Survival and Proliferation of Cells Expressing Caspase-uncleavable Poly(ADP-ribose) Polymerase in Response to Death-inducing DNA Damage by an Alkylation Agent*

(Sabina S. Halappanavar‡§, Yves Le Rhun‡§, Seloua Mounir‡, Luis M. Martins‡, Jacques Huot‡, William C. Earnshaw‡, and Girish M. Shah†**

From the ‡Laboratory for Skin Cancer Research, Laval University Medical Research Center, Centre Hospitalier Universitaire de Québec, Faculty of Medicine, Laval University, Quebec GIV 4G2, Canada, §Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JF, Scotland, United Kingdom, and ¶Centre de Recherche en Cancérologie de l’Université Laval, L’Hôtel-Dieu de Québec, Québec G1K 7P4, Canada

To determine whether caspase-3-induced cleavage of poly(ADP-ribose) polymerase (PARP), a DNA damage-sensitive enzyme, alters the balance between survival and death of the cells following DNA damage, we created stable cell lines that express either caspase-uncleavable mutant or wild type PARP in the background of PARP (−/−) fibroblasts. The survival and apoptotic responses of these cells were compared after exposure to N-methyl-N-nitro-N-nitrosoguanidine (MNNG), a DNA-damaging agent that activates PARP, or to tumor necrosis factor-α, which causes apoptosis without initial DNA damage. In response to MNNG, the cells with caspase-uncleavable PARP were very resistant to loss of viability or induction of apoptosis. Most significantly, ~25% of these cells survived and retained clonogenicity at a level of DNA damage that eliminated the cells with wild type PARP or PARP (−/−) cells. Expression of caspase-uncleavable PARP could not protect the cells from death induced by tumor necrosis factor, although there was a slower progression of apoptotic events in these cells. Therefore, one of the functions for cleavage of PARP during apoptosis induced by alkylating agents is to prevent survival of the extensively damaged cells.

Poly(ADP-ribose) polymerase (PARP),1 EC 2.4.2.30) a DNA repair-associated enzyme is involved in two diametrically opposite responses to DNA damage, i.e. DNA repair along with other survival responses at lower levels of DNA damage and cell death responses after saturating levels of DNA damage. One of the immediate responses to DNA damage in the higher eukaryotic cells is catalytic activation of PARP, which results in consumption of NAD and formation of the polymers of ADP-ribose (pADPr) on PARP itself and a few selected nuclear proteins that are involved in chromatin architecture and DNA-related metabolisms. This rapid reaction by PARP has been implicated in DNA base excision repair and in the maintenance of genomic integrity after DNA damage (reviewed in Refs. 1–5). Recently identified homologs of PARP that can synthesize pADPr raise the possibility that some of the survival functions of full-length PARP may be partially attributable to the PARP homologs (6–11). However, PARP (−/−) mice or cells that possess at least one active PARP homolog (7–9) display high susceptibility to DNA-damaging agents, consistent with a dominant if not exclusive role for full-length PARP in the cellular responses to DNA damage. Two independently created PARP (−/−) mice with interruptions in different exons were shown to be highly susceptible to DNA-damaging agents such as γ-rays and alkylating agents (12, 13). Despite the initial lack of consensus among different studies with PARP (−/−) models, it is now clearly emerging that PARP (−/−) cells have significant deficiencies in response to DNA damage. These cells inherently possess shorter telomeres and unstable chromosomes (14), and in response to genotoxic stress, they exhibit impaired proliferation, increased chromosomal abnormalities, G1/M block in cell cycle, and reduced capacity to repair the DNA damaged by alkylating agents (12, 13, 15, 16) (reviewed in Refs. 17–19). Recently, PARP (−/−) cells were shown to be specifically defective in long patch repair pathways of DNA base excision repair (17). Thus, in response to low or moderate levels of DNA damage, the activity of PARP could help in DNA repair and cell survival. However, PARP may have a completely different function(s) when death responses are initiated.

During the initial phase of apoptotic death, when the majority of the cellular proteins remain intact, PARP is one of the earliest proteins to be specifically cleaved to form two fragments of ~89 and 24 kDa (20). The 89-kDa fragment, although capable of the basal enzymatic activity due to presence of the catalytic domain (21), cannot be stimulated by the DNA strand breaks (20, 22). The apoptosis-specific cleavage of PARP at the DEVD/G site was shown to be due to a protease resembling interleukin-1β-converting enzyme (23) that was identified as CPP32, Yama, or Apopain and later classified as caspase 3 (24–27). Although PARP cleavage has served as a sensitive analytical reporter for the activation of caspases or for the onset of apoptosis in various models of cell death, the physiological role for this cleavage is not known (28, 29). For many of the ~50 other caspase targets, their cleavage serves different functions, such as inactivation of anti-apoptotic factors, activation of proapoptotic factors, or disassembly of cellular struc-
tases (30). However, for some substrates such as PARP, DNA-dependent protein kinase, and DNA replication factor C, it has been assumed that disabling their normal survival functions in DNA repair and replication would facilitate cell death (30, 31). Additionally, the cleaved fragments of PARP might acquire novel functions that could actively contribute to apoptosis. The caspase-induced cleavage of PARP might result in production of an enzyme with altered substrate specificity or subcellular localization. This altered enzyme might actively potentiate the apoptotic response. The 24-kDa fragment of PARP has been shown to bind to apoptotic fragments of DNA (32), and this could facilitate death decisions by competing with any remaining intact PARP or other proteins for binding to strand breaks.

The catalytic function of PARP is also implicated in cell death responses following massive amounts of DNA damage that are encountered during certain pathological conditions. A prolonged and high level of catalytic activation of PARP under these circumstances has been suggested to lead to cell death by energy deprivation due to depletion of NAD and ATP (33). This role of PARP is strongly supported by recent studies demonstrating resistance of PARP (−/−) neuronal, cardiac and pancreatic cells to death caused by extensive DNA damage with ischemia-reperfusion or streptozotocin (34–37). This function of PARP in cell death would be eliminated when it is cleaved by caspasas, because the 89-kDa fragment of PARP cannot be stimulated in the presence of DNA breaks (20, 22).

Thus, PARP has been implicated in both the survival and cell death responses following DNA damage. PARP cleavage by caspasas could play a crucial role in shifting the equilibrium between its two roles, and this can be explored in a model where PARP is rendered resistant to cleavage by caspase 3. The caspase-resistant PARP could strengthen the cellular responses to DNA damage, resulting in delayed cell death and possible survival of the damaged cells. On the other hand, uncleavable PARP could accelerate death by continued depletion of NAD and ATP. To distinguish between these alternatives, we have created stable cell lines that express either caspase-uncleavable mutant or wild type PARP in a PARP (−/−) fibroblast background. Our study for the first time compared the death and survival responses of these stable cell lines after exposure to alkylating DNA damage that activates PARP as part of the survival response or to tumor necrosis factor-α (TNF-α), which can cause apoptosis without initial DNA damage. We hypothesized that response of the cells expressing caspase-uncleavable PARP would be different in these two situations. In response to alkylating DNA damage, uncleavable PARP might have both survival and death functions, whereas in response to TNF-α it might serve only the death functions.

Three recently reported studies used a similar approach and focused mainly on the death responses of cells expressing caspase-uncleavable PARP (38–40). As described later, one of these studies used a transient transfection approach (38), and the other two studies using stable cell lines focused mainly on the death responses after exposure to TNF-α or staurosporine (39, 40), treatments that are not likely to involve survival functions of PARP induced by DNA damage. We report here that in response to moderate levels of DNA damage by the alkylating agent MNNG, cells expressing caspase-uncleavable PARP exhibit reduced cell death and increased cell survival under conditions where cells with wild type PARP are eliminated. When death is induced in the absence of initial DNA damage by TNF-α, the presence of the caspase-uncleavable PARP could not prevent the ultimate apoptotic demise of the cell, and in fact, the progression of apoptosis is slower in cells expressing wild type PARP.
were exposed to 300 C the automodified uncleavable PARP at 113 kDa. B resolved on 15% SDS-polyacrylamide gel electrophoresis and trans- 

buffer (40 mM potassium cacodylate, 40 mM KCl, 0.2 mM EDTA, 0.8 mM A result in clones A12, A18, and C9 (phenotypes. Starting with A1 cells from PARP (Asp214) to alanine (Ala). Several stable clones were isolated by indirect immunofluorescence (data not shown), in agree-

ment with earlier studies (38–40).

The catalytic and DNA damage sensor functions of PARP-

D/A were analyzed from two different perspectives: its potential to make pADPr in vitro by an activity-Western blot method (Fig. 1B) and the actual pADPr synthesis occurring in the cells expressing PARP-D/A by a polymer immunoblot method (Fig. 1C).

In the activity-Western blot (Fig. 1B), a signal for automodified PARP-D/A immobilized at 113 kDa confirmed its basal catalytic function (lane 5), which could be stimulated in the presence of damaged DNA (lane 3). The capacity of PARP-D/A to form pADPr in the oxidant-exposed cells was quite similar to that of PARP-wt, as seen in the ADP-ribose polymer immunoblot (Fig. 1C). Since PARP is the major acceptor for its own reaction, the signal for PARP automodified to varying degrees can be detected in the region of 113–200 kDa (1, 47, 48). Within the first 5–15 min after exposure to 300 μM H2O2, cells expressing PARP-wt or PARP-D/A exhibited a strong signal in the region from 113–200 kDa (Fig. 1C, lanes 7, 8, 12, 13, and 17). In each of these clones, this signal was absent before (Fig. 1C, lanes 6, 11, and 16) or 30 min after exposure to oxidant (lanes 9, 15, and 20). Similar rapid and transient formation of pADPr was detected in the PARP (wt or D/A) clones after exposure to 10–100 μM MNNG (data not shown). In contrast, PARP (−/−) cells exhibited no significant oxidant-induced changes in polymer signal in the region above 113 kDa (lanes 1–5). While examining the PARP (−/−) cells for activity of the PARP homologs, we observed an ~70-kDa polypeptide that was weakly stimulated by DNA in the activity-Western blot (Fig. 1B, lanes 2 versus 4 and 3 versus 5). However, in the polymer immunoblot, the ~70-kDa signal was observed in both untreated control and oxidant-exposed cells (Fig. 1C, lanes 1 and 3). Therefore, this polypeptide may contribute to very low basal levels of pADPr synthesis in PARP (−/−) cells, although, unlike the full-length PARP, it was not stimulated in response to oxidant damage.

In summary, despite the presence of a mutation within its DNA binding domain, caspase-uncleavable D214A mutant PARP was a fully functional enzyme capable of translocating to the nucleus, becoming activated in response to DNA damage and rapidly forming pADPr. Its resistance to cleavage during apoptosis was confirmed in the studies described below.

Resistance of Cells Expressing Caspase-uncleavable Mutant PARP to DNA Damage by an Alkylating Agent—PARP function is likely to have a maximal impact on cell survival after exposure to DNA-damaging agents that activate the enzyme as part of responses involving base excision repair. This was demonstrated by the extreme susceptibility of PARP (−/−) mice to methylmethosurea and irradiation (12). Therefore, cells expressing different forms of PARP were challenged for 2 h with 10–100 μM MNNG, an alkylating agent that activates PARP (49). There was no loss of viability for any of the cell lines immediately after a 2-h exposure to all doses of MNNG (data not shown). However, differences emerged between cells expressing different PARP phenotypes when survival and death responses were monitored over the next 7 days. We would like to emphasize that comparable results were obtained in each experiment, with several different clones for each PARP genotype, indicating that the effects were attributable to the given PARP phenotype.

The differences in susceptibility to MNNG-induced DNA damage among different clones were observed when viability was monitored 24 h after exposure to MNNG (Fig. 2). At the lowest level of DNA damage (10 μM MNNG), ~60% of PARP (−/−) cells lost viability, as compared with only 25 and 10% loss of viability for cells expressing PARP-wt and PARP-D/A, respectively. At higher levels of DNA damage (50–100 μM MNNG), cells with PARP-wt became as sensitive to MNNG as the PARP (−/−) cells. Cells expressing PARP-D/A continued to...
show more resistance, with 50% viability even at 100 μM MNNG. Thus, PARP (-/-) cells were very sensitive to low as well as high doses of MNNG, whereas cells with PARP-wt could comparatively resist lower levels of DNA damage but succumbed to higher levels of DNA damage. In contrast, cells with uncleavable mutant PARP were significantly more resistant across the entire range of MNNG-induced damage.

That PARP catalytic activity contributes to the higher viability observed at 24 h after exposure to 10 μM MNNG in cells expressing PARP-D/A was suggested by experiments using a potent PARP-inhibitor, DHQ (41, 50). Preincubation of cells expressing PARP-D/A for 2 h with 100 μM DHQ made these cells as susceptible to MNNG as the PARP (-/-) cells (Table I). DHQ also abolished the protection observed in the PARP-wt cells (data not shown). The effect of DHQ in MNNG-treated cells was over and above the mild cytotoxicity observed for cells expressing PARP-D/A. Thus, catalytic function of caspase-resistant or wild type PARP provides an early protection against MNNG-induced DNA damage.

To determine if the cells that retained viability at 24 h were in the process of DNA repair and recovery or if they were undergoing a delayed cell death response, we monitored their cell cycle profile from 24 to 72 h after exposure to MNNG (Fig. 3). As anticipated from the viability data, PARP (-/-) cells were most susceptible, and this was evident at 24, 48, and 72 h after exposure to 10 μM MNNG. These cells exhibited a decrease in the G1 peak, accumulation in G2/M, and appearance of hypodiploid apoptotic cells. Cells with PARP-D/A showed no major changes in the cell cycle at 24 h, but by 48–72 h there was a decrease in the G1 peak and a small increase in the G2/M peak accompanied by a large number of hypodiploid apoptotic cells. Therefore, although nearly 75% of PARP-wt cells retained viability at 24 h (see Fig. 2), these cells were beginning to succumb to DNA damage from 48 h after exposure to 10 μM MNNG. In contrast, all three clones expressing PARP-D/A were comparatively resistant to changes in the cell cycle up to 72 h after treatment with 10 μM MNNG. Upon exposure to higher concentrations of MNNG (50 μM), PARP (-/-) and PARP-D/A cells lost virtually the entire G1 peak, whereas PARP-D/A cells exhibited a significant, though reduced, G1 peak (data not shown).

Since cells with PARP-wt were beginning to exhibit death responses from 48 h, we monitored the apoptotic processes occurring in different cells at 48 h after exposure to low (10 μM) and high (100 μM) concentrations of MNNG (Fig. 4). In TUNEL assay, cells with PARP-wt displayed a dose-dependent increase in DNA fragmentation from 10 to 100 μM MNNG (Fig. 4A, top panel). Caspase activation (Fig. 4B, lane 4) and PARP cleavage (Fig. 4C, lanes 4 and 5) had also begun by 48 h in the cells expressing PARP-wt, reflecting the appearance of hypodiploid apoptotic cells during cell cycle analysis. PARP (-/-) cells were most susceptible to MNNG, with a significant loss of DNA and a high degree of TUNEL labeling observed in response to 100 μM MNNG (Fig. 4A, middle panel). These cells also presented the strongest activation of caspase 3 (Fig. 4B, lane 8). As anticipated, neither PARP nor its cleavage products could be seen in the PARP-deficient cells (Fig. 4C, lanes 2 and 3). Cells expressing PARP-D/A also exhibited a dose-dependent increase in DNA fragmentation in response to MNNG (Fig. 4A, bottom panel), suggesting that death responses were initiated in a fraction of these cells. However, the extent of TUNEL reactivity was less severe for PARP-D/A cells as compared with cells with other PARP phenotypes. The lower levels of apoptotic DNA fragmentation observed in PARP-D/A cells at 48 h after exposure to 100 μM MNNG may reflect an ongoing process of DNA repair and delayed apoptosis or both. The reduced susceptibility of cells expressing PARP-D/A was also evident in the negligible levels of activation of caspase 3 detected at 48 h after exposure to 100 μM MNNG (Fig. 4B, lane 6). As expected, the D214A caspase-uncleavable mutant PARP remained intact after MNNG treatment (Fig. 4C, lanes 6 and 7). Thus, cells expressing uncleavable PARP-D/A exhibited a less severe apoptotic response to MNNG as compared with PARP (-/-) or PARP-wt cells.

Next, we examined whether the apparent resistance of cells with uncleavable PARP to MNNG-induced DNA damage was just a highly delayed death response or whether these cells could actually recover, resume proliferation, and form colonies (Fig. 5). After exposure to a low dose of MNNG (10 μM), cells expressing PARP-D/A almost completely lost their clonogenicity (0.2%), whereas nearly 25% of the cells expressing PARP-D/A recovered and formed colonies. In contrast, almost 4% of the PARP (-/-) cells formed colonies after exposure to 10 μM MNNG. At higher MNNG doses of 50 μM, although all the PARP (-/-) and PARP-wt cells lost clonogenicity, about 1% of the PARP-D/A cells formed colonies. Only at the highest dose of 100 μM MNNG, none of the cells with different PARP phenotypes retained clonogenicity (data not shown). We confirmed these results using at least two more cell lines for each of the PARP phenotypes, e.g. PARP (-/-) cells (A1, A11), PARP-wt cells (B57 and F20), and PARP-D/A cells (A12 and A18).

In summary, our results show that PARP (-/-) cells are very sensitive to DNA damage by alkylating agent, whereas cells with wild type PARP initially resist lower levels of DNA damage but eventually succumb once apoptosis is initiated. In contrast, cells with uncleavable PARP retain higher viability and exhibit diminished cell cycle alterations and comparatively reduced apoptotic response, and most significantly, nearly one-
fourth of these cells could recover sufficiently to resume proliferation.

Apoptotic Response in Cells Expressing Uncleavable PARP Exposed to Tumor Necrosis Factor-α—Treatment of cells with TNF-α triggers an apoptotic process that does not begin with DNA damage but instead involves activation of a caspase cascade downstream of membrane receptor ligation (51). To determine whether the effects of uncleavable PARP on apoptosis reflect an alteration in the response to DNA damage or some more general interference with the apoptotic pathway, we analyzed TNF-α-induced apoptosis in this model (Fig. 6). After treatment with 800 units/ml (i.e. 2 ng/ml) TNF-α and 0.25 µg/ml actinomycin D, all the cells from all the clones were nonviable after 24 h (Fig. 6A). This total cell killing was observed even at the lower dose of 100 units/ml TNF-α. This result shows that resistance of cells expressing uncleavable PARP to MNNG was not due to the absence of alternative functional apoptotic pathways. It also confirms that TNF-α-induced apoptosis can be accomplished in absence of PARP or its cleavage products. However, these results do not rule out the participation of PARP in the death process, and this was made evident during the analysis of the early phase of TNF-α-induced apoptosis.

Six h after TNF-α treatment, ~50–60% of the PARP (−/−) cells were viable, whereas only 1% of the cells with PARP-wt and ~10% of the cells with PARP-D/A were viable (Fig. 6A). All the cell types had initiated caspase 3 activation 6 h after treatment with TNF-α (Fig. 6B). PARP-wt cells had completed the processing of procaspase 3 (Fig. 6B, lane 4). Correspondingly, all the PARP was processed to the 89-kDa apoptotic fragment in these cells (Fig. 6C, lane 3). In the cells with PARP-D/A, apoptosis had been initiated, as evident from activation of caspase 3 (Fig. 6B, lane 6). However, mutant PARP, being uncleavable by caspase 3, remained intact at 6 h after treatment (Fig. 6C, lane 5). At 6 h after exposure to TNF-α, no necrosis-specific fragments of the uncleavable PARP were detectable at 50, 40, or 35 kDa, as reported first for the necrotic HL-60 cells (42) and later for the other cell types (52).

DISCUSSION

At present, nearly 50 proteins are known to be cleaved by caspases during apoptotic death. Cleavage of some of these proteins actively promotes death, whereas cleavage of others may be permissive for the death pathway. If cleavage of these substrates were essential for apoptotic progression, then introduction of uncleavable forms of the protein might be expected to exert a significant protective effect. However, no long term protective effects have been reported after introduction of several caspase-resistant substrates, e.g. Bcl-2 (53), RB1 (54), p21Cip1/WAF1 (55), MEKK-1 (56), PAK-2 (57), DFF45 (58), and the lamins (59). Our study demonstrates for the first time that expression of a caspase-uncleavable PARP not only temporarily
Resistance of Cells with Caspase-uncleavable PARP to MNNG

A. PARP cleavage by caspase 3 resulting in formation of the signature 89-kDa fragment was monitored in the cells 48 h after exposure to 10 or 100 μM MNNG. B. PARP cleavage by caspase 3 was detected by immunoblotting for caspase 3. C. PARP cleavage by caspase 3 resulting in formation of the signature 89-kDa fragment was monitored in the cells 48 h after exposure to 10 or 100 μM MNNG. Apoptotic HL-60 cells were used for identification of the 89-kDa fragment of PARP. Identical results were obtained in three independent experiments.

Protection offered by uncleavable PARP to low levels of DNA damage must be subject to influence by other factors, because only 25% of the cells retain clonogenicity (Fig. 5). This partial penetrance could arise if the susceptibility of cells to apoptosis-inducing signals varies as a function of cell cycle position. In many models of cell death, it has been observed that after receipt of a death-inducing signal, the actual execution of individual cells occurs asynchronously. It could be that execution of the cell after receipt of a lethal signal is a random process, or more likely, it may be influenced by other factors, such as cell cycle position of each cell (61). Earlier studies have demonstrated the cell cycle-specific ADP-ribosylation of proteins in response to γ-irradiation (62). Together, our results suggest that rescue of cells by caspase-uncleavable PARP may be efficient only for cells in a specific phase of the cell cycle that receive a low dose of DNA damage of the type that normally activates PARP.

The higher susceptibility of PARP (−/−) cells to MNNG is in agreement with an earlier study that used another alkylating agent, methyl methane sulfonate to induce the DNA damage (38). However, a selective survival advantage for cells expressing uncleavable PARP, shown here, was not observed in that study (38). Apart from limitations in the experimental analyses of cell death in transient transfections, the earlier study also used a very high dose of 500 μM that was lethal within 1 h. In contrast, we used lower doses of MNNG that caused no initial lethality, whereas the use of stable clones permitted us a more reliable survival estimate by analyzing an entire population of cells with an identical PARP phenotype.

Importantly, we found that in cells treated with high levels of MNNG (100 μM), the presence of uncleavable PARP exerted neither a protective nor harmful effect. We did not observe a high stimulation of catalytic function of the uncleavable PARP from 24 to 96 h after exposure to high doses of MNNG (data not shown). It is therefore unlikely that consumption of NAD by catalytic activity of the uncleavable PARP was critical in death caused by higher levels of MNNG-induced DNA damage. It is
interest to compare these results with recent demonstrations of the role of NAD consumption by PARP in cell death caused by ischemia-reperfusion or streptozotocin (34–37). These pathological conditions induce massive amounts of DNA damage and cause death of specific target cells by both apoptosis and necrosis (18, 34, 63). Under these circumstances, all or most of the wild type PARP must remain uncleaved by the caspases, because death of the target cells is linked to NAD and ATP depletion by the highly activated PARP. Together, our results suggest that the extent of DNA damage, type of death-inducing agent, and susceptibility of the target cell might determine whether catalytic or cleavage functions of PARP would be implicated in survival or apoptotic/necrotic death of the cell.

Our results suggest that stimulation of the TNF-α-induced death pathways is more strongly correlated with caspase cleavage of the wild type PARP than with the catalytic function of the uncleavable PARP. This is observed from the highest (50-fold) susceptibility of PARP-wt cells and intermediate (5-fold) susceptibility of PARP-DA cells, as compared with PARP (−/−) cells (Fig. 6a). In contrast to our results, two recent studies reported that cells expressing uncleavable PARP are more prone to TNF-α-induced apoptosis (39, 40) as well as necrosis (39), as compared with PARP-wt or PARP (−/−) cells. However, 6 h after TNF-α treatment, we did not observe necrotic death in the cells expressing uncleavable PARP. Unlike the earlier study (39), we used much lower concentrations of TNF-α (10–160× less) and actinomycin D (4× less), because in our experience treatment with higher doses of actinomycin D (1 μg/ml) was sufficient to elicit death response even in the absence of TNF-α. Thus, a mixed mode of death induced by both actinomycin D and TNF-α in the other studies could explain the differences observed in our studies. It is also possible that together, our studies reflect different snapshots of the TNF-α-treated cells, i.e. reduced apoptosis at 6 h, increased form of necrotic death at 12 h, and complete loss of viability by 24 h.

In conclusion, the most significant advantage associated with expression of uncleavable PARP was observed when DNA damage was low enough for the cells to remain viable for a significant period of time before commitment to either survival or death. At this stage, cells with wild type PARP succumbed, whereas some of the cells with uncleavable PARP survived. Therefore, PARP activation and its cleavage might influence crucial life and death decisions relatively early in the apoptotic pathway.

Acknowledgments—We are thankful to following researchers for providing different reagents: Dr. Z. Q. Wang for PARP (-/-) and (+/+ ) cell lines; Drs. M. Miwa and A. Burkle for monoclonal anti-polymer 10H; Dr. J. H. Kupper for cDNA of human PARP; Dr. G. Poirier for monoclonal anti-PARP C-2–10; and Dr. D. Nicholson for rabbit polyclonal anti-caspase 3-R#MF393. We also thank Dr. Z. Q. Wang for critical reading of the manuscript. M. Dufour at the Flow Cytometry Service of the CHUL Research Center carried out fluorescence-activated cell sorter analysis.

REFERENCES
1. Lindahl, T., Satoh, M. S., Poirier, G. G., and Klungland, A. (1995) Trends Biochem. Sci. 20, 405–411
2. Lautier, D., Lagueux, J., Thibodeau, J., Menard, L., and Poirier, G. G. (1993) Mol. Cell. Biochem. 122, 171–178
3. de Murcia, G., and Menissier-de Murcia, J. (1994) Trends Biochem. Sci. 19, 172–176
4. Hei, Y., Griesenbeck, J., and Schweiger, M. (1997) Rev. Physiol. Biochem. Pharmacol. 131, 127–173
5. Oliver, F. J., Menissier-de Murcia, J., and de Murcia, G. (1999) Am. J. Hum. Genet. 64, 1292–1298
6. Babichuk, E., Cottrill, P. B., Storozhenko, S., Fuangthong, M., Chen, Y., O’Farrell, M. K., Van Montagu, M., Inze, D., and Kushner, S. (1998) Plant J. 13, 635–645
7. Amé, J. C., Rolli, V., Schreiber, V., Niedergang, C., Apiou, F., Decker, P.,
