, induction of λphage, expression of *muc* genes), we constructed the λpR phasmid, a hybrid between the EMBL4 and pR plasmid [8]. This genetic element, which can easily be transfered from one cell to another, was tested for its ability to induce the expression of *muc* genes, which are under the negative control of the *LexA* repressor [5], in the absence of other forms of induction, physical or chemical.

In order to measure the ability of λpR to induce the expression of *muc* genes in *E. coli*, we fused the *muc* genes with the *lacZ* gene [9]. The strain was named bat107, β-galactosidase activity in bat107 increased upon infection with pR. Furthermore the pR plasmid induces the expression of β-galactosidase also in the GY4581 strain where the *lacZ* gene is fused to the *sfiA* gene (filamentous growth) which, in this specific case, was under the control of the cI repressor [10]. The level induced by λpR in bat107 and in the GY4581 strain is comparable to that obtained by Bailone *et al.* [11] by indirect induction with a damaged mini-F.

These results shows that at least two promoters, normally under the negative control of the functionally similar LexA and *cI* repressors, can be activated by infection with the pR plasmid. It is possible to hypothesize, therefore, that a *lexA* and *cI* antagonist is present and coded by the pR plasmid.

DNA-mediated gene transfer permits the introduction of new genetic information into cultured mammalian cells

The pR plasmid, when transfected into mammalian cells, is capable of enhancing their survival to UV light.

LTA cells, co-transformed with ptk1 and pR plasmids were selected in HAT medium. Nine transformed cell lines were established by isolating from separate plates individual clones, which were derived from cells co-transfected with ptk1 and pR DNAs and one cell line from cells transfected with ptk1 alone.

In addition one cell line was also established from an uncloned population of transformants obtained after treatment with both plasmids (TU).

The UV light sensitivity of the above lines was assessed and compared with the sensitivity of the untransformed parental LTA line (Fig. 2). Seven of the 9 co-transformed clonal lines exhibited significantly higher

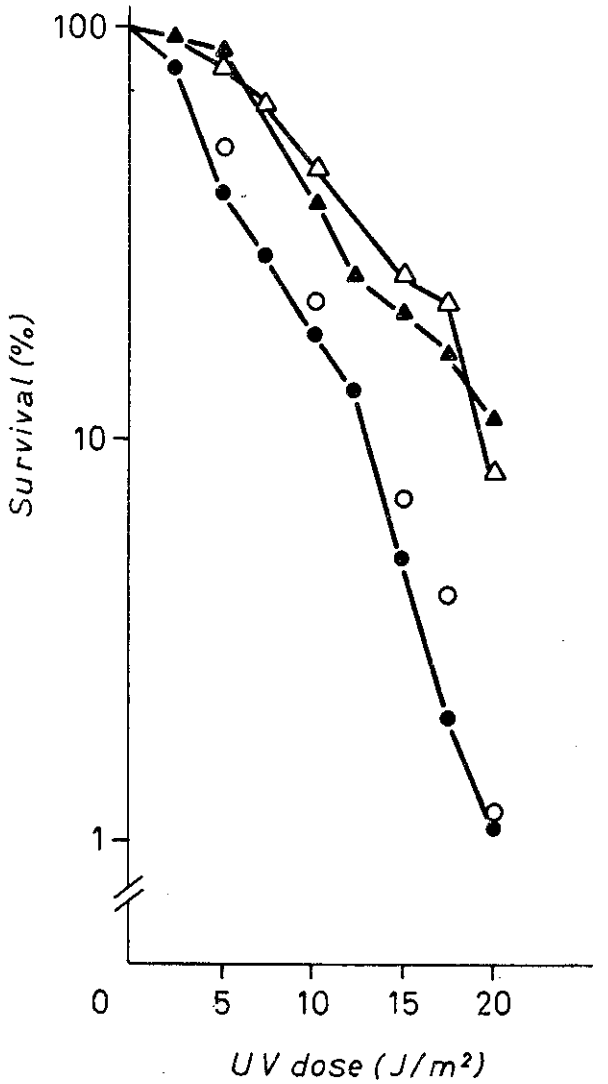

Fig. 2. - UV survival curves of LTA cells co-transformed with ptk1 and with pR plasmid. Cells were irradiated at a dose rate of 1.25 J/m²/s *Symbols:* ● = LTA cells; O = LTA cells transformed with ptk1; ▲ = TU cell line; Δ = clone D.

survival to UV light; similar results were obtained with the uncloned co-transformed cell line (TU). Two of the co-transformed clones as well as the clone transformed only with ptk1, on the other hand, exhibited UV light sensitivity indistinguishable from that of LTA cells.

The characteristics of the resistant lines have persisted over a period of eight months in selective HAT medium. These results show that seven co-transformed clones as well as the TU cell line have acquired an enhanced UV resistance as a consequence of a stable genetic change. In order to relate the acquisition of UV resistance to the presence of pR plasmid, Southern blot hybridizations were performed between DNA from transformed lines and *in vitro* labelled pR DNA. The results of these experiments show that the seven UV resistant clones as well as the uncloned co-transformed TU cell line contain DNA sequences homologous to the pR probe.

The pR plasmid, which enhances the survival of *E. coli* C600 exposed to UV light by induction of the SOS repair regulatory mechanism, showed the same effect when it transformed mouse LTA cells (tk^-, $aprt^-$). With Tn5 insertion mutagenesis, which inactivates UV functions in the pR plasmid, we recognized two different regions of the plasmid, uvpl and *mucAB* genes. These pR UV mutants exhibited the same effect in LTA transformed cells, demonstrating that resistance to UV light, carried by the pR plasmid, was really due to the expression of these two regions, which were also in the mouse cells.

Conclusions and perspectives

Transformation experiments carried out in mouse cells with pR and pR UV mutants have demonstrated that: i) both uvpl and *mucAB* regions are essential for the expression of UV resistent phenotype in mouse cells and in bacteria; ii) this plasmid confers a stable genetic change in the expression of this function in mammalian cells. This trait does not seem to be conferred by pKM101 to LTA cells; in fact, transformation experiments with pKM101 have been unsuccessful in producing expression of a stable UV - resistent phenotype - (unpublished data). Furthermore, Porter *et al.* [12] reported that the introduction of plasmid pKM101-associated *muc* genes in *Saccharomyces cerevisiae* determined a detectable increase in mutagenic effect but not a significant enhancement of UV survival.

The results obtained with plasmid pR both in prokaryotic and mammalian cells suggest that this plasmid may be very suitable for investigating whether an inducible repair pathway is present also in mammalian cells for the following reasons: i) it enhanced UV survival both in bacteria and mouse cells; ii) the *uvpl* and *mucAB* regions are involved in the SOS repair system in bacteria probably amplifying or triggering this process: iii) plasmid pR, although not cloned in a eukaryotic vectors is capable of giving rise to a stable UV^+ phenotype in transformed mouse cells.

The possibility of making genetic and molecular investigations with plasmid pR in prokaryotic cells and the ability of pR to express UV resistance, even in mouse cells, make it possible to compare some SOS functions in bacteria with some inducible responses to DNA damage in mammalian cells.

Furthermore our results give new perspectives on the question concerning the biological significance of SOS system. Until today the SOS system has been considered as a proper bacteria escape mechanism intended to mutate and repair its genome. We revealed that the pR plasmid has a gene *(uvpl)* responsible, together with *mucAB* genes, for an enhanced resistance to UV light in

strains containing it. The pR plasmid has also the bat function which confers to the plasmid the ability to induce the SOS system in absence of any evident DNA damage [8].

All of these results seem to indicate that the pR plasmid is a coordinated set of genes essential for DNA repair process and make it possible to surmize that these genes are necessary for the genome organization and expression of the pR itself rather than to be useful only for some evolutionary process of the bacterial genome.

In our opinion the pR plasmid can be envisaged as a "mini-organism" possessing autonomous coordinate functions. With such a background, we are looking for an unique regulatory function able to rule the vital cycle of pR plasmid.

Review submitted on invitation by the Editorial Board of the *Annali*. Accepted for publication: October 1988.

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