Modulation of DNA Fragmentation Factor 40 Nuclease Activity by Poly(ADP-ribose) Polymerase-1*

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Poly(ADP-ribose) polymerase-1 (PARP-1) influences numerous cellular processes, including DNA repair, transcriptional regulation, and caspase-independent cell death, by utilizing NAD⁺ to synthesize long chains of poly(ADP-ribose) (PAR) on target proteins, including itself. During the apoptotic response, caspases-3 and -7 cleave PARP-1, thereby inhibiting its activity. Here, we have examined the role of PARP-1 activation and cleavage in the latter stages of apoptosis in response to DNA fragmentation. PARP-1 poly(ADP-ribosyl)ation correlated directly with induction of apoptosis by the lipid peroxidation product, 4-hydroxy-2-nonenal. A significant decrease in PAR accumulation was observed upon caspase or DNA fragmentation factor 40 (DFF40) inhibition. Because DNA fragmentation mediated by DFF40 augmented PARP-1 modification status in apoptotic cells, we hypothesized that PARP-1 alters DFF40 function following PAR accumulation. Indeed, PARP-1, in the presence of NAD⁺, significantly decreased DFF40 activity on plasmid substrates. Conversely, PARP-1 enhanced the DNase activity of DFF40 in the absence of NAD⁺. The inhibition of DFF40 activity in the presence of NAD⁺ was reduced by co-incubation with poly(ADP-ribose) glycohydrolase and a PARP inhibitor. Additionally, caspase-cleaved PARP-1, in the presence of NAD⁺, did not inhibit DFF40 activity significantly. Our results suggest that PARP-1 poly(ADP-ribosyl)ation is a terminal event in the apoptotic response that occurs in response to DNA fragmentation and directly influences DFF40 activity.

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Cell death responses triggered by a variety of stimuli often exhibit a continuum from necrosis to caspase-mediated apoptosis (1). Necrosis is biochemically ill-defined, but it is characterized by the depletion of cellular energy stores and membrane rupture (1). Apoptosis, on the other hand, involves the systemic activation of caspases, the cysteine proteases that initiate and execute the death response (1, 2). Many protein targets of active caspases are biologically important apoptotic indicators and give rise to the signature morphological and biochemical changes associated with apoptosis (e.g. cytoskeletal blebbing, nuclear condensation, nucleosomal DNA fragmentation) (2).

Reactive oxygen species are a potentially important group of intracellular apoptotic effectors. Superoxide anion (O₂⁻) and hydroxyl radical (‘OH) can damage most biological macromolecules, including polyunsaturated fatty acids in membranes (3, 4). The ensuing lipid peroxidation reaction gives rise to many compounds, including a series of α,β-unsaturated aldehydes, which are themselves reactive secondary cytotoxins (3–6). 4-Hydroxy-2-nonenal (HNE), a abundant product of lipid peroxidation, causes apoptosis in a wide variety of cell types (7–10). Previously, several laboratories, including ours, have demonstrated that the apoptotic response induced by HNE results from the activation of the intrinsic (i.e. mitochondrial) pathway, whereby cytochrome c and other pro-apoptotic mitochondrial proteins are released (7, 9, 10). Subsequent caspase activation leads to cleavage of apoptotic targets such as poly(ADP-ribose) polymerase-1 (PARP-1), DNA fragmentation factor 45 (DFF45), and DFF35 (2). Both DFF45 and DFF35 function as protein inhibitors of the caspase-activated DNase DFF40 (also called CAD or CPAN), and their cleavage leads to the activation of DFF40 and subsequent DNA degradation (11–15).

PARP-1 is involved in both apoptotic and necrotic responses (16, 17). PARP-1 catalyzes the formation of poly(ADP-ribose) (PAR) using NAD⁺ as an ADP-ribose donor following binding to DNA strand breaks and other distinctive DNA structures (16–18). Under homeostatic conditions, PARP-1 is thought to regulate DNA repair responses and modulate gene transcription (17, 18). Following the initiation of apoptotic signaling, PARP-1 is cleaved and inactivated by caspases-3 and -7 (19–22). Activation of PARP-1 is also believed to occur during apoptosis (17), although its role in regulating nuclear processes has not been extensively studied. During some necrotic responses, rapid stimulation of PARP-1 activity mediates energy depletion and subsequent cell death (23, 24). PARP-1 is considered a mediator of caspase-independent cell death in a wide variety of systems, although its role in caspase-dependent apoptosis is not clearly understood.

Here, we describe the poly(ADP-ribosyl)ation of PARP-1 during the apoptotic response induced by HNE in RKO human colorectal carcinoma cells as a consequence of apoptotic DNA degradation. Because the role of PARP-1 in the latter stages of apoptosis has not been clearly defined, we examined the direct effect of PARP-1 on DFF40 activity. PARP-1 altered DFF40 DNase activity in two distinct ways, depending on whether NAD⁺ was present in the assay. Our results suggest that

1 The abbreviations used are: HNE, 4-hydroxy-2-nonenal; PAR, poly(ADP-ribose); PAR-1, PAR polymerase-1; DFF, DNA fragmentation factor; zVAD-fmk, carbobenzoxy-Val-Ala-Asp-fluoromethyl ketone; zDEVD-fmk, carbobenzoxy-Asp-Glu-Val-Ala-Asp-fluoromethyl ketone; PARG, poly(ADP-ribose) glycohydrolase; dsDNA, double-stranded DNA; DPQ, 3,4-dihydro-5-[4-(1-piperidinyl)-1(2H)-isoquinoline.
PARP-1 can regulate DFF40 activity in vitro, and they provide new insight into the involvement of PARP-1 in the apoptotic DNA fragmentation response.

**EXPERIMENTAL PROCEDURES**

**Materials**—HNE was obtained from Cayman Chemical (Ann Arbor, MI) and was dissolved in methanol. zVAD-fmk was obtained from Promega (Madison, WI). zDEVD-fmk, bovine PARP-1, and PAR were obtained from Calbiochem (La Jolla, CA). Recombinant human caspase-3 was obtained from BD Pharmingen. Bovine thymus poly-(ADP-ribose) glycohydrolase (PARG) was purchased from Biolom (Plymouth Meeting, PA). DPQ was obtained from Sigma.

**Cell Culture**—RKO human colorectal carcinoma cells were grown in McCoy's 5A medium (Invitrogen) supplemented with 10% fetal bovine serum (U.S. Biotechnologies, Parker Ford, PA), 2 mM L-glutamine, and antibiotics at 37 °C and 5% CO2. The total concentration of methanol or Me2SO per culture was ≤0.1% of the total volume. Cells were ~40–60% confluent at the time of treatment.

**Preparation of Cell Lysates and Western Blotting**—Cells were split and treated in 25-cm2 flasks and scraped off into the medium following treatment, centrifuged at 100 × g for 5 min, and washed twice with cold phosphate-buffered saline, pH 7.4. Cell pellets were lysed on ice in buffer containing 1 mM Na2EDTA, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1.0% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 µg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride for 30 min. Debris from lysates was cleared by centrifugation at 16,000 × g for 5 min. The supernatant was recovered, and protein concentrations were quantified using the bicinchoninic acid method (Pierce) with bovine serum albumin as a standard. Proteins were separated by SDS-PAGE under reducing conditions and were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) for 4 h at 0.2 A in a buffer containing 25 mM Tris (pH 8.3), 192 mM glycine, and 20% methanol. Membranes were blocked for 1 h at room temperature in TTBS (100 mM Tris (pH 7.5), 150 mM NaCl, and 0.1% Tween 20) containing 5% nonfat dry milk. Rabbit primary antibodies against human PARP-1 (Cell Signaling Technologies), PAR (Trexigen, La Jolla, CA), and the Myc epitope (Cell Signaling Technologies) and a mouse primary antibody against α-tubulin (Sigma) were diluted in blocking buffer according to the suppliers’ suggestions and were incubated with membranes for 1–2 h at room temperature or overnight at 4 °C. Blots were subsequently washed three times for 10 min with TTBS. Secondary antibodies conjugated to horseradish peroxidase (Amersham Biosciences) were applied at the recommended dilutions for 45 min at room temperature, and the membrane was washed four times with TTBS for 15 min. Enhanced chemiluminescent reagents (Amersham Biosciences) were added for 1–2 min, and blots were autoradiographed.

**DNA Fragmentation Analysis**—The procedure for isolating soluble DNA from cells has been previously described (9).

**Preparation of DFF40 and DFF45 Expression Vectors**—DFF45 cDNA was amplified out of its parental vector pOTB7 using the following primers with their corresponding bases encoding caspase cleavage site aspartate residues was underlined: D117E 5'-CCGATCCCATTTCTGTTGCAGAG-3' and DNA sequencing. Wild-type and D117E/D234E DFF45 cDNAs were cloned into pBluescript-derlined: D117E 5'-CCGATCCCATTTCTGTTGCAGAG-3' and DNA sequencing. Wild-type and D117E/D234E DFF45 cDNAs were cloned into pBluescript (Stratagene) and was subcloned into pCPE4 (Invitrogen).

For Myc epitope tagging, DFF45 was removed from pBluescript-DFF45 by digesting with HindIII and BamHI and ligating the C-terminal-truncated DFF45 into pCDA3.1 (Invitrogen). The C-terminal Myc epitope was added by digesting and inserting an oligonucleotide duplex containing the epitope sequence and the stop codon (5'-AGAGTTTCATCCACAGAGCAAAAAAGGACTTTCAGGAGACCTGTAGGAGCTCGACGGCGG) between the BamHI and XhoI site into pcDNA3.1-DFF45. The tagged DFF45 cDNA was subcloned via the HindIII and XhoI sites into pBluescript SK+ (for sequencing) and pCEP4 (for expression in RKO cells).

**Nuclear Activity and Electrophoretic Mobility Shift Assays**—Myc-tagged DFF45 and wild-type DFF45 were expressed transiently in RKO cells for 48 h. Transfected cells were lysed for 30 min on ice in DFF45 lysis buffer (10 mM HEPES (pH 7.2), 140 mM KCl, 5 mM MgCl2, 1 mM EGTA, 0.2% Nonidet-P40, 0.2 mM phenylmethylsulfonyl fluoride, and protease complete inhibitor tablets (Roche Applied Science)). Protein lysates (1 mg) were immunoprecipitated in spin columns on a rotary mixer overnight at 4 °C using the anti-Myc ProFound immunoprecipitation kit (Plymouth Meeting, PA). Beads were washed twice with lysis buffer. Subsequently, the beads were incubated at room temperature for 30 min with 100 ng of caspase-3 in 90 µl of buffer containing 10 mM HEPES (pH 7.2), 50 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, and 1 mM EGTA. Following incubation with 1 µl of 20 mM zVAD-fmk for 15 min at room temperature, activated DFF45 was eluted from the spin column. Plasmid DNAse assays (30 µl) were performed using 1 µl of eluted DFF45 and 1 µl of control pBluescript SK+ buffer containing 10 mM HEPES (pH 7.2), 50 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, 1 mM EGTA, and 0.5 mg/ml bovine serum albumin at 37 °C for time intervals of 5 min-1 h. PARP-1, NAD+, PARP, and DPQ were added to some reactions at the amounts indicated. Reactions were terminated by addition of 90 µl of 100% EtOH and 3 µl of 3 M sodium acetate (pH 5.4). Samples were frozen overnight, centrifuged to pellet, resuspended in H2O, and electrophoresed on an agarose gel.

**Effect of Caspase Inhibition on PARP-1 Poly(ADP-ribose)ylation**—Poly(ADP-ribose)ylation of PARP-1 in Response to HNE Treatment—in RKO cells treated with HNE for 24 h, full-length PARP-1 underwent a noticeable mobility shift when electrophoresed on a low percentage gel (Fig. 1A), presumably through auto-poly(ADP-ribose)ylation. To confirm an accumulation of negatively charged PAR at the same molecular mass as PARP-1, Western blots using antibodies against PAR were performed. PAR accumulation at the molecular mass of PARP-1 was observed maximally at 45–60 µM HNE (Fig. 1B) and throughout the time course, with levels reaching their highest at 24 h (Fig. 1C). At the same doses and times of PARP-1 poly(ADP-ribose)ylation, PARP-1 cleavage was also observed. These results suggest that, in addition to being inactivated by caspases, some PARP-1 molecules are activated during the late stages of the apoptotic response. Significant accumulation of PAR was not observed on proteins of other molecular mass in appreciable amounts (data not shown).

**Effect of Caspase Inhibition on PARP-1 Poly(ADP-ribose)ylation and DNA Fragmentation Induced by HNE**—Because the time course and dose response of HNE-mediated PARP-1 poly(ADP-ribose)ylation coincided with apoptotic signaling, caspase inhibitors were used to determine whether PARP-1 modification is caspase-dependent. PAR accumulation stimulated by HNE was prevented partially with zDEVD-fmk (a caspase-3 and -7 inhibitor) or completely with zVAD-fmk (a broad spec-
A (30 μM H9262) was significantly reduced in cells expressing mutant DFF45 expressing cells. However, poly(ADP-ribosyl)ation of PARP-1 was seen in vector control and wild-type DFF45-RKO cells. Following HNE treatment, poly(ADP-ribosyl)ation in apoptotic cells.

To clarify whether DFF40, the best characterized apoptotic nuclease, was responsible for the poly(ADP-ribosyl)ation of PARP-1 and DNA fragmentation, wild-type DFF45 was cloned, its caspase-3 cleavage sites were mutated, and both wild-type and mutant DFF45 were expressed in RKO cells. Following HNE treatment, poly(ADP-ribosyl)ation of PARP-1 was seen in vector control and wild-type DFF45-expressing cells. However, poly(ADP-ribosyl)ation of PARP-1 was significantly reduced in cells expressing mutant DFF45 (Fig. 3A). There was no inhibition of overall apoptotic signaling in cells expressing mutant DFF45, as judged by similar levels of cleaved PARP-1 in all cells following HNE treatment. Expression of mutant DFF45, but not wild-type DFF45, caused a pronounced decrease in nucleosomal DNA fragmentation at 24 h (Fig. 3B). Collectively, these results place PARP-1 modification, but not PARP-1 cleavage, downstream of DFF40 activation and suggest that DFF40 is the principal apoptotic DNase in RKO cells.

Disruption of DFF40 Function and Effect on PARP-1 during HNE-mediated Apoptosis—To clarify whether DFF40, the best characterized apoptotic nuclease, was responsible for the poly(ADP-ribosyl)ation of PARP-1 and DNA fragmentation, wild-type DFF45 was cloned, its caspase-3 cleavage sites were mutated, and both wild-type and mutant DFF45 were expressed in RKO cells. Following HNE treatment, poly(ADP-ribosyl)ation of PARP-1 was seen in vector control and wild-type DFF45-expressing cells. However, poly(ADP-ribosyl)ation of PARP-1 was significantly reduced in cells expressing mutant DFF45 (Fig. 3A). There was no inhibition of overall apoptotic signaling...
activity by catalytically active PARP-1 was evident throughout the time course of the assay, suggesting that the synthesis of PAR by PARP-1 had a significant and prolonged impact on DFF40 activity.

Reduction of PARP-1-mediated Inhibition of DFF40 Activity by PARG and DPQ—Given that PARP-1 inhibits DFF40 activity in the presence of NAD⁺, we next tested whether hydrolysis of PAR by PARG or inhibition of PARP-1 activity using DPQ would prevent these effects. DFF40 DNase activity was recovered almost completely in reactions containing PARP-1, NAD⁺/H₁₁₀₀₁, and DPQ and partially by addition of PARG (Fig. 6A). The partial preservation of DFF40 activity by PARG, although modest, is representative of the fact that PARG does not inhibit PARP-1 activity directly but instead hydrolyzes PAR to limit its accumulation.

Western blots of reaction products indicated that pronounced modification (i.e. poly(ADP-ribosyl)ation) of PARP-1 occurred in the presence of NAD⁺, significantly impeding the migration of PARP-1 into the gel (Fig. 6B). However, in the presence of DPQ and PARG, the dramatic change in PARP-1 mobility was reduced. No alteration was observed in the mobility of DFF40 protein, implying that it was not poly(ADP-ribosyl)ated under these experimental conditions. These results collectively suggest that extensive PAR accumulation on PARP-1 is responsible for DFF40 inhibition.

Reduced DFF40 Inhibition by Caspase-cleaved PARP-1—Because PARP-1-mediated inhibition of DFF40 activity was reduced by PARG and DPQ, we hypothesized that PARP-1 cleavage and inactivation by caspases causes similar effects. To test this hypothesis, PARP-1 was cleaved by caspase-3 prior to incubation with DFF40 in the presence or absence of NAD⁺.

A pronounced decrease in DFF40 inhibition was observed when NAD⁺ and cleaved PARP-1 were added, differing greatly from the effects seen with full-length PARP-1 (Fig. 7A). Conversely, cleaved PARP-1 enhances DFF40 activity similarly to full-length PARP-1 in the absence of NAD⁺. Cleavage of PARP-1 and the mobility shift of full-length PARP-1 in the assay were verified by Western blot, providing stronger evidence that inhibition of DFF40 is dependent on PARP-1 catalytic activity (Fig. 7B). Some modification of the caspase-cleaved PARP-1 fragment was observed in the assay, although this was presumably because of the small percentage of full-length PARP-1 remaining in the caspase-
cleaved samples. Nonetheless, these results demonstrate a direct effect of PARP-1 on DFF40 activity in vitro and suggest a potential reason for PARP-1 cleavage during apoptosis, namely to allow for efficient DNA fragmentation by DFF40.

**DISCUSSION**

Numerous biochemical changes take place during the apoptotic response, including the release of specific mitochondrial components into the cytosol, caspase activation, and DNA fragmentation (1, 2, 26). Here, we have explored the effect of PARP-1 activation in response to apoptotic DNA fragmentation. The time course of PARP-1 modification closely resembled the timing of events late in the apoptotic signaling cascade (Fig. 1C). Poly(ADP-ribosyl)ation of PARP-1 indeed occurred downstream of caspase and DFF40 activation (Figs. 2 and 3). In addition, we have discovered that PAR synthesis by PARP-1 can influence DFF40 DNA binding and catalytic activity directly (Figs. 5–8).

PARP-1 can modify itself and other proteins during both necrotic and apoptotic responses (22–24, 28–31). The increased levels of PAR in apoptotic cells have long been associated with DNA fragmentation (32) because PARP-1 typically requires DNA strand breaks for catalysis. Activation of the apoptotic DNase, DFF40, leads to increased PAR levels during apoptosis induced by tumor necrosis factor-α (TNF-α); PAR accumulation is not observed, however, in DFF45-deficient cells, which lack expression of functional DFF40 protein (30, 31). In agreement with these previous findings, overexpression of a caspase-resistant DFF45 significantly reduced both HNE-mediated DNA fragmentation and poly(ADP-ribosyl)ation of PARP-1 (Fig. 3). DNA fragmentation observed in cells expressing this mutant, although present at much lower levels, is potentially the result of some DFF40 activation following cleavage of endogenous DFF45/DFF35 or degradation of DNA by other reported apoptotic nucleases (33–36).
Although PARP-1 poly(ADP-ribosyl)ation occurs following apoptotic DNA fragmentation, the role of its modification and cleavage during apoptosis is still unclear and disputed. Ectopic expression of caspase-resistant PARP-1 mutants has yielded conflicting results about the role of PARP-1 cleavage in apoptosis (37–39), as have experiments where PARP-1 function has been abrogated by pharmacological inhibition, gene disruption, or antisense RNA strategies (28, 29, 40–42). The stimulation of PARP-1 activity during apoptotic responses additionally has several proposed roles. PARP-1 automodification during apoptosis has been suggested to increase its affinity for caspase-7, thereby enhancing its cleavage (22). Another purported role for augmented PARP-1 activity in the apoptotic response is to stimulate a feedback loop with mitochondria and promote increased release of cytochrome c into the cytosol through an undescribed amplification mechanism. In DFF45-deficient cells treated with tumor necrosis factor \( /H_9251 \), the accumulation of PAR was significantly decreased and the timing of apoptotic signaling was drastically slowed (30, 31). However, in our experiments, a pronounced effect on PARP-1 cleavage or overall apoptotic signaling was not observed in cells expressing mutant DFF45 (Fig. 3) or following PARP-1 inhibition.2

We hypothesized, instead, that PARP-1 directly influences nuclear processes during apoptosis and focused our attention on its effects on DNA degradation by DFF40. PARP-1 modulates the activity of several other enzymes involved in DNA damage sensing and metabolism, including Werner syndrome protein, DNA ligase III\(a\), and DNase-\(\gamma/DNAS1L3\) (35, 43–45). In our studies, PARP-1 enhanced DFF40 activity in the absence of NAD\(\gamma\) and markedly decreased DFF40 activity in the presence of NAD\(\gamma\) (Fig. 5). The increase in activity may be due to the alteration of DNA structure by PARP-1, because other DNA-binding proteins like HMG2, histone H1, and topoisomerase II\(\alpha\) promote a significant increase in DFF40 activity through DNA bending and/or direct interaction with DFF40 (46–49). No pronounced enhancement of DFF40 activity was observed using PARP-1 concentrations higher than 50 ng, suggesting that PARP-1 binds extensively to and protects DNA from DFF40 action at these concentrations or that PARP-1 bends DNA to the extent that DFF40 cleavage is reduced.

The decrease in DFF40 activity upon PARP-1 catalysis has several possible explanations. The apoptotic endonuclease DNase-\(\gamma/DNAS1L3\) can be poly(ADP-ribosyl)ated and inhibited by PARP-1 as a means of controlling its activity in non-apoptotic cells (35). However, noticeable modification of DFF40 by PARP-1 was not observed in these assays (Fig. 6B). Another attractive possibility is that DFF40 binds to PAR, which, in turn, results in decreased nuclease activity (Fig. 9). This argument is supported by the presence of a conserved PAR-binding domain in the primary sequence of human DFF40, a region that exhibits considerable homology with the major groove binding helix (i.e. \(\alpha4\)) in the murine DFF40 crystal structure (Fig. 9, B and C) (50, 51). Our experiments, consistent with these reports, suggest that extensive PAR accumulation on PARP-1 may serve to compete DFF40 away from DNA and reduce DFF40 activity (Figs. 6–8). Experiments with PARG, DPQ, or caspase-cleaved PARP-1 (Figs. 6 and 7) support the idea that PAR accumulation on PARP-1 influences DFF40 activity directly, and electrophoretic mobility shift assays indicate that PAR can disrupt the interaction of DFF40 with DNA, albeit with less efficacy than unlabeled dsDNA itself (Fig. 8).

Here, we have demonstrated that stimulation of PARP-1 activity is an event that follows activation of DFF40, the principal apoptotic DNase in RKO cells. We have proposed a mechanism through which PARP-1 automodification influences apoptotic DNA fragmentation by inhibiting DFF40 activity (Fig.

\[\text{FIG. 9. Model of DFF40 activity modulation by PARP-1.} A, \text{ PARP-1 activation and inhibition of DFF40 activity occur in response to initial DNA degradation by DFF40. This effect is prevented by caspase cleavage of PARP-1.} B, \text{ DFF40 primary sequences were aligned from the murine enzyme (\(\alpha4\)-helix 4, \(\alpha4\)) and human enzyme (PAR-binding domain; PBD) as described in Refs. 50 and 51.} C, \text{ the crystal structure of a DFF40 homodimer is depicted. Monomers are represented in blue or red, except for the PBD/\(\alpha4\)-helix in both, which is shown in green. The active site is at the bottom of the cleft, immediately below the PAR-binding domain.} \]
Effects of PARP-1 on DFF40 Activity

9A). Additionally, our experimental results suggest that PARP-1 cleavage by caspases prevents such an effect. PARP-1 is one of several enzymes responsive to DNA strand breaks that are cleaved by caspases and inactivated during apoptosis; others include the kinases DNA-dependent protein kinase-catalytic subunit and ataxia telangiectasia-mutated protein (52–54). Target modification by each of these enzymes often accompanies DNA strand break formation (55, 56), has been observed in apoptotic cells (22, 28, 29, 57, 58), and may alter the accessibility, recruitment, assembly, and/or activity of downstream factors involved in DNA fragmentation through a feedback mechanism. Cleavage of PARP-1, ataxia telangiectasia-mutated protein, and DNA-protein kinase-catalytic subunit may, therefore, serve to prevent inhibition of DFF40 activity. Additionally, their cleavage potentially prevents unnecessary DNA repair. Although such nuclear modifications are not essential for the completion of the apoptotic program in many instances, they likely influence the complete disassembly of genomic DNA in the terminal stages of apoptosis.

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REFERENCES

1. Leist, M., and Jaattela, M. (2001) Nat. Rev. Mol. Cell. Biol. 2, 589–598
2. Earnshaw, W. C., Martins, L. M., and Kauffman, S. H. (1999) Annu. Rev. Biochem. 68, 383–424
3. Sayre, L. M., Smith, M. A., and Ferry, G. (2001) Curr. Med. Chem. 8, 721–738
4. Marnett, L. J., Riggins, J. N., and West, J. D. (2003) Prog. Lipid Res. 42, 318–343
5. Esterbauer, H., Schaur, R. J., and Zollner, H. (1991) Free Radic. Biol. Med. 11, 583–593
6. Uchida, K. (2003) Prog. Lipid Res. 42, 318–343
7. Kruman, I., Bruce-Keller, A. J., Berdesen, D., Waeg, G., and Mattson, M. P. (1997) J. Neurosci. 17, 5089–5100
8. Liu, W., Kato, M., Akhand, A. A., Hayakawa, A., Suzuki, H., Miyata, T., Kurokawa, K., Hotta, Y., Ishikawa, N., and Nakashima, I. (2000) Free Radic. Biol. Med. 30, 801–809
9. Li, Y., Luo, X., and Wang, X. (2001) Nature 412, 95–99
10. Shiokawa, D., Kobayashi, T., and Tanuma, S. (2002) J. Biol. Chem. 277, 10301–10307
11. Liu, X., Li, P., Widlak, P., Zou, H., Luo, X., Garrard, W. T., and Wang, X. (1998) Biochem. Biophys. Res. Commun. 250, 40974–40980
12. Pleschke, J. M., Kiec-Kuczynska, H. E., Strohm, M., and Althaus, F. R. (2000) J. Biol. Chem. 275, 9390–9395
13. Toh, S. Y., Wang, X., and Li, P. (1998) Biochem. Biophys. Res. Commun. 250, 598–601
14. Durrieu, F., Samejima, K., Fortune, J. M., Kandels-Lewis, S., Osheroff, N., and Earnshaw, W. C. (2000) Curr. Biol. 10, 923–926
15. Wielckens, K., Schmidt, A., George, E., Bredehorst, R., and Hitz, H. (1982) Nature 297, 54–57
16. Dawut, L., and Bohr, V. A. (2004) Biochem. Biophys. Res. Commun. 310, 31013–31017
17. Shiokawa, D., Kobayashi, T., and Tanuma, S. (2002) J. Biol. Chem. 277, 537–540
18. Shiokawa, D., Kobayashi, T., and Tanuma, S. (2002) J. Biol. Chem. 277, 10301–10307
19. Lazebnik, Y. A., Keenan, S. H., Derossis, A. M., Poirier, G. G., and Earnshaw, W. C. (1994) Nature 371, 546–547
20. Tewari, M., Quan, L. T., O’Bourke, K., Derossis, A. M., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1995) Cell 81, 801–809
21. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Goreau, Y., Griffin, P. R., Labelle, M., and Lazebnik, Y. A. (1995) Nature 376, 37–43
22. Germain, M., Affar, B. B., D’Amours, D., Dixit, V. M., Salvesen, G. S., and Poirier, G. G. (1999) J. Biol. Chem. 274, 28379–28384
23. Ha, H. C., and Snyder, S. H. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13978–13982
24. Yu, S. W., Wang, H., Poiras, M. F., Coombs, C., Bowers, W. J., Fedoroff, H. J., Poirier, G. G., Dawson, T. M., and Dawson, V. L. (2002) Science 297, 259–263
25. Oberhammer, F., Wilson, J. W., D’Amours, D., Dixit, V. M., Salvesen, G. S., and Poirier, G. G. (1999) J. Biol. Chem. 274, 28379–28384
26. Nagata, S., Nakanishi, K., Kawane, K., Mukae, N., and Fukuyama, H. (2003) Cell Death Differ. 10, 108–116
27. Widlak, P., Lanuszewska, J., Cary, R. B., and Garrard, W. T. (2003) J. Biol. Chem. 278, 26915–26922
28. Yoon, Y. S., Kim, J. W., Kang, K. W., Kim, Y. S., Choi, K. H., and Joe, C. O. (1998) J. Biol. Chem. 273, 9129–9134
29. Simbulan-Rosenthal, C. M., Rosenthal, D. S., Iyer, S., Poirier, G. G., and Smulson, M. E. (1998) J. Biol. Chem. 273, 13703–13712
30. Boulares, A. H., Zolotooka, A. J., Shefer, Z. A., Yakovlev, A., and Smulson, M. E. (2000) Biochem. Biophys. Res. Commun. 270, 796–801
31. Boulares, A. H., Zolotooka, A. J., Yakovlev, A., Xu, M., and Smulson, M. E. (2001) J. Biol. Chem. 276, 38185–38192
32. Ha, H. C., and Snyder, S. H. (1992) Cell Death Differ. 9, 27–39