Caspase-mediated Cleavage of DNA Topoisomerase I at Unconventional Sites during Apoptosis*

(Received for publication, June 22, 1998, and in revised form, November 9, 1998)

Kumiko Samejima,a Phyllis A. Svingen,b Guriqbal S. Basi,c Timothy Kotte,k Peter W. Mesner, Jr.,b Lance Stewart,e,f François Durieu,a Guy G. Poirier,f Emad S. Alnemri,h James J. Champoux,g Scott H. Kaufmann,b,d,h and William C. Earnshawa,b,1

From the *Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland, United Kingdom, the †Division of Oncology Research, Mayo Clinic, Rochester, Minnesota 55905, ‡Athena Neurosciences, Inc., South San Francisco, California 94080, the §Jefferson Cancer Institute, Philadelphia, Pennsylvania 19107-5541, the ¶Centre Hospitalier de l’Université Laval Research Center and Laval University, Sainte-Foy, Quebec G1V 4G2, Canada, and the #Biomolecular Structure Center and the Departments of *Biological Structure and †Microbiology, School of Medicine, University of Washington, Seattle, Washington 98195

Previous studies have demonstrated that topoisomerase I is cleaved late during apoptosis, but have not identified the proteases responsible or examined the functional consequences of this cleavage. Here, we have shown that treatment of purified topoisomerase I with caspase-3 results in cleavage at DDVD146 | Y and EED170 | G, whereas treatment with caspase-6 resulted in cleavage at PEDD123 | G and EED170 | G. After treatment of Jurkat T lymphocytic leukemia cells with anti-Fas antibody or A549 lung cancer cells with topotecan, etoposide, or paclitaxel, the topoisomerase I fragment comigrated with the product that resulted from caspase-3 cleavage at DDVD146 | Y. In contrast, two discrete topoisomerase I fragments that appeared to result from cleavage at DDVD146 | Y and EED170 | G were observed after treatment of MDA-MB-468 breast cancer cells with paclitaxel. Topoisomerase I cleavage did not occur in apoptotic MCF-7 cells, which lack caspase-3.

Cell fractionation and band depletion studies with the topoisomerase I poison topotecan revealed that the topoisomerase I fragment remains in proximity to the chromatin and retains the ability to bind to and cleave DNA. These observations indicate that topoisomerase I is a substrate of caspase-3 and possibly caspase-6, but is cleaved at sequences that differ from those ordinarily preferred by these enzymes, thereby providing a potential explanation why topoisomerase I cleavage lags behind that of classical caspase substrates such as poly(ADP-ribose) polymerase and lamin B1.

Eukaryotic DNA topoisomerase I (topo I),4 an abundant nuclear enzyme (10^2–10^6 copies/nucleus) involved in the regulation of DNA topology and the control of gene expression, is emerging as a protein of considerable medical significance. The enzyme is an important target of camptothecin and related antineoplastic agents (1–5). These agents slow the resealing of DNA complexes within cells (7) and setting into motion events that result in target cell apoptosis (8). In addition, topo I is an important autoantigen in rheumatic disease (9–11). First identified as an ~70-kDa autoantigen termed Scl-70 that reacts with serum from scleroderma patients (12), topo I is recognized by sera from 25% of patients with scleroderma (13) as well as by sera from mice with the TSK (tight skin) model for this disease (14). Although the origin and significance of autoantibodies in rheumatic disease remain controversial, it has been proposed that, at least in systemic lupus erythematosus, autoantibodies may arise as a result of abnormalities in the pathway of cell death by apoptosis (15).

Apoptosis is essential for morphogenesis, tissue homeostasis, and host defense against viruses (16–19). Although the precise biochemical pathways involved in mammalian cell death continue to receive intense scrutiny, it is now clear that cysteine-dependent aspartate-directed proteases (caspases (20)) play important roles in the initiation and execution phases of apoptotic death (19, 21, 22). These proteases have been shown to cleave a wide variety of cellular polypeptides located in both the cytoplasm and the nucleus (reviewed in Refs. 21 and 23–27).

Like a number of other autoantigens (28, 29), topo I is cleaved during apoptosis. However, a contradictory picture has emerged from previous studies of topo I degradation during apoptotic execution. Initial studies indicated that topo I levels markedly diminished during etoposide-induced apoptosis of HL-60 cells without production of a discrete cleavage fragment (8). Compared with apoptotic cleavage of other caspase targets, e.g. poly(ADP-ribose) polymerase (30) or lamin B1 (31), topo I cleavage appeared to be a later event and was typically incomplete (8, 28). In contrast to this result, topo I fragments of 70 kDa have been reported in HeLa cells exposed to UV-B irradiation (28), HL-60 cells treated with etoposide (32), and Jurkat cells exposed to anti-CD95 antibody (29, 32). In this last model system, topo I proteolysis was inhibited by preincubation of cells with the broad spectrum caspase inhibitor benzoyloxycarbonyl-Val-Asp-D-arginine fluoromethyl ketone (10 μM) for 30 min before addition of anti-CD95 antibody. In contrast, during tumor necrosis factor-induced apoptosis in C3HA fibroblasts (33) and necrosis of HL-60 or Jurkat cells induced by HgCl2, ethanol, H2O2, or heat (32), topo I was degraded into small fragments.

* This work was supported in part by National Institutes of Health Grants AG13487 (to E. S. A.), CA69008 (to S. H. K. and W. C. E), and GM49156 (to L. S. and J. J. C.) and by a principal research fellowship from the Wellcome Trust. To whom correspondence should be addressed: Inst. of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland, UK. Tel.: 44-131-650-7101; Fax: 44-131-650-7100; E-mail bill.earnshaw@ed.ac.uk

1 The abbreviations used are: topo I, DNA topoisomerase I; YVAD-cmk, acetyltyrosinylvalinylalanylasparglyl chloride; PAGE, polyacrylamide gel electrophoresis.

© 1999 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
Topoisomerase I Cleavage during Apoptosis

Several questions about the apoptotic cleavage of topo I remain unanswered. 1) Which proteases are responsible for topo I cleavage during apoptosis? 2) Where are the cleavage sites located within the topo I molecule? 3) Are the topo I fragments generated during apoptosis enzymatically active? 4) Are the same topo I fragments invariably generated in different cells undergoing apoptosis? In this study, we have mapped the sites at which caspase-3 and caspase-6 cleave topo I, compared the resulting fragments with those generated in situ in several cell types undergoing apoptosis, demonstrated that the major topo I cleavage fragment retains enzymatic activity, and probed the location of the cleaved fragment within apoptotic cells. The results of this study not only identify topo I as a caspase substrate, but also provide an explanation for its slow cleavage relative to other apoptotic events and demonstrate its variable cleavage in different apoptotic cell types.

EXPERIMENTAL PROCEDURES

Materials—Reagents were obtained from the following suppliers: YVAD-cmk from Bachem (Essex, United Kingdom); Hybrid-C membranes, peroxidase-coupled anti-mouse and anti-human secondary antibody, anesthetics, and chemicals from Amer sham International (Buckinghamshire, UK); agonistic anti-Fas (Fas is the cell-surface death receptor also known as CD95 and Apo-1) antibody CH-11 from Kamiya Biomedico Co. (Seattle, WA); etoposide, paclitaxel (Taxol®), and 5-fluoro-2′-deoxyuridine (41) from MDA-MB-468 cells, MCF-7 cells, and A549 cells treated with 17 µm etoposide or 100 ng/ml CH-11 antibody for 14 h. After the cells were sedimented at 200 × g for 10 min, all further steps were performed at 4 °C. Cells were washed once with RPMI 1640 medium containing 10 µM HEPES (pH 7.4) followed by phosphate-buffered saline, incubated for 20 min in nuclear isolation buffer (10 mM NaCl, 10 mM Tris-HCl (pH 7.4), and 5 mM MgSO4 containing freshly added 1 mM α-phenylmethylsulfonyl fluoride and 100 units/ml aprotinin), and homogenized in a tight-fitting Dounce homogenizer. Nuclei and nuclear fragments were sedimented at 20,000 × g for 15 min. The nuclear supernatant was sedimented at 105,000 × g for 60 min. Protein in the supernatant from this second centrifugation step (cytosol) was precipitated with 10% trichloroacetic acid, washed once with 10% trichloroacetic acid and three times with −20 °C acetone, and dissolved in solubilization buffer.

Immunolocalization of Topoisomerase I in Apoptotic Cells—After treatment with anti-Fas antibody CH-11 or etoposide as described above, Jurkat cells were sedimented onto coverslips, air-dried, fixed in 3.7% (w/v) formaldehyde, and permeabilized with 0.2% (v/v) Nonidet P-40 (40). Topoisomerase I was visualized by indirect immunofluorescence using an antibody I-18 and rhodamine-labeled affinity-purified anti-mouse IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as described previously (44). After the final series of washes, samples were incubated for 3 min with 1 µg/ml Hoechst 33258 in phosphate-buffered saline prior to mounting in Vectashield (Vector Laboratories, Inc., Burlingame, CA). Samples were visualized using a DeltaVision microscope (Applied Precision, Issaquah, WA).

RESULTS AND DISCUSSION

Cleavage of Topoisomerase I in Jurkat Cells Undergoing Apoptosis—Initial studies were performed to confirm that topo I is cleaved to a detectable fragment during apoptosis and to assess the timing of this cleavage relative to other proteolytic events. After incubation of the agonistic anti-Fas antibody CH-11 to Jurkat cells, proteolytic cleavage of procaspase-7 (Fig. 1D) and procaspase-3 (data not shown) was detected within 60 min, the same time frame in which we have previously demonstrated caspase activation using catalytic assays for DEVD-aminotrifluoromethylcoumarin cleavage and affinity labeling (35). Cleavage of poly(ADP-ribose) polymerase at DEVD, G3 (30) to yield a characteristic 89-kDa fragment likewise was detected at 60 min (Fig. 1B). Cleavage of lamin B1 (manifested in Fig. 1C as a decrease in the intensity of the 67-kDa full-length polypeptide) began between 60 and 80 min after addition of CH-11 antibody to the cells. By 180 min, half of the poly(ADP-ribose) polymerase and 75% of the lamin B1 molecules were cleaved in these cells. In contrast, the earliest cleavage of topo I to a detectable fragment was not evident until 140 min after addition of CH-11 antibody to the cells (Fig. 1A). Moreover, <5% of the topo I was cleaved under these conditions. A similar disparity between cleavage of topo I and the other two caspase substrates was observed after treatment of the Jurkat cells with proapoptotic stimuli shown.

These results not only confirmed that topo I was cleaved to a detectable fragment during apoptosis initiated by triggering two discrete pathways in Jurkat cells (35, 45), but also indicated that topo I cleavage was delayed relative to other cleavages.

Cleavage of Topoisomerase I by Cloned Human Caspases—
Examination of the topo I sequence (46) did not reveal any perfect matches to the preferred cleavage sites of the known caspases (47, 48). Accordingly, there were two formal possibilities that could explain the delayed cleavage of topo I. First, topo I might be cleaved by a non-caspase protease that was activated downstream of the caspases. Several groups have presented indirect evidence for such proteases (49–51), two of which have recently been identified (52, 53). Second, it was presented indirect evidence for such proteases (49–51), two of which have recently been identified (52, 53). The four putative nuclear localization signals (NLS) Lys\(^{59}\)–Glu\(^{65}\), Lys\(^{150}\)–Asp\(^{156}\), Lys\(^{174}\)–Asp\(^{180}\), and Lys\(^{192}\)–Glu\(^{198}\) (open circles); the active-site Tyr\(^{272}\); and the domain structure of topo I are also indicated.

Although 12 human caspases have been identified to date, caspase-3 and caspase-6 are the most widely studied with respect to the cleavage of target proteins during apoptosis. To examine the possibility that caspase-3 and/or caspase-6 can cleave topoisomerase I in vitro, purified recombinant human topo I was incubated with extracts prepared from SF9 cells infected with baculoviruses expressing either human caspase-3 or caspase-6. Each of these caspases cleaved topoisomerase I at two sites (Fig. 2A). Caspase-3 generated an 80-kDa major fragment and a 76-kDa minor fragment. In contrast, caspase-6 predominantly generated the 76-kDa fragment plus a minor fragment of 82 kDa. Interestingly, neither cleavage reaction was particularly efficient, with \(\sim 30\) or 50% of the topoisomerase I remaining intact after the 2-h incubation with caspase-3 or caspase-6, respectively. This is to be contrasted with the quantitative cleavage of poly(ADP-ribos) polymerase by caspase-3 within minutes under similar conditions (54). Cleavage of topo I by each caspase was completely inhibited by preincubation of the extracts with the caspase inhibitor YVAD-cmk at 100 \(\mu\)M (data not shown), consistent with the view that the cleavages were caspase-mediated.

To confirm that the slow rate of caspase-mediated topo I cleavage is an intrinsic property of the enzyme-substrate interaction and is not due to other factors present either in vivo or in SF9 cell extracts, the cleavage reaction was reconstituted from purified components. Purified bovine poly(ADP-ribos) polymerase and human topo I were incubated with purified recombinant human caspase-3 in the same tube for various times at 37 °C, denatured in SDS-PAGE sample buffer, and analyzed by immunoblotting (Fig. 2B). Under these conditions, caspase-3 cleaved all of the poly(ADP-ribos) polymerase within 15 min. In contrast, topo I cleavage was undetectable at 30 min, but became evident at later time points.

Collectively, the observations in Fig. 2 (A and B) establish several points. First, topo I is cleaved by both caspase-3 and caspase-6, making it one of a small number of polypeptides that are reportedly cleaved by both caspases (55, 56). Second, caspase-mediated topo I cleavage is relatively inefficient compared with other substrates.

Mapping the Caspase Cleavage Sites in Topoisomerase I—To further explore the caspase-mediated cleavage of topo I, the amino acid sequences of the two cleaved fragments generated
by caspase-3 and the two cleaved fragments generated by caspase-6 were determined (Fig. 2C). The analysis was simplified by the fact that, in each case, caspase cleavage liberated a long carboxyl-terminal fragment with a free N terminus. Thus, N-terminal sequencing of each fragment was sufficient to identify the cleavage site. The four cleaved fragments yielded three distinct cleavage sites that were defined in two cases by eight amino acids of peptide sequence and in one case by seven amino acids of sequence (underlined in Fig. 2C). This analysis revealed sites of cleavage adjacent to Asp₁²¹, Asp₁⁴⁶, and Asp₁⁷⁰. Cleavage occurred in the sequences PEDD₁²²↓G (caspase-6), DDVD₁⁴⁶↓Y (caspase-3), and EEED₁⁷⁰↓G (caspase-3 and caspase-6). Although this analysis cannot rule out the possibility that additional caspase-mediated cleavages occur in the N-terminal domain, our kinetic analysis revealed no proteolyzed species corresponding to cleavages in this domain. Therefore, if such cleavages do occur, they either must take place after the cleavages described above or must produce species that are further processed rapidly to yield those species.

Of the three sequences, only DDVD₁⁴⁶↓Y corresponds to a canonical caspase-3 cleavage site. The other two sequences are unusual and would not be predicted based on current understanding of caspase cleavage specificities. A recent comprehensive study employing a positional scanning substrate combinatorial library (48) demonstrated that the optimal tetrapeptide recognition sequences for caspase-3 and caspase-6 were DEVD and VEID, respectively, in remarkable agreement with the recognition sequences for caspase-3 and caspase-6. Although this analysis cannot rule out the possibility that additional caspase-mediated cleavages occur in the N-terminal domain, our kinetic analysis revealed no proteolyzed species corresponding to cleavages in this domain. Therefore, if such cleavages do occur, they either must take place after the cleavages described above or must produce species that are further processed rapidly to yield those species.

The present finding that PEDD₁²²↓G and EEED₁⁷⁰↓G are in fact used when caspases cleave topoisomerase I not only provides evidence that factors in addition to primary sequence are important in cleavage site selection, but also suggests that studies of caspase cleavage site specificity using small peptides might need be interpreted with caution when drawing conclusions about the cleavage of native protein substrates. This is consistent with recent results showing that caspase cleavage of poly(ADP-ribose) polymerase was significantly increased following phosphatase treatment of apoptotic extracts, whereas cleavage of tetrapeptide substrates was unaffected (58).

Topoisomerase I Cleavage in Various Cell Types—All previous studies demonstrating apoptotic cleavage of human top I were performed using leukemia cell lines. To determine whether top I cleavage is limited to this cell type, we examined several different cell types undergoing apoptosis in response to various stimuli. Topoisomerase I cleavage products were observed not only in Jurkat (Fig. 3A, lane 5) and K562 (data not shown; identical to Jurkat cells) leukemia cells, but also in A549 lung (lanes 8 and 10) and MDA-MB-468 breast (lanes 13 and 15) cancer cells undergoing apoptosis in response to previously described proapoptotic stimuli (31, 41, 42, 59). Interestingly, different patterns of top I cleavage were observed in these model systems. In the Jurkat, K562, and A549 cell lines, the single top I cleavage product comigrated with the major fragment (b) generated by caspase-3 in vitro (cf. Fig. 3A, lanes 2, 5, 8, and 10). In contrast, apoptotic MDA-MB-468 cells contained two top I cleavage products, a larger fragment (b) that comigrated with the major caspase-3 cleavage product and a smaller fragment (c) that comigrated with the major caspase-6 cleavage product.

To determine whether these apparent differences reflect differences between the cell lines as opposed to differences in the apoptotic stimuli, three different cell lines were treated with 100 nm paclitaxel (Fig. 3B). Consistent with the results in Fig. 3A, two topo I fragments were evident in apoptotic MDA-MB-468 cells (Fig. 3B, lane 3). As was the case for etoposide and topotecan treatment, one fragment predominated in A549 cells (Fig. 3B, lane 9). When the exposure of the blot was increased in order to begin to bring up the background, a faint smear was seen beneath the major cleavage product (Fig. 3B, lane 9’). Although this is the region of the gel in which fragment c runs, this smear did not appear upon close inspection to correspond to a discrete band. Thus, there is a clear (although slight) difference in the pattern of top I cleavage between MDA-MB-468 and A549 cells in response to a single initiating response. An even more dramatic difference was observed when top I processing in apoptotic MCF-7 cells was examined. Topoisomerase I cleavage was entirely absent in these cells (Fig. 3B, lane 6), which lack caspase-3 (60). This indicates that top I cleavage is mediated by caspase-3 or a caspase that lies downstream of caspase-3.

Localization of Topoisomerase I Cleavage Products in Apoptosis—Recent results indicate that topo II dissociates from the chromatin during the course of apoptosis (61). To determine whether top I likewise undergoes relocalization, we examined
the distribution of intact topo I and its fragment in apoptotic cells. Topoisomerase I has four predicted nuclear localization signals: Lys\(^{59}\)–Glu\(^{65}\), Lys\(^{150}\)–Asp\(^{156}\), Lys\(^{174}\)–Asp\(^{180}\) and Lys\(^{192}\)–Glu\(^{198}\) (Fig. 2C) (62). Although cleavage at DDVD\(^{146}\) Y would remove one of these potential nuclear localization signals, the resulting carboxyl-terminal fragment would be expected to retain its nuclear targeting function. In fact, a previous study has indicated that Lys\(^{192}\)–Glu\(^{198}\) is apparently sufficient to direct the nuclear transport of topo I (36).

Several approaches were used to examine the localization of topo I and its major fragment during apoptosis. In initial experiments, normal and apoptotic Jurkat cells were examined by indirect immunofluorescence using monoclonal anti-topo I antibody according to protocols previously described (44). In these studies (data not shown), a decrease in topo I staining was observed in many of the apoptotic cells. It appeared that the low levels of remaining detectable antigen were frequently (but not invariably) excluded from condensed apoptotic bodies. We could not, however, rule out the possibility that changes in chromatin structure were masking the topo I epitope in the apoptotic bodies, nor could we ascertain whether the topo I antibody (which detects both full-length polypeptide and cleaved fragments (Figs. 1–3)) was providing information about the topo I that remained intact in the apoptotic cells as opposed to the topo I cleavage fragment.

To circumvent these difficulties, the distributions of topo I and its cleavage product were compared by subcellular fractionation. Jurkat cells were induced to undergo apoptosis by treatment with low levels of agonistic anti-Fas antibody or etoposide for 14 h, lysed by homogenization, and subjected to differential sedimentation to produce a sedimentable fraction (containing nuclei and nuclear fragments) and cytosol. Immunoblotting revealed that both intact and fragmented topo I were recovered exclusively in the sedimentable fraction (Fig. 4A, upper panel). In contrast, procaspase-2 was exclusively cytoplasmic (Fig. 4A, lower panel); and the shuttling protein B23 was found predominantly in the nucleus, but with low levels detected in the cytoplasm (middle panel). Based on these results, it appears that topo I remains associated with nuclei during apoptosis.

### The Major Topoisomerase I Cleavage Fragment Remains Catalytically Active
To further examine the subcellular location of the topo I fragment as well as assess its catalytic function, a band depletion assay was performed. The basis of this assay is described in detail by Kaufmann et al. (43). Under normal conditions, the catalytic intermediate that contains the active-site Tyr\(^{723}\) covalently linked to a 3'-phosphate of the substrate DNA has a short half-life. Recent crystallographic data suggest that the presence of the plant alkaloid camptothecin perturbs the structure of this intermediate such that the free 5'-hydroxyl of the DNA might be displaced by −4.5 Å from the phosphate group that would be the site of attack for religation (63). As a consequence, the religation of this intermediate is slowed; and topo I-DNA covalent complexes accumulate. If cells containing these covalent topo I-DNA intermediates are lysed under denaturing conditions and subjected to SDS-PAGE, the topo I trapped in these complexes migrates as a smear with reduced mobility, resulting in a reduction in the signal for topo I at \(M_r \sim 100,000\).

This band depletion assay is illustrated in Fig. 4B (lanes 1–5). Treatment of control Jurkat cells with increasing concentrations of the topo I poison topotecan resulted in progressive loss of the topo I signal at \(M_r \sim 100,000\). Control experiments revealed that the signal for topo I could be restored within 2 min by exposing the cells to conditions that shift the cleavage-religation equilibrium of the enzyme in favor of free topo I (data not shown) (43). Application of the same assay to Jurkat cells induced to undergo apoptosis by treatment with anti-Fas anti-

---

**Fig. 4. Examination of topo I subcellular distribution and ability to form covalent enzyme-DNA complexes.** A, Jurkat cells incubated without (lanes 1, 4, and 7) or with 100 ng/ml anti-Fas antibody CH-11 (lanes 2, 5, and 8) or 17 µM etoposide (lanes 3, 6, and 9) for 14 h were separated into fractions containing nuclei and nuclear fragments (lanes 4–6) or cytosol (lanes 7–9), subjected to SDS-PAGE, and blotted with monoclonal anti-topo I antibody C-21 (upper panel), polyclonal anti-B23 antibody (middle panel), or monoclonal anti-procaspase-2 antibody (lower panel). Gels were loaded with 50 µg of protein/lane. Note that procaspase-2 partitioned exclusively with the cytosol fraction, whereas topo I partitioned exclusively with the nuclei and nuclear fragments. B, control Jurkat cells (lanes 1–5) or Jurkat cells treated with 500 ng/ml anti-Fas antibody CH-11 for 4 h (lanes 6–10) were incubated for 45 min at 37 °C with 0 (lanes 1 and 6), 3 (lanes 2 and 7), 10 (lanes 3 and 8), 30 (lanes 4 and 9), or 100 (lanes 5 and 10) µM topotecan. At the completion of the incubation, samples were sedimented at 3200 × g for 1 min and immediately lysed under denaturing conditions as recently described (43). After SDS-PAGE and transfer to nitrocellulose, blots were probed with monoclonal anti-topo I antibody C-21 (upper panel) or monoclonal anti-poly(ADP-ribose) polymerase (PARP) antibody C-II-10. Lanes 6–10 are longer exposures of lanes 6–10 so that effect of topotecan on the topo I fragment can be better discerned. Note that the 80-kDa topo I fragment (Frag) behaved just like full-length topo I in this assay. Additional experiments, incubation of cells at 48 °C for 2 min prior to lysis completely restored the topo I signal (data not shown).

---

body (Fig. 4B, lanes 6–10) or etoposide (data not shown) revealed that signals for topo I and the cleavage product were both attenuated as the concentration of topotecan increased. These observations indicate not only that the topo I fragment remains in the vicinity of DNA, but also that the fragment remains catalytically active in situ, a result that is consistent with the previous finding that the amino-terminal 200 amino acids are dispensable for topo I activity (36, 46).

**Conclusions**—Recent results indicate that treatment of HeLa cells with topo I poisons results in down-regulation of topo I polypeptide levels as a result of ubiquitin-mediated proteolysis (64, 65). In contrast, we observed topo I cleavage to one or two relatively stable fragments in cells undergoing apoptosis.
Topoisomerase I Cleavage during Apoptosis

topoisomerase I cleavage indicates that caspases have been activated, but does not
indicate that cleavage in situ is mediated by caspase-3 or a protease downstream of
caspase-3, e.g. caspase-6 (66).

In complementary experiments, we demonstrated that topo I is a substrate of two
different caspases, caspase-3 and caspase-6 (Fig. 2A). Although two other polypeptides
have recently been reported to be dual substrates of these two caspases (55, 56), topo I
is the first substrate for which the cleavage sites have been identified. Two of the three
cleavages mediated by caspase-3 and caspase-6 occurred at sites that differed from
those reportedly preferred by these enzymes (Fig. 2C).

These results have several implications. On the one hand, the demonstration of cleavage at disfavored sequences indicates
that cleavage site predictions based solely on examination of tetrapeptide substrates might not identify all caspase cleavage
sites in native protein substrates. On the other hand, the use of kinetically less favorable sites might also explain the relatively
slow rate of topo I cleavage by recombinant caspases (55, 56), topo I is the first substrate for which the cleavage sites
have been identified. Two of the three cleavages mediated by

caspase-6 (66).

caspase-6 is a substrate of two different caspases, caspase-3 and

caspase-3, indicates that topo I cleavage in situ is mediated by caspase-3 or a protease

downstream of caspase-3, e.g. caspase-6 (66).

In complementary experiments, we demonstrated that topo I is a substrate of two different caspases, caspase-3 and
caspase-6 (Fig. 2A). Although two other polypeptides have recently been reported to be dual substrates of these two

REFERENCES

1. Slichter, W. J., Rosenwaks, E. K. and Kaufmann, S. H. (1993) J. Natl. Cancer Inst. 85, 271–279.
2. Costin, D. and Potmesil, M. (1994) Adv. Pharmacol. 29, 51–72.
3. Chen, A. Y. and Potmesil, M. (1994) Ann. N.Y. Acad. Sci. 748, 191–218.
4. Gupta, M., Fujimori, A. and Pommier, Y. (1995) Biochem. Biophys. Acta 1262, 1–14.
5. Rothenberg, M. L. (1997) Cancer Res. 57, 1647–1681.
6. Armstrong, D. K., Isacs, J. T., Ottaviano, Y. L. and Davidson, N. E. (1992) Curr. Opin. Cell. Biol. 4, 570–578.
7. Goldner, H. H. (1986) Chromosoma (Berl.) 94, 132–138.
8. Mou, G., Bannister, A. J., Zou, J. H. and Reinberg, D. (1997) EMBO J. 16, 6626–6636.
9. Macgregor, S. A., Verma, I. M. and Harris, B. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 5145–5149.
10. Leibson, P. J., Leibson, C. M. and Eischen, C. M. (2002) Cancer Res. 62, 1717–1723.
11. Wright, S. C., Schellenberger, U., Wang, H. W. and You, J. J. (2002) J. Biol. Chem. 277, 4573–4576.
12. Armstrong, S. and Douglas, J. R. (1997) Cancer Res. 57, 515–519.
13. Rosen, A. (1997) Nature 387, 357–358.
14. Raff, C. (1992) Science 256, 1456–1462.
15. Sasaki, T., Sakamoto, T., Kato, H., Yamaoka, Y., Umemoto, Y., Kato, Y. and Mizutani, J. (2000) J. Biol. Chem. 275, 14083–14089.
16. Kurosawa, R., Miura, S., Kato, H., Yamaoka, Y., Umemoto, Y., Kato, Y. and Mizutani, J. (1999) J. Biol. Chem. 274, 10847–10854.
17. Armstrong, S. and Douglas, J. R. (1997) Cancer Res. 57, 515–519.
18. Armstrong, S. and Douglas, J. R. (1997) Cancer Res. 57, 515–519.
19. Armstrong, S. and Douglas, J. R. (1997) Cancer Res. 57, 515–519.
20. Armstrong, S. and Douglas, J. R. (1997) Cancer Res. 57, 515–519.
21. Armstrong, S. and Douglas, J. R. (1997) Cancer Res. 57, 515–519.
Caspase-mediated Cleavage of DNA Topoisomerase I at Unconventional Sites during Apoptosis

Kumiko Samejima, Phyllis A. Svingen, Guriqbal S. Basi, Timothy Kottke, Peter W. Mesner, Jr., Lance Stewart, Françoise Durrieu, Guy G. Poirier, Emad S. Alnemri, James J. Champoux, Scott H. Kaufmann and William C. Earnshaw

J. Biol. Chem. 1999, 274:4335-4340.
doi: 10.1074/jbc.274.7.4335

Access the most updated version of this article at http://www.jbc.org/content/274/7/4335

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 71 references, 42 of which can be accessed free at http://www.jbc.org/content/274/7/4335.full.html#ref-list-1