Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 157–162, January 1997
Cell Biology

Resistance of actin to cleavage during apoptosis

QIZHONG SONG*†, TIE WEI*, SUSAN LEES-MILLER‡, EMAD ALNEMRI§, DIANNE WATTERS*, AND MARTIN F. LAVIN*¶

*Queensland Cancer Fund Research Unit, Queensland Institute of Medical Research, Bancroft Centre, PO Royal Brisbane Hospital, Herston, 4029 Brisbane, Queensland, Australia; ¶Department of Surgery, University of Queensland, Brisbane, Queensland, Australia; ‡Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Canada; and §Department of Pharmacology and the Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA 19107

Communicated by Jerome Gross, Harvard Medical School, Charlestown, MA, October 28, 1996 (received for review September 6, 1996)

ABSTRACT A small number of cellular proteins present in the nucleus, cytosol, and membrane fraction are specifically cleaved by the interleukin-1β-converting enzyme (ICE)-like family of proteases during apoptosis. Previous results have demonstrated that one of these, the cytoskeletal protein actin, is degraded in rat PC12 pheochromocytoma cells upon serum withdrawal. Extracts from etoposide-treated U937 cells are also capable of cleaving actin. It was assumed that cleavage of actin represented a general phenomenon, and a mechanism coordinating proteolytic, endonucleolytic, and morphological aspects of apoptosis was proposed. We demonstrate here that actin is resistant to degradation in several different human cells induced to undergo apoptosis in response to a variety of stimuli, including Fas ligation, serum withdrawal, cytotoxic T-cell killing, and DNA damage. On the other hand, cell-free extracts from these cells and the ICE-like protease CPP32 were capable of cleaving actin *in vitro*. We conclude that while actin contains cleavage sites for ICE-like proteases, it is not degraded *in vivo* in human cells either because of lack of access of these proteases to actin or due to the presence of other factors that prevent degradation.

Apoptosis or programmed cell death is a physiological mechanism responsible for the elimination of unwanted cells during the process of development and the removal of self-reactive lymphocytes (1, 2). The biochemical mechanisms underlying apoptosis remain unclear, but several genes implicated in the process have been identified (reviewed in refs. 3 and 4). It is evident from recent results that a growing family of cysteine proteases which share similarity with the interleukin-1β-converting enzyme (ICE) play a central role in the execution phase of apoptosis (reviewed in refs. 5 and 6). Identification of this family of ICE-like proteases is largely due to investigations of the regulation of cell death in *Caenorhabditis elegans* (7). In these studies several genes controlling cell death, including *ced3*, *ced4*, and *ced9*, have been identified. Yuan *et al.* (8) demonstrated significant sequence homology between Ced3 and ICE, which cleaves inactive pro-interleukin-1β to an active form (9), and overexpression of this protein in fibroblasts resulted in apoptosis (10). To date, eight homologs of ICE have been isolated, including Nedd2/Ich-1 (11, 12), CPP32/Apopain/Yama (13–15), TX/ICE$_{rel}$II (16, 17), TY/ICE$_{rel}$III (17, 18), Mch 2 (19), Mch 3/ICE-LAP3 (20, 21), Mch 4 (22), and FLICE (23). Those proteases share similar structural features, contain an active site QACRG pentapeptide, and are unique in their requirement for an Asp residue at the P1 position in the cleavage site (6). Further support for a role for ICE-like proteases in apoptosis are the observations that some of these enzymes are activated by different stimuli that cause apoptosis (21, 24, 25); overexpression of mutated or truncated forms fail to induce apoptosis (12, 13, 15); and specific inhibitors, designed on the substrate cleavage sites, prevent apoptosis (14).

Recent evidence points to a cysteine protease cascade in apoptosis acting at two levels, initially interacting with and cleaving each other in an apparently hierarchical fashion for activation (22, 26), followed by degradation of a small number of protein substrates that play important roles in cell function or structure (5, 6). The best-described substrate is poly(ADP-ribose) polymerase (PARP), which is activated by DNA damage to poly(ADP-ribosyl)ate a number of nuclear proteins and facilitate DNA repair (27, 28). PARP is cleaved by CPP32 and Mch3 (14, 20, 29) and with lower efficiency by Nedd2, ICE, TX, and Mch2 (19, 30). The degradation of PARP is expected to interfere with DNA repair and facilitate DNA fragmentation. Degradation of a second enzyme involved in the recognition and repair of DNA damage, DNA-dependent protein kinase (DNA-PK) is also observed during apoptosis (31–34). Other proteins thought to be targets for ICE-like proteases include the U1 70-kDa protein component of the small ribonucleoprotein U1 SnRNP (35); topoisomerases (36, 37), nuclear lamins (36, 38, 39); the transcription factors SREBP-1 and 2 (40), Gas 2 (41), and protein kinase Cδ (42).

Cleavage of another substrate, actin (43), and the actin-associated protein fodrin (44) may contribute to the morphological changes characteristic of apoptosis, and it has been suggested that cleavage of actin may allow for the activation of DNase 1 and the subsequent fragmentation of DNA (43). However, while actin has been shown to be a substrate of ICE and ICE-like proteases, these data were largely acquired with lysates prepared from cells undergoing apoptosis (43, 45). Degradation of actin was demonstrated in response to serum withdrawal in rat PC12 cells at 24 and 48 h after withdrawal (43), but the pattern of cleavage differed from that *in vitro*. We have carried out an extensive investigation of the fate of actin in different cell types in response to a variety of stimuli that cause apoptosis, and we failed to observe degradation. As in previous studies, we observed that cell-free extracts prepared from cells undergoing apoptosis and recombinant ICE-like proteases cleave this substrate. These results demonstrate that while a protein may contain recognition sites for an ICE-like protease and be cleaved *in vitro*, enzyme access or other factors may prevent its degradation during apoptosis.

MATERIALS AND METHODS

Reagents. An anti-human Fas monoclonal antibody (clone CH-11) was from Upstate Biotechnology (Lake Placid, NY).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Copyright © 1997 by THE NATIONAL ACADEMY OF SCIENCES OF THE USA
0027-8424/97/94157-6$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: ICE, interleukin-1β-converting enzyme; PARP, poly-(ADP-ribose) polymerase; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, catalytic subunit of DNA-PK; CTL, cytotoxic T lymphocyte; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

†To whom reprint requests should be addressed. e-mail: qizhongS@qimr.edu.au.

Rabbit skeletal muscle actin and an anti-actin polyclonal antibody against the C-terminal 11 amino acids of actin were obtained from Sigma. An anti-actin monoclonal antibody against amino acid region 23–34 of actin was from Boehringer Mannheim. Apoptosis-inducing agents (etoposide, EDTA, EGTA) were obtained from Sigma. DPK1, a polyclonal antibody against amino acid region 2018–2136 of DNA-PKcs, the catalytic subunit of DNA-PK, was used for immunoblotting of this protein.

Cell Lines and Culture Conditions. The Burkitt lymphoma cell lines BL30A, BL30K, BM13674, WW2, and BL18 (33), the monocytic cell line U937, the lymphocytic leukemia Molt-4 cell line, and HeLa cells were maintained in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum at 37°C under a humidified atmosphere containing 5% CO_2. Cells were induced to undergo apoptosis by exposure to etoposide (40 μM), EGTA (5 mM), or EDTA (10 mM); by exposure to 20 Gy of γ-irradiation from a ^{137}Cs source (4 Gy/min); by withdrawal of serum for 24 h; by Fas ligation for 10 h (50 ng/ml anti-Fas antibody); or by cytotoxic T-cell killing. Apoptosis was determined by change in cell morphology, which was evaluated by using fluorescence microscopy with cells stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (1 μg/ml).

Cytotoxic T lymphocyte (CTL)-Mediated Apoptosis. The CTL clone LC13 is HLA B8-restricted and recognizes the epitope FLRGRAYGL. In this system CTLs act as both effector and target cells (46). For CTL-mediated cytolysis, peptide FLRGRAYGL (10 μM) was added to U-shaped microtiter wells containing 10^5 cells and incubated for 15 min at 37°C prior to centrifugation at 200 × *g* and further incubation at 37°C for the times indicated in individual experiments, prior to harvesting and extraction of proteins. An irrelevant peptide, EENLLDFVRE (10 μM), was used as a control. An HLA B44-restricted clone, but not clone LC13, recognizes this Epstein–Barr virus epitope (46).

Immunoblotting. After the various treatments, cells were pelleted at 200 × *g*, washed twice in ice-cold PBS at pH 7.2, and lysed in buffer (50 mM Tris·HCl/150 mM NaCl/2 mM EDTA/2 mM EGTA/25 mM NaF/25 mM β-glycerol phosphate, pH 7.5) containing 0.2% Triton X-100, 0.3% Nonidet P-40, 0.1 mM sodium vanadate, 0.1 mM phenylmethanesulfonyl fluoride (PMSF), leupeptin (5 μg/ml), and aprotinin (5 μg/ml). Electrophoresis was performed on 20 μg of protein sample in loading buffer on SDS/8% or 14% polyacrylamide gels, proteins were transferred to poly(vinylidene difluoride) (PVDF) membranes, and blots were probed with different antibodies.

Preparation of Bacterial Lysates and Assay of Protease Activity. Mch2α, TX, and CPP32 bacterial lysates were prepared as described (20). In brief, exponentially growing bacteria carrying the protease expression plasmids were induced with 1 mM isopropyl 1-thio-β-D-galactopyranoside for 3–6 h and then lysed by sonication in a lysis buffer containing 25 mM Hepes at pH 7.5, 5 mM EDTA, 2 mM DTT, and 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). The lysates were centrifuged at 16,000 × *g* for 10 min, and the clear bacterial extracts were collected. To confirm that the recombinant peptides were active, 4-μl aliquots of the extracts were incubated with 5 μl of the fluorogenic peptide DEVD-AMC (final concentration, 50 μM) for different periods of time, and the release of 7-amino-4-methylcoumarin (AMC) was measured by spectrofluorometry as described previously (47).

***In Vitro* Actin Cleavage by CPP32, Mch2α, and TX Proteases.** Recombinant forms of the ICE-like proteases CPP32, Mch2α, and TX were prepared as bacterial lysates as described above, and activities of these lysates were determined. For cleavage of actin, 10-μl aliquots of the bacterial extracts were incubated with 0.4 μg of purified protein in a 20-μl reaction volume containing 25 mM Hepes at pH 7.5, 5 mM EDTA, 5 mM DTT, and 0.1% CHAPS for 1 h at 37°C. The reactions were terminated by addition of 5 μl of 5× loading buffer followed by SDS/PAGE and immunoblotting with the appropriate antibody.

Preparation of Extracts for Actin Cleavage *in Vitro*. Cell extracts were prepared from BL30A cells treated with 40 μM etoposide, 6 h after treatment. Cells were pelleted at 200 × *g*, washed twice in ice-cold PBS at pH 7.2, and then lysed by sonication in lysis buffer containing 25 mM Hepes at pH 7.5, 5 mM EDTA, 2 mM DTT, 0.1% CHAPS, 1 mM phenylmethanesulfonyl fluoride, and aprotinin, leupeptin, and pepstatin at 2 μg/ml each. The lysates were centrifuged at 16,000 × *g* for 10 min, and the supernatants were collected and stored at −20°C until required. For cleavage of actin, extracts (20 μg of total protein) were incubated with 0.4 μg of purified protein in 20-μl reaction volumes containing 25 mM Hepes at pH 7.5, 5 mM EDTA, 5 mM DTT, and 0.1% CHAPS for 1 h at 37°C prior to separation by SDS/PAGE and immunoblotting with antibody.

RESULTS

Cleavage of Actin by ICE-Like Proteases *in Vitro*. Previous studies have shown that both ICE and an unidentified ICE-like protease are capable of cleaving actin in cell lysates (43, 45). We have utilized a number of recombinant ICE-like proteases

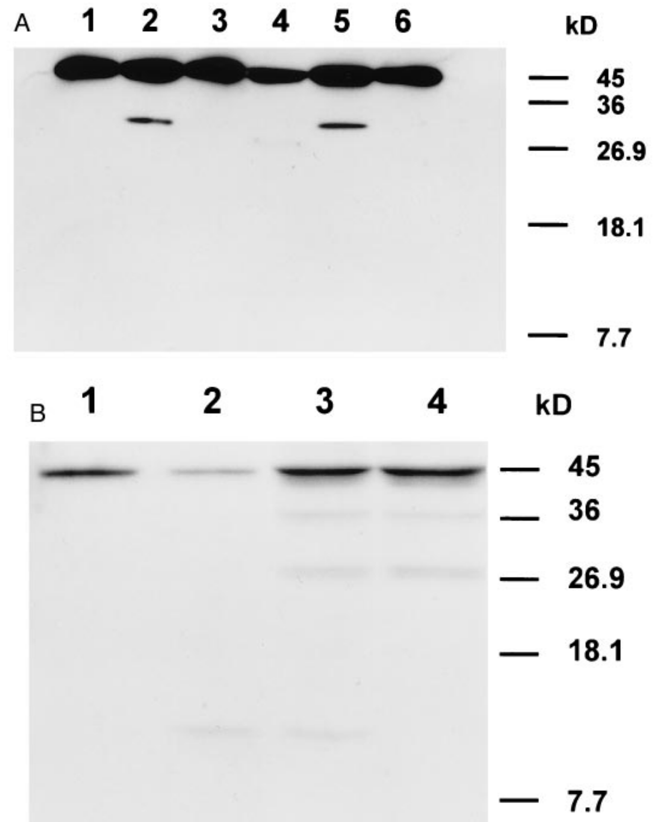

FIG. 1. Cleavage of actin by ICE-like proteases and extracts from etoposide-treated BL30A cells. (*A*) Immunoblotting with a monoclonal antibody against the N terminus of actin, amino acids 23–34. Lane 1, actin alone; lane 2, CPP32 + actin; lane 3, TX + actin; lane 4, Mch2α + actin; lane 5, extracts from cells undergoing apoptosis + actin; and lane 6, extracts from cells undergoing apoptosis. (*B*) Immunoblotting with a polyclonal antibody against the C-terminal 11 amino acids of actin. Lane 1, actin alone; lane 2, CPP32 + actin; lane 3, extracts from cells undergoing apoptosis + actin; and lane 4, extracts from cells undergoing apoptosis.

to investigate the cleavage of actin in more detail. Use of a monoclonal antibody recognizing amino acid residues 23–34 of actin demonstrated that CPP32 cleaved actin into one major fragment of 30 kDa (Fig. 1*A*, lane 2); at longer periods of incubation the amount of the 30-kDa fragment increased and further degradation of this fragment was evident (results not shown). Degradation to the 30-kDa fragment was identical to that observed when extracts from a Burkitt lymphoma cell line (BL30A), induced to undergo apoptosis with etoposide (40 μM), were incubated with purified actin (Fig. 1*A*, lane 5). In this case also the 30-kDa fragment was further degraded with time (results not shown). Mashima *et al* (45) have previously reported a similar pattern of actin degradation in extracts from etoposide-treated U937 cells. Very little cleavage was obtained when actin was treated with recombinant Mch2α and the fragment was less than 30 kDa (Fig. 1*A*, lane 4), and no cleavage was detected with TX protease (lane 3) or when extracts only were used (lane 6). Since the monoclonal antibody was directed against the N terminus of actin, we also employed a polyclonal antibody against the C-terminal 11 amino acids of actin that, on the basis of the cleavage pattern described elsewhere (43) and in Fig. 1*A*, was predicted to detect a 14-kDa fragment. This antibody detected a fragment of the expected size, when either the recombinant enzyme CPP32 (Fig. 1*B*, lane 2) or extracts from BL30A cells undergoing apoptosis (lane 3) were incubated with actin. The additional bands in lanes 3 and 4 (extracts only) are due to cross-reacting bands, since they are also present in untreated extracts (results not shown). It is also clear from lane 4 that incubation of extracts alone does not lead to degradation of endogenous actin (largely polymerized), indicating that actin is susceptible to cleavage only in the unpolymerized G form. This observation agrees with a recent report, in which several

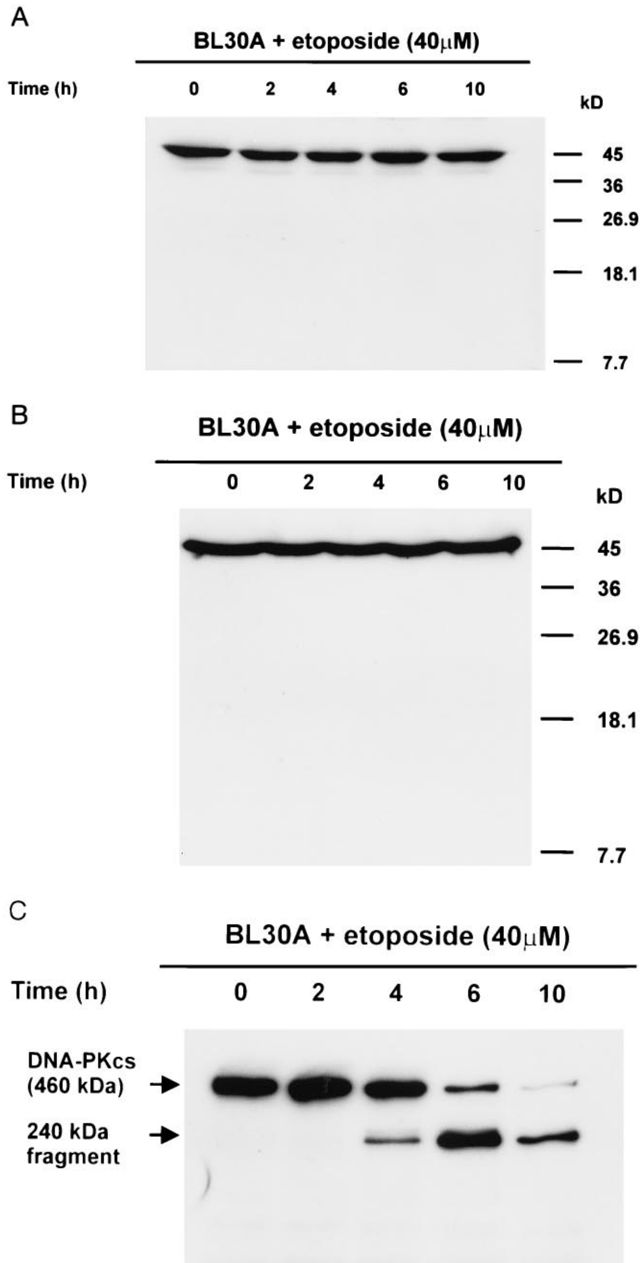

FIG. 2. Lack of cleavage of actin during apoptosis induced by etoposide in BL30A cells. (*A*) A polyclonal antibody against the C-terminal 11 amino acids of actin was used for the immunoblot. (*B*) Immunoblotting with a monoclonal antibody against amino acid region 23–34 of actin. (*C*) Time course of cleavage of DNA-PKcs after exposure of BL30A cells to 40 μM etoposide. DNA-PKcs was detected by using a polyclonal antibody against amino acid region 2018–2136 of DNA-PKcs (DPK1).

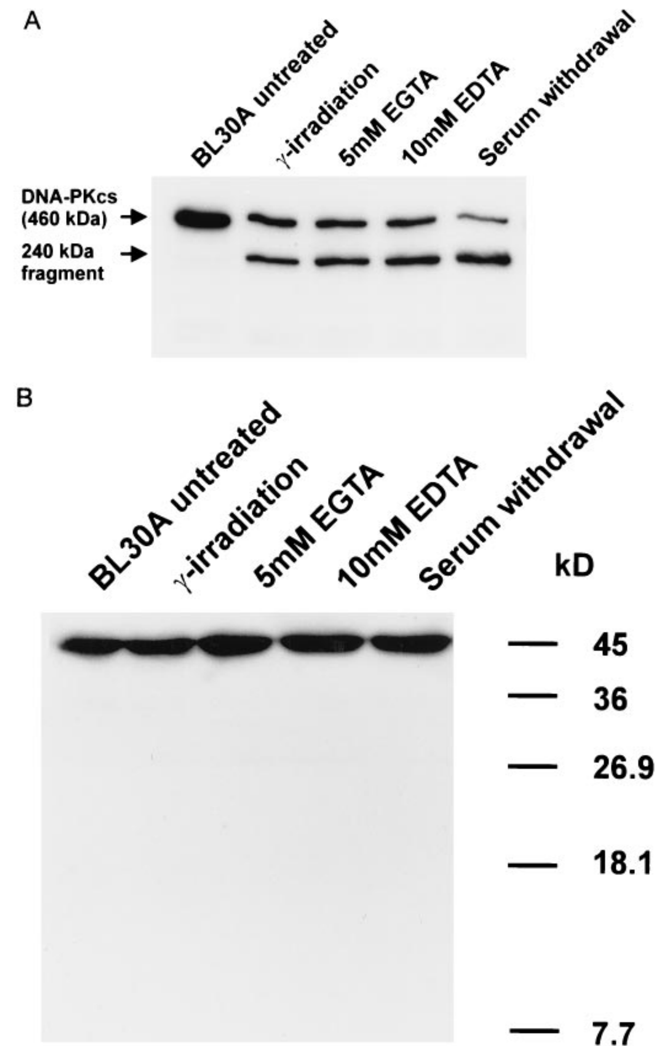

FIG. 3. Lack of degradation of actin during apoptosis of BL30A cells induced by a variety of agents and serum withdrawal. (*A*) Effect of different agents and serum withdrawal on the cleavage of DNA-PKcs. Cells were treated with γ-irradiation (20 Gy) and incubated for 8 h; EGTA (5 mM) for 8 h; EDTA (10 mM) for 6 h; or serum was withdrawn for 24 h. In all cases, ≈40-60% of the cells were undergoing apoptosis. (*B*) Lack of degradation of actin after treatment of BL30A cells with the same agents and serum withdrawal. A polyclonal antibody against the C-terminal 11 amino acids of actin was used for the immunoblot.

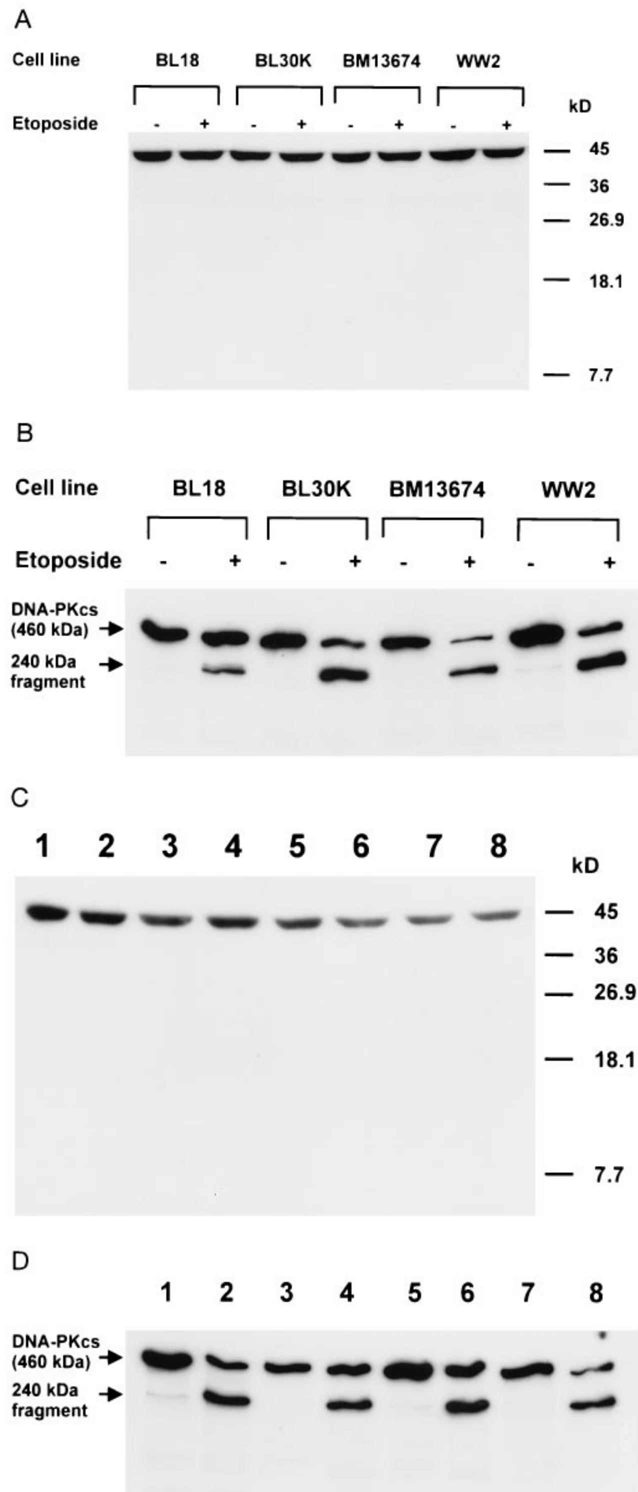

FIG. 4. Lack of actin cleavage in different cell types by a variety of agents causing apoptosis. (*A*) Lack of cleavage of actin in different Burkitt lymphoma cells after exposure of cells to 40 μM etoposide. A polyclonal antibody against the C-terminal 11 amino acids of actin was used for immunoblotting. (*B*) Cleavage of DNA-PKcs during apoptosis induced by etoposide (40 μM) in different Burkitt lymphoma cells. DNA-PKcs was detected with DPK1 antibody. (*C*) Lack of degradation of actin in HeLa, Molt-4, U937, and CTL cells undergoing apoptosis induced by different agents. For HeLa cells, extracts were prepared from detached cells after 8 h of treatment with etoposide (40 μM). For Molt-4 cells, cells were irradiated (20 Gy) and extracts were prepared 8 h after irradiation. For U937 cells, cells were pretreated with interferon γ (200 international units/ml) for 24 h and then incubated with anti-Fas antibody at 50 ng/ml for 10 h prior to preparation of extracts. CTLs were incubated with specific peptide for 4 h prior to preparation of extracts. Lane 1, Molt4 untreated; lane 2, Molt4 exposed to 20 Gy of γ-irradiation; lane 3, HeLa untreated; lane 4, HeLa + 40 μM etoposide; lane 5, CTL untreated; lane 6, CTL + specific peptide; lane 7, U937 untreated; lane 8, U937 + 50 ng/ml anti-Fas antibody. A polyclonal antibody against the C-terminal 11 amino acids of actin was used for immunoblotting. (*D*) Cleavage of DNA-PKcs during apoptosis induced by different agents in HeLa, Molt-4, U937, and CTL cells. DNA-PKcs was detected with DPK1 antibody.

protein substrates were cleaved when extracts from Jurkat cells were incubated with nuclei in the presence of granzyme B or CL granules, but there was no cleavage of actin observed within these extracts (48).

Fate of Actin in Cells Undergoing Apoptosis. The data obtained with lysates suggest that CPP32 is responsible for the cleavage of actin in cells undergoing apoptosis, as appears to be the case for other substrates, PARP (29), DNA-PKcs (33), and U1 70-kDa protein (35). To investigate the degradation of actin during apoptosis, we used BL30A cells, which had previously been shown to be highly susceptible to apoptosis induced by a variety of agents (33, 49). We found no evidence for actin degradation up to 10 h after treatment with etoposide using the two antibodies (Fig. 2*A* and *B*), at which time more than 80% of BL30A cells are undergoing apoptosis (results not shown). Under these conditions degradation of DNA-PKcs is detected by 4 h, and it is extensive by 10 h (Fig. 2*C*). When different agents were used under conditions that gave rise to 50% apoptosis, DNA-PKcs was degraded to approximately 50% (Fig. 3*A*, lanes 2–5). However, there was no evidence for cleavage of actin under the same conditions (Fig. 3*B*). Contrary to a previous report showing that in PC12 cells deprived of serum, degradation of actin occurred 24–48 h later (43), we saw no evidence for such degradation over the same time period in BL30A cells when the same polyclonal antibody was used (Fig. 3*B*, lane 5).

Use of Different Cell Lines. To rule out that the resistance of actin to degradation in BL30A cells was a peculiarity of that cell line, we chose four other Burkitt cell lines, known to undergo apoptosis in response to different agents (33, 49). In all cases, we failed to detect cleavage of actin (Fig. 4*A*). Under these conditions 40–60% of cells were undergoing apoptosis (results not shown). However, as observed previously, DNA-PKcs was again degraded in response to treatment with the different agents in all the cell lines used (Fig. 4*B*). Non-Burkitt cells were also used to check for actin degradation. No cleavage was evident in HeLa cells treated with 40 μM etoposide (Fig. 4*C*, lane 2); in U937 cells treated with anti-Fas antibody (lane 8); in γ-irradiated (20 Gy) Molt 4 cells (lane 4); and in Epstein–Barr virus-specific CTLs (clone LC13) (lane 6), which we have demonstrated previously to be effective targets for their own lysis in the presence of specific epitope (46). In all these cell types with the different apoptosis-inducing stimuli, DNA-PKcs was degraded as expected (Fig. 4*D*).

DISCUSSION

It is becoming increasingly obvious that activation of a family of ICE-like proteases is common to apoptosis induced by a variety of stimuli (5, 6). On the basis of substrate preference and order of activation, it seems likely that an amplifiable protease cascade exists involving several activation pathways (22, 26). One such pathway involves the activation of Mch4, which in turn cleaves and activates CPP32 and Mch3 (22). Once activated, these proteases target a number of important substrates involved in functional and structural roles in the cell (5, 6). Since the majority of proteins, as determined by Coomassie blue staining, remain intact in cells undergoing apoptosis, it is evident that cleavage by the ICE-like proteases

is highly selective, certainly at the earlier stages of the process (5, 33). Several of these targets are present in the nucleus, where fragmentation of DNA has been a classical indicator of apoptosis (reviewed in ref. 50). Nuclear events occur at several levels, including degradation of lamins, which would be expected to lead to chromatin collapse (38, 39); cleavage of two key DNA repair enzymes, DNA-PKcs and PARP, which would facilitate the fragmentation of DNA (13–15, 31–34, 37); the loss of a number of proteins involved in DNA processing and maintaining chromatin structure (36, 37, 51); and the degradation of proteins implicated in splicing and maturation of mRNA (19, 35). Outside the nucleus the specific degradation of fodrin, a major component of the cortical cytoskeleton during apoptosis induced by different agents, has been linked to the characteristic morphological changes such as membrane blebbing and condensation of the cytoplasm (44). In support of this, Kayalar *et al*. (43) have shown that actin, which associates with fodrin, is degraded at 24 h after withdrawal of serum from rat PC12 cells. This cleavage would also be expected to alter cell morphology as a result of depolymerization of F-actin. In addition, actin cleaved with ICE *in vitro* has a reduced capacity to inhibit DNase 1, an enzyme implicated in DNA fragmentation during apoptosis (52, 53), and the cleaved product is diminished in its ability to polymerize (43). On the basis of these *in vitro* data and the observation that actin is degraded in PC12 cells undergoing apoptosis, Kayalar *et al*. (43) have proposed a model in which actin normally inhibits DNase 1, but on degradation of actin by an ICE-like protease, this endonuclease is free to enter the nucleus and fragment DNA. In addition, the degradation of actin would disrupt the cytoskeleton and contribute to the morphological changes seen in apoptosis.

While this hypothesis is attractive, it depends very much on the universality of actin cleavage. The major outcome of the present study was the observation that actin was not degraded during the process of apoptosis in a variety of human cells, nor was it degraded when treated extracts were incubated at 37°C. Degradation was observed only when exogenous unpolymerized actin was added to treated extracts. This agrees with the resistance of polymerized actin to degradation in whole cells. We have investigated several stimuli that induce apoptosis, including DNA-damaging agents, Fas ligation, serum withdrawal, and CTL-mediated killing, and in no case did we obtain evidence for any degradation of actin, but another known substrate, DNA-PKcs, was cleaved in response to all the different agents. On the other hand, when extracts were prepared from cells undergoing apoptosis and incubated with actin, a monoclonal antibody against the N terminus detected a 30-kDa fragment. This pattern was supported by the use of a C-terminal antibody that would be expected to detect a fragment of approximately 14 kDa. These observations are compatible with the predicted site of cleavage at Asp-244 to generate the 30-kDa product (43). Kayalar *et al*. (43), using purified ICE incubated with actin, demonstrated a similar pattern of cleavage of actin, and Mashima *et al*. (45) observed the same patterns when they used actin incubated with cytosolic extracts from U937 cells undergoing apoptosis. Use of peptide inhibitors prevented cleavage of actin, indicating that it was a substrate from an unidentified ICE-like protease (45). These results demonstrate that while cytosolic extracts are useful to identify potential substrates in apoptosis (14, 15, 20, 22, 43, 45), this extrapolation cannot always be made. Actin is a typical example of the latter, being a good substrate for extracts and recombinant ICE-like proteases *in vitro* but resistant to degradation *in vivo*.

Ann Knight is acknowledged for typing the manuscript. We thank the Australian National Health and Medical Research Council and the Queensland Cancer Fund for support.

1. Wyllie, A. H., Kerr, J. F. R. & Currie, A. R. (1980) *Int. Rev. Cytol.* **68,** 251–306.
2. Steller, H. (1995) *Science* **267,** 1445–1449.
3. White, E. (1996) *Genes Dev.* **10,** 1–15.
4. Vaux, D. L. & Strasser, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2239–2244.
5. Martin, S. J. & Green, D. R. (1995) *Cell* **82,** 349–352.
6. Kumar, S. & Lavin, M. F. (1996) *Cell Death Differ.* **3,** 255–267.
7. Ellis, R. E., Yuan, J. & Horvitz, H. R. (1991) *Annu. Rev. Cell Biol.* **9,** 663–698.
8. Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M. & Horvitz, H. R. (1993) *Cell* **75,** 641–652.
9. Thornberry, N. A., Bull, H. G., Calaycay, J. R., Chapman, K. T., Howard, A. D., *et al*., (1992) *Nature (London)* **356,** 768–774.
10. Miura, M., Zhu, H., Rotello, R., Hartwig, E. A. & Juan, J. (1993) *Cell* **75,** 653–660.
11. Kumar, S., Kinoshita, M., Noda, M., Copeland, N. G. & Jenkins, N. A. (1994) *Genes Dev.* **8,** 1613–1626.
12. Wang, L., Miura, M., Bergeron, L., Zhu, H. & Yuan, J. (1994) *Cell* **78,** 739–750.
13. Fernandes-Alnemri, T., Litwack, G. & Alnemri, E. S. (1994) *J. Biol. Chem.* **269,** 30761–30764.
14. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., Munday, N. A., Raju, S. M., Smulson, M. E., Yamin, T. T., Yu, V. L. & Miller, D. K. (1995) *Nature (London)* **376,** 37–63.
15. Tewari, M., Quan, L. T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S. & Dixit, V. M. (1995) *Cell* **81,** 801–809.
16. Faucheu, C., Diu, A., Chan, A. W. E., Blanchet, A. M., Miossec, C., Herve, F., Collard-Dutilleul, V., Gu, Y., Aldape, R. A., Lippke, J. A., Rocher, C., Su, M. S. S., Livingston, D. J., Hercend, T. & Lalanne, J. L. (1995) *EMBO J.* **14,** 1914–1922.
17. Munday, N. A., Vaillancourt, J. P., Ali, A., Casano, F. J., Miller, D. J., Milineaux, S. M., Yamin, T. T., Yu, V. L. & Nicholson, D. W. (1995) *J. Biol. Chem.* **270,** 15870–15876.
18. Faucheu, C., Blanchet, A. M., Collard-Dutilleul, V., Lalanne, J. L. & Diu-Hercend, A. (1996) *Eur. J. Biochem.* **236,** 207–213.
19. Fernandes-Alnemri, T., Litwack, G. & Alnemri, E. S. (1995) *Cancer Res.* **55,** 2737–2742.
20. Fernandes-Alnemri, T., Takahashi, A., Armstrong, R., Krebs, J., Fritz, L., Tomaselli, K. J., Wang, L., Yu, Z., Croce, C. M., Earnshaw, W. C., Litwack, G. & Alnemri, E. S. (1995) *Cancer Res.* **55,** 6045–6052.
21. Duan, H., Chinnaiyan, A. M., Hudson, P. L., Wing, J. P., He, W. W. & Dixit, V. M. (1996) *J. Biol. Chem.* **271,** 1621–1625.
22. Fernandes-Alnemri, T., Armstrong, R., Krebs, J., Srinivasula, S. M., Wang, L., Bullrich, F., Fritz, L. C., Trapani, J. A., Tomaselli, K. J., Litwack, G. & Alnemri, E. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 7464–7469.
23. Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J. D., Zhang, M., Gentz, R., Mann, M., Krammer, P. H., Peter, M. E. & Dixit, V. M. (1996) *Cell* **85,** 817–827.
24. Hasegawa, J. I., Kamada, S., Kamiike, W., Shimizu, S., Imazu, T., Matsuda, H. & Tsujimoto, Y. (1996) *Cancer Res.* **56,** 1713–1718.
25. Chinnaiyan, A. M., Hanna, W. L., Orth, K., Duan, H., Poirier, G. G., Froelich, C. J. & Dixit, V. M. (1996) *Curr. Biol.* **6,** 897–899.
26. Kumar, S. (1996) *Int. J. Biochem. Cell. Biol.*, in press.
27. Althus, F. R. & Ritcher, C. (1987) *Mol. Biol. Biochem. Biophys.* **37,** 1–126.
28. Satoh, M. S. & Lindahl, T. (1992) *Nature (London)* **356,** 356–358.
29. Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G. & Earnshaw, W. C. (1994) *Nature (London)* **371,** 346–347.
30. Gu, Y., Sarnecki, C., Aldape, R. A., Livingston, D. J. & Su, M. S.-S. (1995) *J. Biol. Chem.* **270,** 18715–18718.
31. Casciola-Rosen, L. A., Anhalt, G. J. & Rosen, A. (1995) *J. Exp. Med.* **182,** 1625–1634.
32. Casciola-Rosen, L. A., Anhalt, G. J. & Rosen, A. (1996) *J. Exp. Med.* **183,** 1957–1964.
33. Song, Q., Lees-Miller, S. P., Kumar, S., Zhang, N., Chan, D. W., Smith, G. C. M., Litwack, G., Khanna, K. K. & Lavin, M. F. (1996) *EMBO J.* **15,** 3238–3246.
34. Song, Q., Burrows, S. R., Smith, G., Lees-Miller, S. P., Kumar, S.,

Chan, D. W., Trapani, J. A., Alnemri, E., Litwack, G., Lu, H., Moss, D. J., Jackson, S. & Lavin, M. F. (1996) *J. Exp. Med.* **184,** 619–628.
35. Casciola-Rosen, L. A., Miller, D. K., Anhalt, G. J. & Rosen, A. (1994) *J. Biol. Chem.* **269,** 30757–30760.
36. Kaufmann, S. H. (1989) *Cancer Res.* **49,** 5870–5878.
37. Voekel-Johnson, C., Entingh, A. J., Wold, W. S. M., Gooding, L.-R. & Laster, S. M. (1995) *J. Immunol.* **154,** 1707–1716.
38. Neamati, N., Fernandez, A., Wright, S., Kiefer, J. & McConkey, D. J. (1995) *J. Immunol.* **154,** 3788–3795.
39. Lazebnik, Y. A., Takahashi, A., Moir, R. D., Goldman, R. D., Poirier, G. G., Kaufmann, S. H. & Earnshaw, W. C. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 9042–9046.
40. Wang, X., Zelenski, N. G., Yang, J., Sakai, J., Brown, M. S. & Goldstein, J. L. (1996) *EMBO J.* **15,** 1012–1020.
41. Brancolini, C., Benedetti, M. & Schneider, C. (1995) *EMBO J.* **14,** 5179–5190.
42. Emoto, Y., Manome, Y., Meinhardt, G., Kisaki, H., Kharbanda, S., Robertson, M., Ghayur, T., Wong, W. W., Kamen, R., Weichselbaum, R. & Kufe, D. (1995) *EMBO J.* **14,** 6148–6156.
43. Kayalar, C., Örd, T., Testa, M. P., Zhong, L. T. & Bredesen D. E. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2234–2238.
44. Martin, S., O'Brien, G. A., Nishioka, W. K., McGahon, A. J., Mahboubi, A., Saido, T. C. & Green, D. R. (1995) *J. Biol. Chem.* **270,** 6425–6428.
45. Mashima, T., Naito, M., Fujita, N., Noguchi, K. & Tsuruo, T. (1995) *Biochem. Biophys. Res. Commun.* **217,** 1185–1192.
46. Moss, D. J., Burrows, S. R., Baxter, G. D. & Lavin, M. F. (1991) *J. Exp. Med.* **173,** 681–686.
47. Thornberry, N. A., Peterson, E. P., Zhao, J. J., Howard, A. D., Griffin, P. R. & Chapman, K. T. (1994) *Biochemistry* **33,** 3934–3940.
48. Martin, S. J., Amarante-Mendes, G. P., Shi, L., Chuang, T.-H., Casiano, C. A., O'Brien, G. A., Fitzgerald, P., Tan, E. M., Bokoch, G. M., Greenberg, A. H. & Green, D. R. (1996) *EMBO J.* **15,** 2407–2416.
49. Khanna, K. K., Wie, T., Song, Q., Burrows, S. R., Moss, D. J., Krajewski, S., Reed, J. C. & Lavin, M. F. (1996) *Cell Death Differ.*, in press.
50. Ashkenas, J. & Werb, Z. (1996) *J. Exp. Med.* **183,** 1947–1951.
51. Baxter, G. D., Smith, P. J. & Lavin, M. F. (1989) *Biochem. Biophys. Res. Commun.* **162,** 30–37.
52. Ucker, D. S., Obermiller, P. S., Eckhart, W., Apgar, J. R., Berger, N. A. & Meyers, J. (1992) *Mol. Cell Biol.* **12,** 3060–3069.
53. Peitsch, M. C., Polzar, B., Stephen, H., Crompton, T., MacDonald, H. R., Mannherz, H. G. & Tschopp, J. (1993) *EMBO J.* **12,** 371–377.