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The pR UV$^+$ plasmid, transfected into mammalian cells, enhances their UV survival

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ABSTRACT

It has been recently reported that the pR plasmid enhances the UV survival in E.coli C600.

In order to test whether this function may be expressed also in mammalian cells, LTA (tk$^-$ aprt$^-$) mouse cells were cotransformed with pR plasmid DNA and ptk1 plasmid as selectable marker. Tk$^+$ transformants were analyzed for their UV survival and for the presence of pR DNA sequences by blot-hybridization.

The results show a correlation between the enhanced UV survival and presence of pR DNA sequences in cotransformed LTA mouse cells.

INTRODUCTION

DNA-mediated gene transfer permits the introduction of new genetic information into cultured mammalian cells (1). Transformed cells expressing exogenous DNA coding for selectable markers may be used to test the expression of other DNA sequences simultaneously integrated at high frequency (1). Recently Mulligan and Berg (2) have transformed mammalian cells with an E.coli gene that codes for xanthine-guanine phosphoribosyltransferase (XGPRT) cloned in SV40 DNA-based vectors, resulting in the synthesis of readily measurable quantity of the bacterial enzyme. We have reported the construction of pR plasmid carrying a gene coding for a protein involved in error prone process and responsible for UV resistance in E.coli (3).

In this paper we present evidence that this plasmid, not containing eukaryotic promoters, when transfected into mammalian cells, is capable of enhancing their survival to UV light.

MATERIALS AND METHODS

Cells and plasmids

LTA mouse cells (tk$^-$, aprt$^-$) were obtained from S. Bacchetti

and maintained in α-MEM containing 10% fetal calf serum.

Plasmid ptk1 (kindly supplied by S. Bacchetti) is a selectable marker carrying ampicillin (Ap) resistance and thymidine kinase (tk) gene; it is derived by 3.4 kb fragments of Herpes virus type 1 cloned in BamHI site of pB322 (4). The pR plasmid (Ap^+, UV^+) is a R46 derivative constructed as previously described (3).

Transformation, co-transformation and selection

Transformation of LTA tk^- cells to tk^+ phenotype was performed with purified ptk1 DNA (50 ng) using the calcium phosphate method (5) modified by Wigler et al. (1). In co-transformation experiments, uncleaved ptk1 (50 ng), co-precipitated with 50 ng of HindIII cleaved pR UV^+ DNA in presence of 5 µg salmon sperm carrier DNA and calcium phosphate, was added to sub-confluent monolayers ($5x10^5$ cell/60 mm dish) of LTA cells growing in α-MEM +10% fetal calf serum. The medium was changed after 12 hours at 37°C and tk^+ transformants were selected in α-MEM containing hypoxantine (0.1 mM), aminopterine (1 µM) and thymidine (40 µM) (HAT) and 10% fetal calf serum. After two weeks in selective medium (HAT) tk^+ transformants were observed and isolated colonies were picked and grown into mass cultures.

UV irradiation

The source of UV light was a 30 watt germicidal lamp emitting over 90% of its energy at 256 nm and shielded to give a dose rate of 1.25 J m^{-2} sec^{-1}. The fluences were measured with a Latarjet UV-meter. Cells (500/60 mm dish) were plated 24 hrs prior to irradiation and irradiated after removal of the growth medium. Colonies were stained and counted after 15 days of growth in α-MEM ($\pm$ HAT) and 20% of fetal calf serum. Plating efficiencies for each dose were obtained from the average of 3 plates.

Extraction and restriction digestion of DNA

High molecular weight DNA from cultured cells was prepared as described by Wigler et al. (1). Plasmid DNA were isolated from cultures of E.coli C600 as described by Humphreys et al. (6). Cellular or plasmid DNAs were digested with various restriction endonucleases under the conditions recommended by the supplier (New England Biolabs). Digestions were performed at an enzyme/DNA ratio of 2-3 U/µg for 4 hrs at 37°C.

Filter hybridization

High molecular weight DNA (10-15 µg) from transformed and control cells was digested with restriction endonucleases BglII EcoRI, BamHI and electrophoresed through 1% agarose slab gel in Tris-borate buffer pH8.2. The DNA was denatured *in situ* and transferred to nitrocellulose filters according to Southern (7). ^{32}p labeled pR UV^+ DNA probe (sp.act. $7x10^7$ cpm/µg) was prepared *in vitro* by nick-translation (8). Hybridization was at 65°C in 10x Denhardt, 2xSSC, 0.2% SDS over-night, following which the filters were washed 5 timesfor 1 hr in 2xSSC, 1xSSC and 0.1SSC at 65°C. 10 days exposure on Fuji X-ray films was used.

RESULTS

Experimental design

Mouse LTA cells deficient in thymidine kinase activity (tk^-) can be stably transformed to a tk^+ phenotype by transformation with plasmid ptk1 followed selection in HAT medium. Ptk1 can also be used as a selectable marker in co-transformation experiments to test uptake by the cells of other gene for which no direct selection method is available. To obtain co-transformants, cultures were exposed to the ptk1 DNA in presence of pR plasmid DNA. Tk^+ transformants were randomly isolated and scored for their survival to UV light and for the presence of pR DNA sequences by molecular hybridization.

Co-transformation of mouse cells with pR UV^+ DNA

LTA cells, co-transformed with ptk1 and pR plasmids (see Materials and Methods) were selected in HAT medium. Tk^+ transformation efficiency was 1.10^{-5}. Nine transformed cell lines were established by isolating from separate plates individual clones, which were derived from cells co-transfected with ptk1 and pR UV^+ DNAs (named with alphabetic letters from A to I) and one cell line from cells transfected with ptk1 alone (indicated with T). In addition one cell line was also established from an uncloned population of transformants obtained after treatment with both plasmids (TU).

Expression of UV resistance in co-transformed cells

The UV light sensitivity of the above lines was assessed and compared with the sensitivity of the untransformed parental LTA

Table 1 - Quantitative parameters for the UV survival curves

Cell line	Transforming DNA	$\tilde{D}_0$ a) $\pm$ S.E. J/m^2	$\tilde{D}_q$ a)	$\tilde{n}$ a)
LTA b)	--	3.9 $\pm$ 0.3	2.5	1.9
TU b)	pTK1+pRUV$^+$	7.1 $\pm$ 0.4	3.8	1.7
A	"	6.6 $\pm$ 1.0	4.2	1.9
B	"	4.0 $\pm$ 0.3	3.0	2.1
C	"	4.1 $\pm$ 0.3	2.8	2.0
D	"	7.0 $\pm$ 0.7	4.5	1.9
E	"	5.6 $\pm$ 0.4	4.4	2.2
F	"	6.9 $\pm$ 0.9	4.1	1.8
G	"	7.0 $\pm$ 0.2	4.1	1.8
H	"	7.1 $\pm$ 0.5	4.9	2.0
I	"	7.0 $\pm$ 1.3	4.1	1.8
T	pTK1	3.7 $\pm$ 0.5	2.6	2.0

a) From an operational point of view, the $\tilde{D}_0$ value can be described as the dose reducing the surviving fraction by a factor 1/e., measured on the exponential portion of the survival curve fitted by the least squares method. The values of $\tilde{n}$ are obtained by extrapolation of the straight line portion of the curve to the ordinates at zero dose. The quasi-threshold dose $\tilde{D}_q$, which is another parmeter used to describe the shoulder width, was derived from the formula $\tilde{D}_q = \tilde{D}_0 \ln \tilde{n}$ (9).
b) refers to uncloned lines.

line. Seven of the 9 co-transformed clonal lines (A,D,E,F,G,H and I) exhibited significantly higher survival to UV light; similar results were obtained with the uncloned co-transformed cell line (TU). Two of the co-transformed clones (B and C) as well as the clone transformed only with ptk1 (T), on the other hand, exhibited UV light sensitivity indistinguishable from that of LTA cells. Quantitative parameters for the UV survival curves of the cell lines tested are listed in Table 1.

On the average the D_0 of the resistant lines was 1.7 times that of the untransformed parental line, whereas the $\tilde{n}$ values are similar in the cell lines tested. The slopes of the UV survival curves of "resistant" clones versus "non resistant" cells are significantly different ($p<0.01$) as determined by the Student t test.

Fig.1 shows typical examples of survival curves obtained for

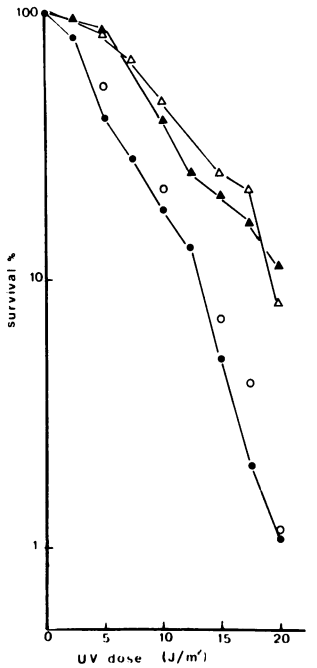

Fig.1. UV survival curves of LTA cells co-transformed with ptk1 and with pR plasmid DNAs. The transformants were selected in HAT medium. The UV survival of the cells was determined as described in Materials and Methods. ●LTA cells; o Clone B; ▲Tu cell line; △Clone D.

co-transformed and control cell lines. The characteristics of the resistant lines have persisted over a period of 8 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.

Physical presence of the pR UV^+ plasmid DNA in co-transformed cells

In order to relate the acquisition of UV resistance to the presence of pR UV^+ plasmid Southern blot hybridizations were performed between DNA from transformed lines and *in vitro* labelled pR UV^+ DNA. Genomic DNA from transformed cells was digested with restriction endonucleases BglII, EcoRI and BamHI which cleave the pR plasmid as indicated in Fig.2. Following agarose gel electrophoresis and transfer to nitrocellulose paper, hybridization was performed as described in Materials and Methods. 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 UV^+ probe.

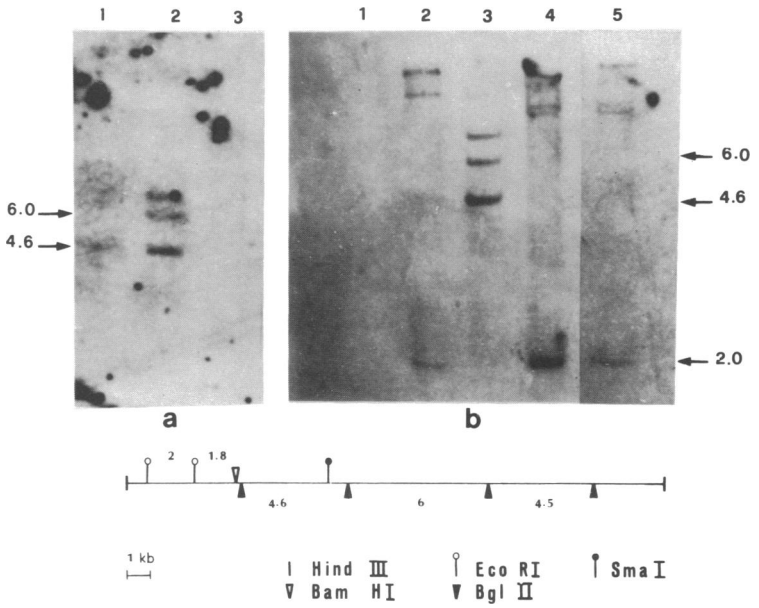

Fig.2. Identification of pR sequences in representative clones and TU cell line.
a) 10 µg of DNA from each cell line were digested with BglII; lane 1: LTA control cell line; lane 2: TU cell line; lane 3: clone T. Only TU cell line shows molecular hybridization with pR DNA. b) 10 µg of DNA from three independently isolated clones are digested with BglII, EcoRI and EcoRI/BamHI. Lane 1 clone B (EcoRI digestion); lane 2,3 and 4: clone A digested with EcoRI/BamHI, BglII and EcoRI respectively (the digestion with EcoRI was performed with 15 µg of clone A DNA); lane 5: clone D digested with EcoRI. Only clone B (lane 1) lacks detectable pR sequences. Arrows indicate the molecular weight in kb of the pR plasmid internal fragments.

In Fig.2 the hybridization patterns of clones A and D (Fig. 2b lane 2-5) and TU cell line (Fig.2a lane 2) are shown. On the contrary, clones which don't show enhanced UV survival whether transformed with ptk1 alone (T) or co-transfected with both plasmids, are negative for these sequences (Fig.2a lane 3; Fig. 2b lane 1).

The hybridization pattern observed when clones A and D were digested with EcoRI/BamHI and EcoRI (Fig.2b lane 2,4,5) shows the 2 kb band corresponding to the internal EcoRI fragment (see map Fig.2). The 6.0 and 4.6 kb bands detected after digestion with

BlgII (Fig.2a lane 2; Fig.2b lane 3) correspond to the three internal fragments of the pR UV^+ plasmid (6.0, 4.6 and 4.5 which co-migrate, see map Fig.2). Other bands of higher molecular weight are also observed which may represent pR sequences adjacent to cellular DNA. Alternatively these patterns may be consistent with the recircularisation of pR plasmid or else with the formation of pR oligomers. In both cases it appears that the cells have acquired the fragments containing the prokaryotic gene for UV resistance. By our preliminary results this function is localized in the 4.6 BglII internal fragment. The analysis of both UV resistance and presence of pR DNA in all cell lines examined demonstrates that no clones are to be found whose cells contain pR but are not UV resistant, and that there are no UV-resistant colonies of cells which lack the pR plasmid DNA.

It is therefore possible to correlate the acquisition of UV resistance with the presence in the cells of pR UV^+ DNA sequences. The presence of pR plasmid sequences is indeed supported by the observed stability of the resistant phenotype of transformants.

DISCUSSION

The results we have obtained through the analysis of the UV survival curves and of the hybridization patterns, indicate that a) UV function is enhanced only in those co-transformed clones in which pR DNA sequences are detected by molecular hybridization , and b) the T cell line and the clones that have acquired only the ptk1 plasmid sequences, are negative for the pR DNA and show the same UV survival of LTA cells.

The pR plasmid used in these co-transformation experiments, contains a gene which codes for a protein involved in error prone system in E.coli (3). It is thus tempting to speculate that expression of this gene is responsible for the enhanced UV survival, similarly to what we have observed in bacteria (3). This could indicate that repair processes in prokaryotes and eukaryotes might be at least in part carried out by similar molecules.

At present it is impossible to distinguish whether the pR plasmid DNA is integrated in eukaryotic genome or if it replicates as autonomous circular molecules.

Two hypotheses can be advanced to explain the expression of

pR UV^+ function in mouse cells: the pR UV^+ DNA contains genetic signals needed to express the UV^+ function; alternatively, enhanced UV survival might be the result of integration of plasmid sequences into a site that allows the expression of this function in the host genome.

Besides its importance in the study of the repair mechanism, this plasmid may be also considered as a suitable vector in which it is possible to clone gene specifying products whose expression could be engineered in mouse cells.

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REFERENCES

1. Wigler,M., Sweet,R., Sim,G.K., Wold,B., Pellicer,A., Lacy,E., Maniatis,T., Silverstein,S. and Axel,R. (1979) Cell 16, 777-785.
2. Mulligan,R.C. and Berg,P. (1980) Science 209, 1422-1427.
3. Gigliani,F., Elli,R., Marcucci,L. and Battaglia,P.A. (1981) Nucleic Acids Res. 9, 623-631.
4. Graham,F.L., Bacchetti,S., McKinnon,R., Stanners,C., Cordell Goodman,H.M. (1980) Introduction of Macromolecules into viable Mammalian Cells, pp.3-25, Alan R.Liss Inc.
5. Graham,F.L. and van der Eb,A.J. (1973) Virology 52, 456-467.
6. Humphreys,G.O., Willshaw,G.A. and Anderson,E.S. (1975) Biochim. Biophys. Acta 383, 457-463.
7. Southern,E.M. (1975) J. Mol. Biol. 98, 503-517.
8. Rigby,P.W.J., Dieckmann,M., Rodes,C. and Berg,P. (1977) J. Mol. Biol. 113, 237-251.
9. Elkind,N.M. and Whitmore,G.F. (1967) The Radiobiology of Cultured Mammalian Cells. Gordon and Breach Publ., New York.