Functional Competition between Poly(ADP-ribose) Polymerase and Its 24-kDa Apoptotic Fragment in DNA Repair and Transcription*

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Poly(ADP-ribose) polymerase is a 113-kDa nuclear enzyme that binds to both damaged DNA and to RNA associated with actively transcribed regions of chromatin. Binding of poly(ADP-ribose) polymerase to DNA lesions activates it, catalyzing the covalent addition of multiple ADP-ribose polymers to the enzyme (autonomous modification). During apoptosis, poly(ADP-ribose) polymerase is cleaved by caspase-3, resulting in the formation of an N-terminal 24-kDa fragment, containing the DNA binding domain, and a C-terminal 89-kDa catalytic fragment. The functional relevance of this cleavage is not well understood. We therefore prepared a recombinant 24-kDa poly(ADP-ribose) polymerase fragment and investigated the role of this fragment in DNA repair and transcription. The 24-kDa fragment retained its binding affinity for both DNA breaks and RNA. In an in vitro cell-free DNA repair assay, this fragment inhibited rejoining of DNA breaks and suppressed ADP-ribose polymerase formation by competing with poly(ADP-ribose) polymerase in binding to DNA breaks. With regard to transcription, it has recently been demonstrated that binding of poly(ADP-ribose) polymerase to transcribed RNA reduces the rate of transcript elongation and that autonomous modification of poly(ADP-ribose) polymerase bound to DNA breaks results in up-regulation of transcription. We tested the 24-kDa fragment for its ability to suppress transcript elongation, and we found that it competed against the up-regulation of transcription mediated by full-length poly(ADP-ribose) polymerase. The ability of the 24-kDa fragment to inhibit DNA repair, ADP-ribose polymerase formation, and damage-dependent up-regulation of transcription may contribute to the apoptotic shift from cell survival to cell death mode.

Poly(ADP-ribose) polymerase (PARP)† is a highly abundant nuclear enzyme present at about 2 × 10⁵ molecules per nucleus (1). This enzyme is composed of an N-terminal DNA binding domain, containing two zinc finger motifs, a C-terminal NAD⁺ binding domain, catalyzing the synthesis of ADP-ribose polymers from its substrate, NAD⁺, and an autonomic modification site, which unites the N-terminal and C-terminal domains (2). Poly(ADP-ribose)ylation by PARP at the modification site of the protein is initiated by the binding of the zinc fingers to DNA breaks (3, 4). As a consequence of this modification, the binding affinity of PARP for DNA is reduced, resulting in dissociation of PARP from DNA breaks (5) and thereby allowing the DNA repair machinery to access the sites of DNA damage (6).

In cells where DNA breaks are generated by DNA-damaging agents, PARP is activated and autolysed (3, 4), leading to the conclusion that PARP is involved in the cellular response to genetic damage, particularly in the repair of damaged DNA (3). However, PARP has been shown to lack DNA repair activity in itself (6, 7). Alternatively, it has been suggested that PARP is involved in chromatin stabilization (8), in DNA replication (9, 10), and in transcription (11–13), although the roles played by PARP in these processes are not yet understood.

In nuclear localization experiments, PARP is observed in clear foci, associated both with regions of chromatin actively transcribed by RNA polymerase II as well as with nucleoli where rRNA is synthesized by RNA polymerase I (14). Dispersal of the foci upon treatment of cells with the transcription inhibitors actinomycin D or 5,6-dichloro-1-β-ribofuranso-ybenzimidazole suggests an involvement of PARP in transcription (15). In addition, such foci are also dispersed by treatment of isolated nuclei with RNase (16). These observations thus suggest an interaction between PARP and transcribed RNA. Recently, we demonstrated that RNA-bound PARP reduces the rate of RNA elongation by RNA polymerase II and that autonomous modification of PARP in response to DNA damage up-regulates transcription (17). Since DNA-damaging agents induce RNA damage as well, we proposed that this up-regulation allows cells to compensate for the loss of damaged RNA that occurs collaterally with DNA damage and that this pathway is required for cell survival following exposure to DNA-damaging agents (17).

When cells are exposed to sufficiently high levels of DNA-damaging agents, they commit to cell death by inducing either apoptosis or necrosis. During apoptosis PARP is cleaved by the apoptosis-specific protease, caspase-3, resulting in the formation of an N-terminal 24-kDa fragment, containing the DNA binding domain, and a C-terminal 89-kDa catalytic domain, containing the autonomic modification site (18–20). Recently, Halapanavar et al. (21) and Oliver et al. (22) reported that, in PARP knockout cells, expression of uncleavable PARP, lacking the caspase-3 recognition sequence, causes delayed induction of DNA damage-induced apoptosis. This observation suggests

* This work was supported in part by the National Cancer Institute of Canada for the Terry Fox Run and the Canadian Institutes of Health Research; The Canada Foundation for Innovation and the Quebec government provided infrastructure support. The costs of publication of this article were defrayed in part by the payment of page charges. This advertisement is an advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: PARP, poly(ADP-ribose) polymerase; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; ELISA, enzyme-linked immunosorbent assay; TUNEL, terminal dUTP nick-end labeling; DMEM, Dulbecco’s modified Eagle’s medium.
that cleavage of PARP has a role in damage-induced apoptosis. In addition, Herceg and Wang (23) and Boulaires et al. (24) suggested that cleavage of PARP is also required for tumor necrosis factor-α-induced cell death. However, Herceg and Wang (23) found increased cell death by necrosis, whereas Boulaires et al. (24) demonstrated a promotion of apoptotic, rather than necrotic, cell death by expressing uncleavable PARP in PARP knockout cells.

Since the 24-kDa fragment contains the DNA binding domain, which is capable of binding to DNA breaks (25), it has been speculated that the 24-kDa fragment possibly counteracts functions of PARP and promotes the process of apoptosis (19). However, biochemical characteristics of the 24-kDa fragment remain to be elucidated. Thus, we prepared the 24-kDa apoptotic fragment of PARP and asked whether the 24-kDa fragment competes against the functions of PARP in DNA repair, ADP-ribose polymer formation, and transcription.

**MATERIALS AND METHODS**

Cell Line and Antibody—GMO1953A lymphoblastoid cells were obtained from NIGMS Human Mutant Cell Repository (Camden, NJ). The C2-B subline was used against the autoregulatory domain of PARP and the F1–23 antibody against the DNA binding domain of PARP (zinc finger 2) (26) were kindly provided by Dr. G. G. Poirier.

Expression of Recombinant PARP and the 24-kDa Fragment—Full-length PARP cDNA (bases 1–3039) or the sequence corresponding to the 24-kDa DNA binding domain of PARP (bases 1–654), which is found in apoptotic cells (18–20), was cloned into pET3a (Novagen) and used to transform HMS 174 de3 cells (Novagen) together with pLySE (Novagen). After overnight pre-culture, the Escherichia coli were propagated in 2 liters of Luria-Bertani medium in the presence of 34 μg/ml chloramphenicol and 100 μg/ml ampicillin for 3 h, and expression of PARP or the 24-kDa fragment was induced in the presence of 0.4 mM isopropyl-β-D-thiogalactoside for 3 h at 37 °C. The bacteria were then spun down at 3500 × g for 10 min, and the pellet was washed in phosphate-buffered saline and spun down. The resulting pellet was resuspended in 20 ml of buffer containing 0.1 M NaCl, 50 mM Tris-HCl, pH 8.0, 12% glycerol, 2 mM MgCl₂, and 0.1 mM phenylmethylsulfonyl fluoride (Buffer CB), and PARP or the 24-kDa fragment was extracted by sonication. After a 30-min centrifugation at 35,000 × g (at 4 °C), the supernatant was used for purification of PARP or the 24-kDa fragment.

**Purification of PARP—E. coli lysate (750 mg) was applied to a phosphocellulose column (10 mm diameter and 2-ml bed volume) equilibrated with Buffer CB. PARP was eluted using a linear gradient of Buffer CB containing 0.1–2.0 M NaCl. The fractions of interest were pooled and used for the renaturation of PARP or the 24-kDa fragment in the presence or absence of 2 mM NaCl under reaction conditions described previously (27).

Analysis of Poly(ADP-ribosylation) —To determine the amount of ADP-ribose polymers produced in the cell-free DNA repair assay, 1.3 μCi of [32P]NAD⁺ and 0.25 mM NAD⁺ were added to the reaction described above. Reactions were terminated by addition of trichloroacetic acid, and insoluble [32P] activity retained on a GF/C filter was counted as described previously (28).

**Pulse-Chase Elongation Assay—**Pulse-chase elongation assays were carried out as described previously (17). Briefly, a pGEl plasmid was linearized by digestion using ScaI, creating a 90-base G-less sequence at one end of the DNA. A-C-tail was added to allow loading of RNA polymerase II from DNA break ends. The DNA was then incubated with RNA polymerase II (1.0 units) (provided by Dr. H. Serizawa) (29) in the presence of RNasin, ATP, UTP, CTP, and [α-32P]CTP (ATP 37 °C for 30 min as described previously (30). A chase was initiated by addition of GTP and excess CTP in the presence or absence of PARP or the 24-kDa fragment. After incubation for 24 h at 37 °C, DNA was isolated, and then 20 min at 37 °C, and then 20 min at 22 °C. The annealed double-stranded oligodeoxyribonucleotide was then precipitated with ethanol and ammonium acetate, and the resulting pellet was dissolved in 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. The binding reaction was carried out using 1 pmol of the 32P-labeled double-stranded oligodeoxyribonucleotide with varying amounts of PARP or the 24-kDa fragment in a buffer containing 5 mM Tris-HCl, pH 8.0, and 5 mM MgCl₂ for 15 min at 30 °C in a 15-μl reaction mixture. Samples were then fractionated by native 6% polyacrylamide gel electrophoresis, and the gel was dried and exposed to x-ray film for autoradiography or used for quantitation by AlphaImager (Packard Instrument Co.).

**Cell-free DNA Repair Assay—**Cell-free extracts were prepared from GMO1953A lymphoblastoid cells following the method of Manley et al. (27). The cell-free DNA repair assay was carried out using 50 μg of extract, 300 ng of γ-irradiated plBluescript II KS⁺ (pBS, 3 kilobase pairs) containing an average of one single-stranded DNA break per molecule (6), and varying amounts of the 24-kDa fragment in the presence or absence of 2 mM NaCl under reaction conditions described previously (27). After purification of the DNA, unrepaird pBS (open circular) and repaired pBS (closed circular) were resolved by ethidium bromide, 1% agarose gel electrophoresis.

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and antibiotics (complete DMEM) was added; HeLa S3 cells were cultured for 24 h after which the medium was replaced with serum-free DMEM, and the cells were exposed to N\(-\)methyl-N\(^9\)-nitro-N\(^\prime\)-nitrosoguanidine (MNNG) (50 \(\mu\)M). After 20 min of treatment at 37 °C, the medium was replaced with complete DMEM, and cells were cultured for 2 h. TUNEL staining (fluorescein \textit{in situ} cell death detection kit, Roche Molecular Biochemicals) was then carried out after fixing the cells in 3% paraformaldehyde and permeabilizing them with 0.1% Triton X-100 and 0.1% sodium citrate according to the supplier’s instructions (Roche Molecular Biochemicals). TUNEL-positive cells were visualized by fluorescence microscopy.

RESULTS

Recombinant PARP and the 24-kDa Fragment—To investigate the effect of the 24-kDa PARP fragment on DNA repair and transcription, we first prepared the recombinant 24-kDa fragment and full-length PARP. As shown in Fig. 1, the 24-kDa fragment, which migrated to an apparent molecular mass of about 30 kDa on SDS-polyacrylamide gels, was purified to over 99% homogeneity as described under “Materials and Methods”. The purity of full-length recombinant PARP was about 95%, with several truncated products observed (Fig. 1). Quantitation of the 24-kDa fragment and PARP was carried out by direct ELISA using the F1–23 antibody (26), which recognizes the zinc finger 2 motif of PARP and the 24-kDa fragment.

Binding of the 24-kDa Fragment and PARP to DNA Breaks—\(^{32}\)P-Labeled double-stranded oligodeoxynucleotide (50 base pairs) was incubated with either the 24-kDa fragment or PARP. If the zinc finger motifs found in PARP and the 24-kDa fragment bind to DNA ends (2, 31), the mobility of the \(^{32}\)P-labeled DNA probe should be reduced on a native gel. As shown in Fig. 2, discrete retarded bands were in fact observed when the labeled probe was incubated with the 24-kDa fragment. Migration of the probe was similarly retarded when PARP was used instead of the 24-kDa fragment, with the probe migrating slightly more slowly than in the case of the 24-kDa fragment. In addition, the labeled probe was reproducibly found at the origin of the lane. Addition of excess unlabeled double-stranded oligodeoxynucleotide inhibited retardation of the probe, confirming that formation of the retarded labeled material was due to binding of the 24-kDa fragment or PARP to the DNA probe (data not shown). The data in Fig. 2 reveal a linear relationship between \(^{32}\)P activity associated with the retarded fraction and the amount of the 24-kDa fragment or PARP and allow us to determine that the 24-kDa fragment has about 25% of the binding activity of full-length PARP. Thus, even after cleavage of PARP by caspase-3, the resulting 24-kDa fragment retains significant DNA binding activity.

Inhibition of DNA Repair by the 24-kDa Fragment—In the absence of PARP’s substrate, NAD\(^{+}\), PARP binds to and persists on DNA breaks, thereby inhibiting DNA repair (6). Thus, dissociation of PARP from DNA breaks by automodification is a prerequisite for DNA repair (6). Since the 24-kDa fragment is capable of binding to DNA breaks (Fig. 2) but lacks the automodification site, these fragments should persist on DNA breaks and inhibit DNA repair even in the presence of NAD\(^{+}\). To test this hypothesis, a cell-free DNA repair assay was carried out using open circular pBS containing \(\gamma\)-ray-induced single-stranded DNA breaks, cell-free extracts, and varying amounts of the 24-kDa fragment in the presence or absence of NAD\(^{+}\). As shown in Fig. 3, only 7% of DNA breaks were rejoined in the absence of NAD\(^{+}\) due to inhibition of DNA repair by bound PARP. By contrast, when poly(ADP-ribosyl)ation and dissociation of PARP from DNA breaks was initiated by addition of NAD\(^{+}\), about 30% of DNA breaks were repaired. This NAD\(^{+}\)-promoted DNA repair was significantly inhibited by addition of the 24-kDa fragment.
A 6.6-fold molar excess of the 24-kDa fragment relative to PARP was sufficient to inhibit NAD$^+$-promoted DNA repair by 80% (Fig. 3, A and B). 15 pmol of PARP derived from extract versus 100 pmol of the 24-kDa fragment), consistent with the hypothesis that the 24-kDa fragment, unlike full-length PARP, binds to and persists on DNA breaks in the presence of NAD$^+$.

We then measured the amount of ADP-ribose polymers generated in the cell-free assay in the absence or presence of the recombinant 24-kDa fragment. As previously observed (28), incubation of cell-free extracts with DNA breaks caused transient formation of ADP-ribose polymers (Fig. 3C). However, addition of the 24-kDa fragment significantly inhibited ADP-ribose polymer formation, suggesting that the 24-kDa fragment effectively competed with PARP in binding to DNA breaks and thereby reduced the overall level of poly(ADP-ribosylation) in the presence of NAD$^+$.

Since ADP-ribose polymers are synthesized from NAD$^+$, inhibition of poly(ADP-ribose) formation should reduce the consumption of NAD$^+$. To quantify the amount of NAD$^+$ present in the reaction mixture following the cell-free DNA repair assay, $^{32}$P-NAD$^+$ (32 nM) was added to the assay and the reaction mixtures were applied to native 6% polacrylamide gels. The $^{32}$P activity was visualized by autoradiography, and the $^{32}$P activity in the retarded fraction was quantified with InstantImager (Packard); relative activity is shown (arbitrary units).
particular with actively transcribed regions; this association has been shown to occur by direct binding of PARP to transcribed RNA (14–16). We recently demonstrated that binding of PARP to RNA stem-loop structures reduces the rate of RNA elongation by RNA polymerase II and that formation of DNA breaks and subsequent automodification of PARP removes the transcriptionally inhibitory PARP molecules, thus up-regulating RNA synthesis (17).

To investigate whether the 24-kDa fragment binds to RNA stem-loops, uniformly $^{32}$P-labeled synthetic stem-loop RNA was prepared by transcribing the TAR sequence from human immunodeficiency virus, type I (see "Materials and Methods"), and was mixed with either the 24-kDa fragment or PARP. As shown in Fig. 4, PARP reduced the mobility of stem-loop RNA on a native polyacrylamide gel, generating a discrete band. The 24-kDa fragment also reduced the mobility of TAR stem-loop RNA (Fig. 4), suggesting that the fragment, like full-length PARP, is capable of binding to RNA stem-loops. The data in Fig. 4 reveal a linear relationship between $^{32}$P activity associated with the retarded fractions and the amount of 24-kDa fragment or PARP, enabling us to conclude that the 24-kDa fragment has about 15% of the binding activity of full-length PARP.

**Suppression of Transcript Elongation by the 24-kDa Fragment**—We then tested whether the 24-kDa fragment was capable of reducing elongation of RNA transcripts using a reconstituted pulse-chase elongation assay. Linearized pAGF1, with a 90-base G-less sequence at one end, was prepared (17) and used as a template. During the pulse, RNA polymerase II was loaded onto linearized pAGF1 from the end where the G-less sequence was located and allowed to transcribe the G-less sequence in the absence of GTP but in the presence of ATP, UTP, CTP, and [α-$^{32}$P]CTP, resulting in the formation of $^{32}$P-labeled 90-base transcripts (Fig. 5A). The chase was initiated by adding GTP, excess unlabeled CTP, and either the 24-kDa fragment or PARP. As shown in Fig. 5A, the formation of discrete labeled transcripts generated by pausing of RNA polymerase II at putative pause sites was used to follow transcript elongation. Consistent with our previous report showing that PARP suppresses transcript elongation (17), addition of PARP inhibited the production of longer transcripts (Fig. 5A). The addition of the 24-kDa fragment likewise resulted in fewer of the longer transcripts (Fig. 5A), suggesting that the 24-kDa fragment also acted to suppress RNA synthesis by RNA polymerase II.

RNA stem-loop structures have been demonstrated to result in pausing of RNA polymerase II during transcription (33). In our previous report (17), we suggested that the reduced rate of transcript elongation in the presence of PARP occurs as the result of PARP binding to and stabilizing these stem-loop structures. Upon DNA damage, automodification of PARP promotes the resolution of PARP–RNA complexes, thereby up-regulating transcription (17). On the other hand, the 24-kDa fragment lacks the automodification site and may therefore compete with full-length PARP and inhibit its ability to up-regulate transcription downstream of DNA damage. To test this hypothesis, we carried out run-off transcription assays using HeLa nuclear extracts in the presence or absence of the 24-kDa fragment. Reactions were carried out using 1.8 transcription units of HeLa nuclear extract that contained about 2 pmol of PARP. As shown in Fig. 5B, run-off transcripts 400 bases long were produced from linearized pCMV-Luc. Upon addition of NAD$^+$, PARP that was bound to the ends of linearized template DNA became automodified, and an increase in transcripts was observed, consistent with our previous report (17). However, the addition of the 24-kDa fragment to the run-off reactions (i.e. in addition to PARP and NAD$^+$) resulted in the production of fewer transcripts (Fig. 5B). A 15-fold molar excess of the 24-kDa fragment relative to PARP was sufficient to reduce the NAD$^+$-promoted transcription by 80% (Fig. 5, B and C, 2 pmol of PARP derived from HeLa nuclear extracts versus 30 pmol of the 24-kDa fragment), indicating that these fragments in fact inhibit PARP-mediated up-regulation of RNA synthesis.

**Increased Induction of Apoptosis in Cells Expressing the 24-kDa Fragment**—To investigate whether expression of the 24-kDa fragment biases cells toward apoptosis, HeLa S3 cells were transiently transfected with pcDNA 3.1–/AF24 for 24 h. The transfected cells were exposed to 50 μM MNNG for 20 min and then incubated in normal medium for an additional 2 h, after which apoptosis was analyzed by TUNEL assay. Control cells were transfected with pcDNA 3.1–/AF24 and treated with MNNG showed a further and dramatic increase in such TUNEL signals (Fig. 6H, 76% positive cells).

Quantitative Western blotting of transfected cells (using either the C-II-10 monoclonal antibody, which recognizes the automodification domain of PARP, or the F1–23 antibody against zinc finger 2 of both PARP and the 24-kDa fragment (26)) revealed a PARP concentration of 28 fmol per 1 × 10⁶ cells versus 180 pmol of the 24-kDa fragment per 1 × 10⁶ cells. Since the transfection efficiency was estimated to be greater than 90% (as determined by the number of green fluorescent cells following transfection with pcDNA 3.1–/GFP), we calculated that the ratio of 24-kDa fragment to PARP in HeLa S3 cells expressing the 24-kDa fragment was 7:1. This ratio appears sufficient, therefore, to sensitize cells to the apoptotic effects of MNNG.

**DISCUSSION**

During apoptosis, the abundant nuclear enzyme PARP is cleaved by caspase-3, generating a 24-kDa fragment containing the DNA binding domain (18–20). In this report, we have demonstrated that the 24-kDa fragment, like full-length PARP, is capable of binding to both DNA breaks and RNA, although the affinity of the 24-kDa fragment for DNA breaks and RNA was reduced to 25 and 15% relative to full-length PARP, respectively. However, given the high affinity of PARP for DNA breaks (31) and RNA (34), the reduced affinity is still significant and consistent with a functional role for the 24-kDa fragment in apoptosis.

Since apoptosis can be induced in PARP knockout cells (35–37), cleavage of PARP may not be required for apoptosis per se. However, it has been suggested that PARP functions to bias cells toward survival (21, 37, 38). If so, then a reduction in the level of full-length PARP as a consequence of cleavage by caspase-3 may counter this normal bias. In addition, the fact that the resulting 24-kDa fragment retains the DNA binding domain of PARP but lacks the automodification site may enable it to act as a dominant negative factor in relation to full-length PARP, since automodification regulates both the DNA binding activity and its poly(ADP-ribosyl)ation activity of PARP (5). In fact, we have demonstrated here that the 24-kDa fragment inhibited DNA repair, ADP-ribose polymer formation, and damage-dependent up-regulation of transcription mediated by PARP, all of which could shift the cell bias from survival to death through apoptosis.

Inhibition of DNA repair by the 24-kDa fragment can be accounted for by the fact that the 24-kDa fragment, unlike
full-length PARP molecules, persists on DNA breaks, thus preventing DNA repair enzymes from gaining access to sites of damage. DNA repair can similarly be inhibited under conditions where PARP automodification is suppressed (6), since automodification is essential for driving the dissociation of PARP from DNA breaks. Alternatively, overexpression in mammalian cells of a 42-kDa PARP fragment containing the DNA binding domain and automodification site but lacking the catalytic domain also inhibits DNA repair due to persistence of the 42-kDa fragments on DNA breaks (39). As expected, such inhibition increases the sensitivity of cells to DNA-damaging agents (3, 39). Thus, inhibition of DNA repair by binding of the 24-kDa fragment to DNA breaks may also sensitize cells to DNA-damaging agents.

Fig. 5. Effect of the 24-kDa fragment on transcription. A, pulse-chase transcription assay was carried out with purified RNA polymerase II as described under “Materials and Methods.” During the pulse, RNA polymerase II was loaded onto a G-less sequence located at one end of the linear template in the presence of α-32PCTP, CTP, ATP, and UTP but not GTP. RNA polymerase stalls at the first G, generating a 90-base transcript. The chase was initiated by adding excess CTP and GTP in the presence of the 24-kDa fragment (1 pmol) or PARP (0.1 pmol). Samples were applied to a 6% acrylamide, 8 M urea gel, and transcripts were visualized by autoradiography. B, a cell-free run-off transcription assay was carried out for 1 h at 30 °C with HeLa nuclear extracts and the 24-kDa fragment in the presence or absence of 0.25 mM NAD+ in a 10-µl reaction mixture. Transcripts were then fractionated on a 3% polyacrylamide, 8 M urea gel, and the gel was exposed to x-ray film to visualize the 400-base run-off transcripts. The relative proportions of the 24-kDa fragment and PARP (2 pmol derived from HeLa nuclear extracts) are also shown. C, produced run-off transcripts expressed in arbitrary units.

death by necrosis (23, 40–43). In fact, prevention of NAD+ and ATP depletion stimulates apoptosis and suppresses induction of necrosis (23, 41–43). Consistent with these observations, our biochemical results also support the possibility that the 24-kDa fragment allows cells to enter an apoptotic, rather than necrotic, pathway by preventing over-consumption of NAD+.

The 24-kDa fragment caused inhibition of PARP-mediated up-regulation of transcription (Fig. 5). We recently reported that PARP reduces the rate of transcript elongation by RNA polymerase II and that activation and automodification of PARP, as occurs in response to DNA damage, relieve this inhibition, thereby resulting in up-regulation of transcription (17). Since DNA-damaging agents induce RNA damage as well, we proposed that such up-regulation allows cells to compensate for the collateral loss of RNA following exposure to DNA-damaging agents, and that this pathway is likely required for cell survival. The inhibition of such a regulatory pathway by the
24-kDa PARP fragment may therefore sensitize cells to DNA-damaging agents.

Following expression of the 24-kDa fragment in HeLa cells, Kim et al. (43) found a 50% reduction in ADP-ribose polymer synthesis after exposure of the cells to UV light, as well as increased induction of apoptosis. In the biochemical analysis reported here, a 50% reduction in ADP-ribose polymer formation occurred in the presence of about 120 pmol of the 24-kDa fragment (based on data in Fig. 3C) or about an 8-fold excess of 24-kDa fragment relative to full-length PARP (15 pmol per reaction). In addition, consistent with the report from Kim et al. (43), we also found increased induction of apoptosis by MNNG in HeLa cells expressing a 7-fold excess of 24-kDa fragment relative to full-length PARP (Fig. 6). Furthermore, our biochemical analysis shows that addition of a 7-fold excess of 24-kDa fragment relative to full-length PARP inhibited both NAD−-promoted DNA repair (see Fig. 3B, 15 pmol of full-length PARP derived from cell-free extracts versus 100 pmol of the 24-kDa fragment) and PARP-mediated up-regulation of transcription (see Fig. 5C, 2 pmol of full-length PARP derived from HeLa nuclear extracts versus 13 pmol of the 24-kDa fragment) by 80 and 60%, respectively. Thus, the 24-kDa fragment, by competing with and acting in opposition to PARP, may contribute to the process by which cells irreversibly commit to apoptosis (44).

Acknowledgments—We thank G. G. Poirier for providing the C-II-10 and the F1–23 antibodies; C. W. Ward for editing and comments; and Sachiko Sato for technical advice.

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