FEBS Letters 538 (2003) 178–182 FEBS 27062

Ribosome-inactivating proteins depurinate poly(ADP-ribosyl)ated poly(ADP-ribose) polymerase and have transforming activity for 3T3 fibroblasts

Luigi Barbieri, Maurizio Brigotti, Paolo Perocco[1], Domenica Carnicelli, Marialibera Ciani, Laura Mercatali, Fiorenzo Stirpe*

Dipartimento di Patologia sperimentale, Università di Bologna, Via San Giacomo 14, I-40126 Bologna, Italy

Received 15 January 2003; accepted 11 February 2003

First published online 24 February 2003

Edited by Lev Kisselev

Abstract It has been known that ribosome-inactivating proteins (RIPs) from plants damage ribosomes by removing adenine from a precise position of rRNA. Subsequently it was observed that all tested RIPs depurinate DNA, and some of them also non-ribosomal RNAs and poly(A), hence the denomination of adenine polynucleotide glycosylases was proposed. We report now that ricin, saporin-L2, saporin-S6, gelonin and momordin depurinate also poly(ADP-ribosyl)ated poly(ADP-ribose) polymerase (auto modified enzyme), an enzyme involved in DNA repair. We observed also that all RIPs but gelonin induce transformation of fibroblasts, possibly as a consequence of damage to DNA and of the altered DNA repair system.

Key words: Ribosome-inactivating protein; Poly(ADP-ribose) polymerase; Adenine polynucleotide glycosylase; Cell transformation

1. Introduction

Ribosome-inactivating proteins (RIPs) are either type 1, single-chain proteins, or type 2, consisting of an A chain with enzymatic activity linked to a B chain, a sugar-binding lectin, or type 3, consisting of a type 1-like N-terminated peptide prolonged by a C-terminated domain (reviewed in [1,2]). Interest in RIPs is growing, due to their antiviral properties, which render transfected plants less sensitive to viruses (reviewed in [3]), and to their use as conjugates with antibodies (immunotoxins), employed to eliminate specific types of cells (reviewed in [4,5]).

RIPs were found to remove adenine from rRNA [6], thus being denominated rRNA N-glycosidases (rRNA *N*-glycohydrolases, EC 3.2.2.22). In addition, all of them depurinate DNA and some of them also poly(A) [7,8], and the denomination of polynucleotide adenine glycosylase was proposed for these proteins [9].

Several lines of evidence suggest that base removal from DNA triggers poly(ADP-ribose) polymerase (PARP)-catalysed poly(ADP-ribosyl)ation of several nuclear proteins involved in chromatin architecture and DNA metabolism, resulting in the expression of signals that activate DNA repair (reviewed in [10]). In the nucleus one of the acceptors of poly(ADP-ribose) is PARP itself (auto modified PARP).

The broad substrate specificity of RIPs led us to investigate whether they could act on a different nucleic acid, namely poly(ADP-ribose), in the form of poly(ADP-ribosyl)ated PARP (auto modified PARP). The investigation was performed with type 1 RIPs, selected on the basis of their activity on various substrates or among those used for the preparation of immunotoxins, and with ricin, as a representative of type 2 RIPs.

2. Materials and methods

2.1. Materials

RIPs were purified as described in the appropriate references reported in [1]. BALB/c 3T3 cells, clone A31, were originally obtained from American Type Culture Collection (Rockville, MD, USA). Exponentially growing cells were seeded in 60-mm culture dishes in Dulbecco's minimal essential medium supplemented with nutrient mixture F-12 (Gibco, Paisley, UK) and 10% newborn calf serum (complete medium).

2.2. Cytotoxicity and transformation

Four hundred cells/plate were seeded in four plates for each treatment. Cells were cultured in the presence of RIPs for 72 h, and then cultures were grown for further 7–10 days in complete medium without RIPs. The formation of cell colonies (larger than 50 cells) was evaluated by staining culture plates with 10% aqueous Giemsa and the determination of clonal efficiency was performed.

The transforming activity induced by the various RIPs was evaluated from the formation of cell transformation foci. According to the protocols used in previous work [11], a level-II transformation test, allowing the amplification of the cell transforming activity of treatments, was performed [12,13]. Briefly, cells were seeded at 1×10^4 cells/plate in four replicates for each treatment, and were exposed to scalar concentrations of the various RIPs for 72 h. Plates were washed with phosphate buffered saline, and cells were cultured in complete medium for about 2 weeks until confluence was attained. At this time, level-II transformation plates were set up by pooling the contents of the four confluent plates for each treatment, and reseeding 1×10^5 cells/plate in 12 replicates per treatment to ensure determination of a valid test [11,12]. After about 6 further weeks, cultures were arrested, plates were washed with PBS, stained with 10% aqueous Giemsa and scored for assessing the formation of transformation foci.

*Corresponding author. Fax: (39)-51-20 94746.
E-mail address: stirpef@alma.unibo.it (F. Stirpe).

[1] Deceased 7 September, 2002.

Abbreviations: RIP, ribosome-inactivating protein; PARP, poly(ADP-ribose) polymerase; PARG, poly(ADP-ribose) glycohydrolase; hsDNA, herring sperm DNA

0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00176-5

Transformation foci were counted, considering as positive those deeply basophilic, showing a dense layer formation and a random orientation of cells at the edges of foci and ≥1 mm (type III foci) [12].

2.3. Preparation of poly(ADP-ribosyl)ated PARP

The 2251 bp DNA (10.5 μg) obtained by PCR amplification of the 731–2981 region of pBR322 [14] was treated with 100 nM saporin-L2 in 1.75 ml of 50 mM sodium acetate, pH 4. After 40 min at 30°C, the deadenylated 2251 bp DNA was phenol-extracted at pH 7.8 and precipitated with ethanol. The pellet was dissolved in 10 mM Tris–HCl (pH 7.5)/0.1 mM EDTA. The extent of deadenylation (82%) was assessed in parallel samples containing [^{3}H]2251 bp DNA, labelled in the purine ring of adenine, as described in [14]. Poly(ADP-ribosyl)-ated PARP was prepared essentially as described by Shah et al. [15]. The 1.7 ml-reaction mixture (100 mM Tris–HCl, pH 8, 10 mM $MgCl_2$, 1.5 mM dithiothreitol, 10% glycerol) contained 1.7 μg of deadenylated 2251 bp DNA, 1 mM NAD^+, 340 μl of 50% ethanol (added drop wise with constant mixing) and 100 μl of nicotinamide [2,8-^{3}H]adenine dinucleotide (32.3 Ci/mmol, 100 μCi/ml, NEN Dupont). The radiolabelled NAD^+ was included in the mixture to monitor the poly(ADP-ribosyl)ation reaction on small aliquots as described by Shah et al. [15]. After 2 min at 30°C, 85 μl of bovine PARP (0.09 U/μl, Calbiochem) were added and the mixture was further incubated for 30 min. Then, the sample was brought to a final volume of 2 ml by adding 215 μl of 1.3 M NaCl and was applied to a PBS-equilibrated PD-10 column (Pharmacia). The [^{3}H]poly(ADP-ribosyl)ated PARP eluted between 1 and 2.5 ml, giving a final yield of 255 pmol of ADP-ribose/pmol of PARP. To prepare ^{32}P-labelled poly-(ADP-ribosyl)ated PARP, 20 μl of [^{32}P]NAD^+ (1000 Ci/mmol, Amersham) was used to substitute [^{3}H]NAD^+ in the same reaction mixture. The ^{32}P-labelled molecule was employed to evaluate the integrity of the poly(ADP-ribose) chains of PARP after treatment with RIPs (see below). In each preparation a parallel control reaction mixture was performed omitting PARP enzyme.

2.4. Determination of glycosylase activity

Released adenine from herring sperm DNA (hsDNA) was measured after separation by HPLC. Reaction was in a final volume of 50 μl containing 100 mM KCl, 50 mM sodium acetate buffer, pH 4.0, 10 μg of hsDNA, and scalar amounts of each RIP sufficient to release detectable amounts of adenine. Reaction was stopped in ice by addition of 200 μl of cold BA buffer (50 mM sodium acetate, pH 4.0, 20 mM KCl), and then the reaction products were applied to sample preparation columns (Bond Elut® NH2, from Varian, Harbor City, CA, USA) previously equilibrated at 2°C with BA buffer. Adenine was eluted in 400 μl of BA buffer by briefly centrifuging at 200×*g*, whereas phosphate-containing nucleic acids were retained; samples were in duplicate.

[^{3}H]- or [^{32}P]poly(ADP-ribosyl)ated PARP (0.85 pmol corresponding to 216.75 pmol of ADP-ribose monomers) was incubated in the absence or in the presence of 100 nM RIPs in a 50 μl-reaction mixture containing 100 mM KCl and buffer. Experiments were performed either in 50 mM sodium acetate (pH 4) or in 6 mM sodium phosphate buffer, pH 7.5. Incubation was at 37°C, at the times indicated in the legends to figures.

Adenine was measured by LC/MS on a Waters Alliance/zq apparatus. Chromatography to separate adenine was on a Waters XTerra MS C18 column (2.1×50 mm, 2.5 μm beads) equilibrated and eluted in 10 mM ammonium acetate (solvent A)/methanol (solvent B) at 0.3 ml/min at 15°C. Equilibration was in 98% A, after sample injection (100 μl) the column was eluted (2 min) with equilibration solvents, and then with 90/10 (A/B) for 5 min. Tightly bound material was then eluted with 20/80 (acetonitrile/A) (0.6 min) and equilibration was obtained with 1.2 min of 90/10 followed by 9 min of 98/2 (A/B). MS analysis was in positive electrospray with single ion recording (135+1 *m/z*) on a split flow of approximately 50 μl/min. Parameters were optimised manually for maximum sensitivity in present column elution conditions. Duplicate chromatograms were combined with Micromass MaxLynx software to reduce noise. Data are the mean of two experiments after subtraction of adenine spontaneously released from the substrate. Samples containing standard adenine (from 1 to 30 pmol) were incubated and processed together with the experimental samples. A standard curve was fitted by linear regression analysis and released adenine from experimental samples was determined by plotting against the standard curve.

2.5. Treatment of poly(ADP-ribosyl)ated PARP with PARG

Treatment with poly(ADP-ribose) glycohydrolase (PARG; 1 U/ml, Alexis Biochemicals) was performed where indicated (see Fig. 3) at the end of the incubation described above. The 50 μl-reaction mixtures were brought to 70 μl by the addition of 1.4×10^{-3} U of PARG and 2-mercaptoethanol to the final concentration of 10 mM. In the case of treatment with saporin-L2 at pH 4, the reaction mixtures were back-titrated to neutral pH by adding 15 μl of 1 M Na_2HPO_4. Incubation was for 10 min at 37°C. After treatment with saporin-L2 and/or PARG, the integrity of [^{32}P]poly(ADP-ribose) chains of PARP was analysed on a 15-cm-long, 0.75-mm-thick 5% polyacrylamide gel in 16 mM HEPES–KOH, pH 7.5, 16 mM Na acetate, 0.1 mM EDTA. Electrophoresis was at room temperature for 3 h at 300 V. The same samples were also analysed on the polyacrylamide gel described above containing 1% SDS. Electrophoresis was at room temperature for 2 h at 300 V. Autoradiographies were performed using an intensifying screen.

3. Results and discussion

We have obtained auto modification of PARP when a synthetic DNA extensively deadenylated by saporin-L2 was employed as a source of damaged DNA in poly(ADP-ribosyl)ation assays in vitro (see Section 2).

All RIPs tested depurinated auto modified PARP (Fig. 1). The activity of saporin-L2 was almost linear with time for at least 3 h, whereas that of other RIPs slowed down considerably after 1–2 h (Fig. 2). This indicates that saporin-L2 can remove more adenine residues than other RIPs do. A similar difference was observed in the effects of RIPs on ribosomes, from which saporin-L2 and other saporins removed several adenine residues per ribosome, and other RIPs only one [16]. These results suggest a different substrate specificity of the various RIPs, possibly linked to the sequence of the bases in the vicinity of adenosine or to different accessibility of the different adenosine residues.

The release of adenine from auto modified PARP prompted us to investigate the effect of deadenylation on the integrity of poly(ADP-ribose) chains of, and consequently on the level of, auto modified PARP. The saporin-L2-catalysed deadenylation of ^{32}P-labelled auto modified PARP clearly induced a reduction of the intensity of PARP bands both at pH 7.5 (Fig. 3a, lane 3) and pH 4 (Fig. 3b, lane 3). Presumably this was due to degradation of the poly(ADP-ribose) chains linked to PARP, since a similar reduction was obtained by the action of PARG

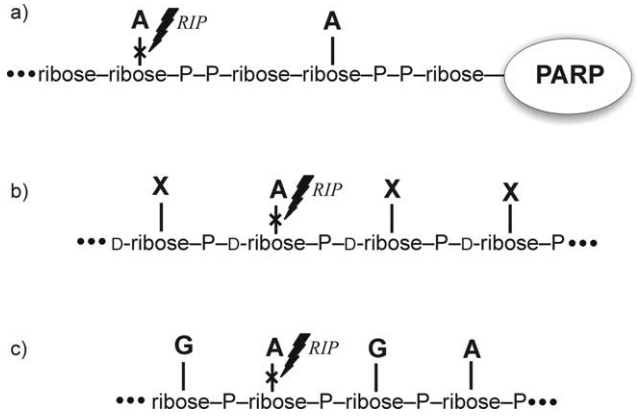

Fig. 1. Schematic representation of the action of RIPs on different substrates. a: Poly(ADP-ribosyl)ated PARP; b: DNA (X represents any base); c: RNA (Endo's sequence).

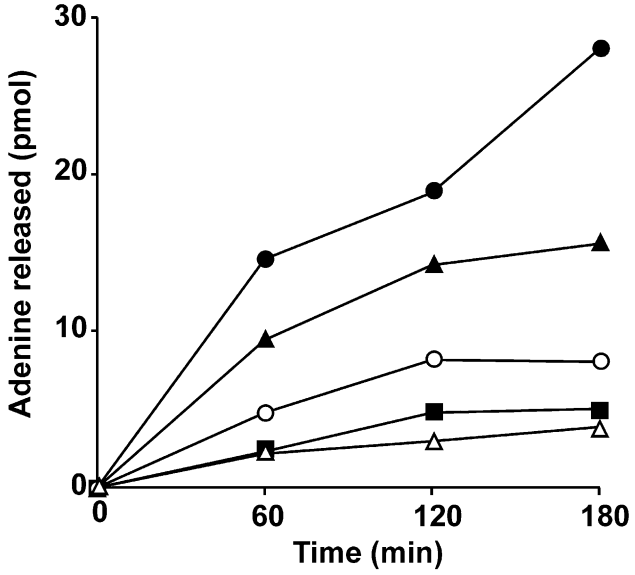

Fig. 2. RIP-catalysed release of adenine from poly(ADP-ribosyl)ated PARP. [^{3}H]Poly(ADP-ribosyl)ated PARP (0.85 pmol) was incubated in the absence or in the presence of 100 nM (5 pmol) RIPs (● saporin-L2, ○ ricin, ■ saporin-S6, △ gelonin, ▲ momordin I) in 50 μl-reaction mixtures containing 50 mM sodium acetate, pH 4, and 100 mM KCl. After incubation at 37°C, at the indicated times the adenine released was measured by LC/MS on a Waters Alliance/zq apparatus as previously described [21]. The PARP-free control reaction mixtures obtained during the preparation of poly(ADP-ribosyl)ated PARP (see Section 2) when treated with RIPs gave values similar to the background release of adenine.

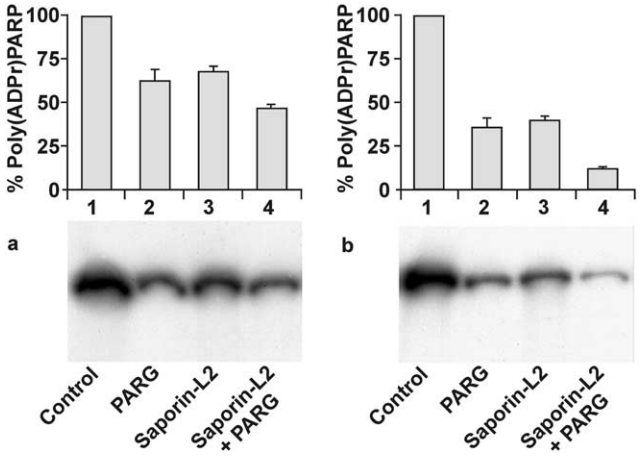

Fig. 3. PAGE analysis of poly(ADP-ribosyl)ated PARP after RIP and/or PARG treatment. [^{32}P]Poly(ADP-ribosyl)ated PARP was treated (3 h at 37°C) with saporin-L2 in (a) 6 mM sodium phosphate buffer (pH 7.5), 100 mM NaCl or in (b) 50 mM sodium acetate, pH 4, 100 mM NaCl. Treatment (10 min at 37°C) with PARG (0.02 U/ml, Alexis Biochemicals) was performed at pH 7.5 at the end of the previous incubation as described by Shah et al. [15]. The integrity of the poly(ADP-ribose) chains of PARP was analysed on 5% polyacrylamide gel in 16 mM HEPES–KOH, pH 7.5, 16 mM Na acetate, 0.1 mM EDTA. The intensities of bands from three independent experiments were quantified and mean data are depicted in the histogram. Error bars represent standard deviation. SDS–PAGE analysis performed on the same samples gave very similar results.

on the same substrate (Fig. 3a, lane 2; Fig. 3b, lane 2). In the experiments carried out at physiological pH, concentrations of saporin-L2 20-fold higher had to be used to induce the same release of adenine obtained at pH 4 (see Fig. 2), i.e. about 10% of total adenine residues present in the assay. The breakdown of poly(ADP-ribose) chains, more evident at pH 4 rather than at pH 7.5 (Fig. 3a, lane 3 vs. Fig. 3b, lane 3), might be due to a weakening of the pyrophosphate linkage of the polymer and/or to a loosening of the α(1″–2′) or α(1‴–2″) ribosyl–ribose glycosidic bonds after adenine removal. A direct action of saporin-L2 on the latter glycosidic bonds appears unlikely, since RIPs acting on DNA and rRNA specifically cleave an *N*-glycosidic bond [6,7].

The major mechanism to repair abasic sites in mammalian cells is the long-patch base excision repair (BER) pathway [17]. The role of PARP during DNA repair is now clearly established [18], since NAD^+-dependent auto modification

Table 1
Cytotoxicity to, and transformation of BALB/c 3T3 cells by RIPs

Additions		Colonies/plate	Clonal efficiency	Media foci/plate	$P \leq$	Transformation frequency	$P \leq$
None		65.5 ± 1.2	0.164	0.16 ± 0.11	*	1.0 ± 0.7	*
DMSO	0.5%	53.5 ± 5.7	0.134	0.11 ± 0.11	*	0.8 ± 0.8	*
3-Methylcholanthrene	2.5 μg/ml	39.0 ± 2.8	0.100	1.08 ± 0.37	0.04	10.8 ± 3.8	0.02
Saporin-L2	2.5×10^{-9} M	45.2 ± 0.8	0.113	0.60 ± 0.22	*	5.3 ± 2.0	0.04
	2.5×10^{-8} M	22.2 ± 1.8	0.055	1.41 ± 0.22	< 0.001	25.8 ± 4.1	0.002
	2.5×10^{-7} M	10.5 ± 1.6	0.026	7.90 ± 0.90	< 0.001	304.2 ± 34.9	< 0.001
Saporin-S6	10^{-10} M	65.0 ± 1.4	0.162	0.08 ± 0.08	*	0.5 ± 0.5	*
	10^{-9} M	65.7 ± 4.1	0.164	0.10 ± 0.10	*	0.6 ± 0.6	*
	10^{-8} M	23.7 ± 1.9	0.060	1.08 ± 0.33	0.01	18.0 ± 5.6	0.01
Gelonin	10^{-11} M	72.2 ± 0.7	0.180	0.36 ± 0.15	*	2.0 ± 0.8	*
	10^{-10} M	52.2 ± 0.8	0.130	0.41 ± 0.19	*	3.2 ± 1.5	*
	10^{-9} M	40.2 ± 0.6	0.100	0.33 ± 0.14	*	3.3 ± 1.4	*
Momordin I	5×10^{-10} M	62.2 ± 3.7	0.155	0	*	0	*
	5×10^{-9} M	37.0 ± 3.0	0.082	0.33 ± 0.18	*	3.6 ± 2.0	*
	5×10^{-8} M	8.5 ± 0.6	0.021	0.60 ± 0.24	*	28.6 ± 11.6	0.03
Ricin	10^{-14} M	59.2 ± 4.2	0.148	0.25 ± 0.13	*	1.7 ± 0.9	*
	10^{-13} M	68.5 ± 3.9	0.171	1.08 ± 0.43	0.05	6.3 ± 2.5	*
	10^{-12} M	64.0 ± 0.7	0.160	1.00 ± 0.30	0.01	6.3 ± 1.9	0.001

*$P > 0.05$. Experimental conditions are described in the text.

Table 2
Depurination of hsDNA by RIPs

RIPs	Depurination of hsDNA (pmol adenine released/pmol enzyme/40 min)
Type 1	
Saporin-L2	622.9
Saporin-S6	12.2
Gelonin	8.6
Momordin I	0.5
Type 2	
Ricin	5.0

Experimental conditions were: 10 μg hsDNA, the appropriate amount of RIPs, 50 mM sodium acetate, pH 4, 100 mM KCl, in a final volume of 50 μl for 40 min at 37°C. Reaction was stopped and adenine measured by LC/MS on a Waters Alliance/zq apparatus. Other experimental details are described in the text.

of PARP is required for BER to be completed [19,20]. In view of this, the removal of adenine from DNA, together with the deadenylation of auto modified PARP, suggest that, if these modifications occurred in vivo, RIPs could cause cell transformation. This was tested in parallel with the cytotoxicity (Table 1). Indeed, all RIPs tested but gelonin induced transformation of 3T3 cells, sometimes at concentrations causing little (saporin-L2) or even no cytotoxicity at all (ricin). The transforming activity of the various RIPs, in most cases higher than that of 3-methylcholanthrene, was not related to their effect on DNA (Table 2) but, approximately, to their activity on auto modified PARP (Fig. 2). Additional damage may come from the deadenylation of other proteins, since RIPs seem to deadenylate efficiently poly(ADP-ribosyl)ated unfractionated whole histones (unpublished results from our laboratory).

We have previously shown that toxic RIPs, such as ricin, induce precocious damage to nuclear DNA in cultured cells, in addition to the well-known inactivating effect on ribosomes [21]. The nature of the nuclear DNA injury is consistent with the enzymatic activity (adenine release) of the toxins on RNA-free chromatin and on naked DNA in vitro, involving mainly the formation of abasic sites. Present results strongly suggest that the deadenylation of auto modified PARP may concur to the damage. The activity of PARP was enhanced in the course of ricin-induced apoptosis, causing the depletion of NAD^+ and subsequently of ATP, prevented by an inhibitor of PARP [22]. The deadenylation of auto modified PARP induced by RIPs could make more substrate PARP available, thus leading to further ADP-ribosylation of PARP and increased depletion of NAD^+ and ATP. This, together with the impaired repair of damaged DNA, would lead to the necrosis induced by lethal amounts of ricin and other RIPs.

Besides concurring to the cytotoxicity, the RIP-induced deadenylation of DNA together with the damage to the auto modified PARP, an enzyme involved in the DNA repair system, may lead to cell transformation. It has been reported that a single molecule of ricin and other type-2 RIPs can kill a cell [23]. Present results indicate that this cannot be assumed for all RIPs, since the transformation is the result of a cell damage, which obviously is non-lethal. RIPs are present in edible plants, some of which, e.g. spinach [24], are sometimes eaten raw. However, the risk that they might be dangerous due to their transforming activity seems remote because these proteins are present at low level, and presumably are largely hydrolysed in, and absorbed to a limited extent from, the gastrointestinal tract. Equally, the transforming activity should not prevent the use of RIPs-containing immunotoxins, advocated in the therapy of cancer [4], autoimmune and viral diseases [5], as most approved anti-tumour drugs have transforming and actually carcinogenic activity.

Some RIPs, known as Shiga toxins (reviewed in [25]), are produced by pathogenic bacteria (*Shigella dysenteriae* and some strains of *Escherichia coli*). In view of the present results obtained with plant RIPs, the possibility that Shiga toxins may induce transformation of target cells during human infections needs to be carefully investigated.

An adenine glycosylase activity, apparently similar to that of RIPs, seems to exist in animal tissues, in which, like in plants [26], it is enhanced after adverse stimuli (stress or viral infection) [9]. Thus it is conceivable that in animal tissues some stimuli may enhance an enzyme activity that may cause cell transformation.

Acknowledgements: This study was supported by the University of Bologna, Funds for Selected Research Topics, the Ministero dell'Istruzione, dell'Università e della Ricerca, the Ministero della Salute, the Associazione Nazionale per la Ricerca sul Cancro, and the Pallotti's Legacy for Cancer Research.

References

[1] Barbieri, L., Battelli, M.G. and Stirpe, F. (1993) Biochim. Biophys. Acta 1154, 237–282.
[2] Van Damme, E.J.M., Hao, Q., Chen, Y. and Barre, A. (2001) Crit. Rev. Plant Sci. 20, 395–465.
[3] Wang, P. and Tumer, N.E. (2000) Adv. Virus Res. 55, 325–355.
[4] Frankel, A.E., Kreitman, R.J. and Sausville, E.A. (2000) Clin. Cancer Res. 6, 326–334.
[5] Knechtle, S.J. (2001) Philos. Trans. R. Soc. Lond. B 356, 681–689.
[6] Endo, Y. and Tsurugi, K. (1987) J. Biol. Chem. 262, 8128–8130.
[7] Barbieri, L., Gorini, P., Valbonesi, P., Castiglioni, P. and Stirpe, F. (1994) Nature 372, 624.
[8] Barbieri, L., Valbonesi, P., Bonora, E., Gorini, P., Bolognesi, A. and Stirpe, F. (1997) Nucleic Acids Res. 25, 518–522.
[9] Barbieri, L., Valbonesi, P., Bondioli, M., Alvarez, M.L., Dal Monte, P., Landini, M.P. and Stirpe, F. (2001) FEBS Lett. 505, 196–197.
[10] D'Amours, D., Desnoyers, S., D'Silva, I. and Poirier, G.G. (1999) Biochem. J. 342, 249–268.
[11] Perocco, P., Paolini, M., Mazzullo, M., Biagi, G.L. and Cantelli-Forti, G. (1999) Mutat. Res. 440, 83–90.
[12] Schechtman, L.M. (1985) in: Transformation Assay of Established Cell Lines: Mechanisms and Application (Kakunaga, T. and Yamasaki, H., Eds.), pp. 165–184. IARC Scientific Publications 67, International Agency for Research on Cancer, Lyon.
[13] Lubet, R.A., Kouri, R.E., Curren, R.A., Putman, D.L. and Schechtman, L.M. (1990) Environ. Mol. Mutagen. 16, 13–20.
[14] Brigotti, M., Barbieri, L., Valbonesi, P., Stirpe, F., Montanaro, L. and Sperti, S. (1998) Nucleic Acids Res. 26, 4306–4307.
[15] Shah, G.M., Poirier, D., Duchaine, C., Brochu, G., Desnoyers, S., Lagueux, J., Verreault, A., Hoflack, J.C., Kirkland, J.B. and Poirier, G.G. (1995) Anal. Biochem. 227, 1–13.
[16] Barbieri, L., Ferreras, J.M., Barraco, A., Ricci, P. and Stirpe, F. (1992) Biochem. J. 286, 1–4.
[17] Dogliotti, E., Fortini, P., Pascucci, B. and Parlanti, E. (2001) Prog. Nucleic Acid Res. Mol. Biol. 68, 3–27.
[18] Bürke, A. (2001) BioEssays 23, 795–806.
[19] Satoh, M.S., Poirier, G.G. and Lindahl, T. (1994) Biochemistry 33, 7099–7106.
[20] Herceg, Z. and Wang, Z.-Q. (2001) Mutat. Res. 477, 97–110.
[21] Brigotti, M., Alfieri, R., Sestili, P., Bonelli, M., Petronini, P.G.,

Guidarelli, A., Barbieri, L., Stirpe, F. and Sperti, S. (2002) FASEB J. 16, 365–372.
[22] Komatsu, N., Nakagawa, M., Oda, T. and Muramatsu, T. (2000) J. Biochem. 128, 463–470.
[23] Eiklid, K., Olsnes, S. and Pihl, A. (1980) Exp. Cell Res. 126, 321–326.
[24] Gasperi-Campani, A., Barbieri, L., Battelli, M.G. and Stirpe, F. (1985) J. Nat. Prod. 48, 446–454.
[25] Paton, J.C. and Paton, A.W. (1998) Clin. Microbiol. Rev. 11, 450–479.
[26] Stirpe, F., Barbieri, L., Gorini, P., Valbonesi, P., Bolognesi, A. and Polito, L. (1996) FEBS Lett. 382, 309–312.