Actin Stabilization by Jasplakinolide Enhances Apoptosis Induced by Cytokine Deprivation*

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S. Celeste Posey‡§§ and Barbara E. Bierer‡¶**†††

From the ‡Department of Pediatric Oncology, Dana-Farber Cancer Institute, the §Committee on Immunology, Division of Medical Sciences, and the ¶¶Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115 and the ¶¶¶NHLBI, National Institutes of Health, Bethesda, Maryland 20892

Participation of the actin cytoskeleton in the transmission of proliferative signals has been established through the use of compounds that disrupt the cytoskeleton. To address the possibility that actin also participates in the transmission of an apoptotic signal, we have studied the response of the murine interleukin 2 (IL-2)-dependent T cell line CTLL-20 to treatment with the actin-binding compound jasplakinolide upon IL-2 deprivation. Like phallolidin, jasplakinolide stabilizes F-actin and promotes actin polymerization. Treatment of CTLL-20 cells with jasplakinolide, in the presence or absence of recombinant human IL-2, altered actin morphology as assessed by confocal fluorescence microscopy. Jasplakinolide was not toxic to CTLL-20 cells, nor was apoptosis induced in the presence of exogenous recombinant human IL-2. However, actin stabilization at the time of IL-2 deprivation enhanced apoptosis by changing the time at which CTLL-20 cells committed to the apoptotic pathway. This effect of jasplakinolide correlated with its ability to stabilize polymerized actin, as treatment with a synthetic analog of jasplakinolide with a greatly reduced ability to bind actin, jasplakinolide B, did not enhance apoptosis. The enhancement occurred upstream of the induction of caspase-3-like activity and could be inhibited by the overexpression of the anti-apoptotic protein Bcl-xL. These data suggest that the actin cytoskeleton plays an active role in modulating lymphocyte apoptosis induced by cytokine deprivation.

The actin cytoskeleton appears to be intricately involved in lymphocyte signal transduction (1, 2). It has long been recognized that control of the actin cytoskeleton must be coordinated with control of cell cycle events (3) and that actin-related events, such as adhesion, receptor clustering, and receptor internalization, can affect mitogenic signals (4–6). However, the role of the actin cytoskeleton in transmission of intracellular signals leading to apoptosis in lymphocytes remains poorly defined. Apoptosis is a regulated process by which a cell undergoes a form of cell death characterized by cell shrinkage, membrane blebbing, DNA cleavage, and nuclear condensation (7, 8). Elucidation of apoptotic signal transduction pathways has focused largely upon members of the Bcl family and upon the activation of caspases; the mechanisms by which cellular damage results in the changes in mitochondrial membrane potential thought to be required to initiate apoptosis are relatively unexplored.

There is compelling evidence to suggest that the disruption of actin-based, integrin-mediated adhesion events is sufficient to trigger apoptosis in endothelial and epithelial cell lines (reviewed in Ref. 9). However, in a model of lymphocyte apoptosis, suspension cells can be used to segregate the role of the actin cytoskeleton in apoptotic signal transduction from actin-dependent, integrin-mediated survival signals. To study the possibility that the actin cytoskeleton is involved in the transmission of an apoptotic signal triggered by growth factor deprivation, we treated the nonadherent, IL-2-dependent T cell line CTLL-20 with the novel actin-binding cyclodepsipeptide jasplakinolide upon withdrawal from IL-2. Similar to phallloidin, jasplakinolide binds to and stabilizes actin microfilaments and can promote actin polymerization in vitro (10). Jasplakinolide differs from phallloidin, however, in that it is permeant across cell membranes and can therefore be easily used in vivo (10). Previous studies reported that jasplakinolide inhibited the growth of prostate carcinoma cell lines in vitro (11), sensitized Lewis lung carcinoma to radiation in vivo (12), and prevented the self-renewal of acute myeloid leukemia cells (13). In each of these reports, the effects of jasplakinolide were associated with its ability to stabilize F-actin. None of these studies, however, addressed whether the inhibition of growth or sensitization was secondary to induction or enhancement of apoptosis.

We report that the addition of the actin-stabilizing compound jasplakinolide to CTLL-20 cells enhanced apoptosis induced by IL-2 cytokine deprivation. Jasplakinolide was not toxic to cells, nor was apoptosis induced in the presence of recombinant human IL-2 (rhIL-2). The enhancement of apoptosis was time- and concentration-dependent, occurred upstream of caspase activation, and could be attenuated by the overexpression of the anti-apoptotic protein Bcl-xL. Furthermore, actin stabilization accelerated the time at which CTLL-20 cells committed to the apoptotic program. Taken together, these data suggest that modification of the actin cytoskeleton impacts upon signal transduction leading to apoptosis.

MATERIALS AND METHODS

Reagents—Jasplakinolide and its analog jasplakinolide B were provided by the Drug Synthesis and Chemistry Branch, Developmental

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‡‡ To whom correspondence should be addressed: NHLBI, Bldg. 10, Rm. 5D49, 10 Center Dr., Bethesda, MD 20892. Tel.: 301-402-6786; Fax: 301-480-1792; E-mail: biererb@nih.gov.

† The abbreviations used are: IL, interleukin; rhIL-2, recombinant human IL-2; cRPMI-10%, complete RPMI-10%; TUNEL, terminal deoxynucleotide transferase-mediated nick end labeling; FITC, fluorescein isothiocyanate.
Therapeutics Program, Division of Cancer Treatment, NCI, National Institutes of Health (Bethesda, MD). Jasplakinolide and jasplakinolide B were stored in Me₃SO at -20 or -80 °C and diluted into media immediately prior to use. rhIL-2 was kindly provided by Hoffman-LaRoche (Nutley, NJ).

Cell Lines and Cell Culture—The IL-2-dependent murine cell line CTLL-20 (14) was grown in RPMI 1640 medium (MediaTech, Herndon, VA) supplemented with 10% heat-inactivated fetal calf serum (Serma), 10 mm HEPES (Sigma), 2 mm l-glutamine (MediaTech), and 50 μm 2-mercaptoethanol (Sigma) (termed complete RPMI-10% (cRPMI-10%)) to which had been added 1–2% IL-2-containing supernatant conditioned on concanavalin A-stimulated rat spleenocytes. Forty-eight hours prior to an experiment, CTLL-20 cells were expanded in cRPMI-10% supplemented with 100 units/ml rhIL-2. A CTLL-2 clone transfected with pSFFVNeo-bcl-X₀ and consequently overexpressing the Bcl-X₀ protein (BcL-X₀, CTLL-2) was kindly provided by Dr. Craig Thompson (University of Chicago, Chicago, IL) (15). Overexpression of the Bcl-X₀ protein was confirmed by Western blot analysis (data not shown). Bcl-X₀-CTLL-2 cells were grown in cRPMI-10% supplemented with 100 units/ml rhIL-2. All cell lines were free of mycoplasma as determined monthly by mycoplasma PCR Primer Set, Stratagene, La Jolla, CA). For all apoptosis assays, CTLL-20 cells were washed three times in cRPMI-10% supplemented at a density of 2 × 10⁶ cells/ml in cRPMI-10% without rhIL-2 to which either drug or vehicle had been added. Washed cells resuspended in cRPMI-10% with rhIL-2 (100 units/ml) as杀死 controls. The final concentration of Me₃SO in all treatment groups ranged from 0.02 to 0.1%, as indicated.

Immunofluorescent Staining of Cytoskeleton—Cells (1 × 10⁶/sample) treated as indicated were fixed in 4% paraformaldehyde and cytospun (Cytospin 3 cell preparation system, Shandon Scientific Ltd., Cheshire, United Kingdom) onto glass slides. Cells were incubated with 50 μm NH₄Cl for 5 min, permeabilized with a solution of 0.1% Triton X-100 in phosphate-buffered saline for 2.5 min, and blocked with a 2% solution of bovine serum albumin. Cells were then incubated with 30 μg/ml anti-actin monoclonal antibody N350 (Amersham Pharmacia Biotech) for 30 to 45 min at room temperature. Cells were washed three times and incubated with 15 μg/ml Texas Red-conjugated anti-mouse IgM antibody (Jackson ImmunoResearch, West Grove, PA) for 30 min at room temperature. All cells were stained with 1 μg/ml Hoechst 33258. Cyto- spin samples were mounted in a 0.1% (w/v) solution of phenyldiethanolamine in 90% glycerol. Slides were viewed by confocal fluorescence microscopy (model TC514/DMIRBE, Leica Inc., Deerfield, IL) equipped with an argon and argon-krypton lasers for UV (351–364 nm) and red (568 nm) excitation.

Hoechst Staining—Cells were fixed and stained as described (16). In brief, cells (2 × 10⁵/sample) were fixed in an excess volume of 3:1 (v/v) methanol/acetic acid, dried onto glass slides, and stained with a 1 μg/ml Hoechst 33258 (Sigma) solution in double distilled H₂O blocked with nonfat dried milk. In one set of experiments, a 20 or 50 μg/ml solution of pan caspase (Sigma) with 0.5 μg/ml RNase (Boehringer Mannheim) also blocked with nonfat dried milk was used instead of Hoechst as a nuclear stain. The percentage of cells undergoing apoptosis was determined by counting the number of cells with and without condensed nuclei by fluorescence microscopy (Zeiss Axioskop MC100; Carl Zeiss, Thornwood, NY, or Olympus Bmax BX50, Olympus America, Melville, NY). Between 300 and 700 cells were counted for each sample. The 95% confidence interval for each sample within each experiment was determined according to the statistical definition of the variance of a proportion in a binomial experiment (17). The variance was computed as pq/n, where p is the proportion apoptotic, q = 1 - p, and n is the number of cells counted.

For the dose-response curve of jasplakinolide, the results of three experiments were averaged and the data plotted as the mean ± S.D. The IC₅₀ was determined by logit analysis.

RESULTS

Jasplakinolide Promoted Cell Shape Change and Redistribution of Actin in CTLL-20 Cells—It has previously been shown that jasplakinolide enhances actin polymerization and alters the cellular actin cytoskeleton (10, 11). To confirm that jasp- plakinolide altered the actin cytoskeleton in CTLL-20 cells, cells were treated with jasplakinolide (100 nM) or Me₃SO (0.02%) for 8 h in the absence of IL-2. Cytospin preparations were stained by indirect immunofluorescence using an anti-actin monoclonal antibody and with Hoechst 33258 to assess nuclear morphology and analyzed by confocal fluorescence microscopy. Jasplakinolide caused both cell shape change and a redistribution of the actin cytoskeleton (Fig. 1). CTLL-20 cells were observed to be typically smooth and round with an even peripheral distribution of actin. Treatment with jasplakinolide induced protrusions of the cell surface and resulted in a patchy appearance of cortical actin. In some cells treated with jasp- plakinolide, cortical actin appeared only around the nucleus (Fig. 1). The change in actin cytoskeletal distribution was not a result of the apoptotic process, as it occurred prior to commitment to apoptosis (see below), prior to the appearance of condensed or apoptotic nuclei (Fig. 1), and in the presence of rhIL-2 when few, if any, cells were entering or undergoing apoptosis (data not shown).

Stabilization of the Actin Cytoskeleton Enhanced Apoptosis of CTLL-20 Cells in Response to IL-2 Deprivation—Previous reports have suggested that the actin cytoskeleton may play a role in transducing proliferative signals in lymphocytes (see, e.g. Refs. 18–20). To determine whether actin might also be involved in transducing an apoptotic signal, CTLL-20 cells were incubated in the presence or absence of rhIL-2 with jasplakinolide or with vehicle, Me₃SO. Additionally, cells were also treated with jasplakinolide B (Fig. 2A, inset), an analog of jasplakinolide that has been reported to lack actin-modulating activity (11) at a concentration of 1 μM, jasplakinolide B had no demonstrable effect on cell shape or actin redistribution in CTLL-20 cells (data not shown). Cells were harvested after 12 h of incubation with drug, and the percentage of apoptotic cells was quantified by nuclear morphology (Fig. 2A). The number of cells with condensed nuclei after IL-2 deprivation (27%) increased 2-fold (56%) after the addition of jasplakinolide at a concentration of 100 nM. At the concentrations used, jasp- plakinolide was not toxic to cells, as the percentages of apoptotic CTLL-20 cells incubated with rhIL-2 were similar in the absence (1.1%) and presence (2.6%) of drug, a difference that was not statistically significant. Furthermore, the effect of jasplakinolide on apoptosis was dependent upon its ability to bind actin, as the non-actin binding analog jasplakinolide B did not show a similar effect, even at a 10-fold higher concentration (Fig. 2A). In addition, 100 nM jasplakinolide also enhanced...
apoptosis of the pre-B, IL-3-dependent Ba/F3 cell line in response to IL-3 deprivation (data not shown), demonstrating that the effect of drug on apoptosis was not specific to the CTLL-20 cell line nor to dependence on IL-2.

TUNEL staining and subsequent flow cytometry confirmed the results obtained with Hoechst staining (Fig. 2B). The TUNEL assay labels the 3'-hydroxyl ends of DNA remaining after cleavage by endonucleases (21); thus, cells that are stained FITC-bright are considered apoptotic. In the experiment shown (Fig. 2B), the percentage of FITC-bright cells in the jasplakinolide-treated population (~70%) was greater than that in the Me2SO-treated population (~40%) after 15 h of IL-2 deprivation. Again, treatment with jasplakinolide did not cause cell death in the presence of rhIL-2, confirming that jasplakinolide is not nonspecifically toxic to the cells. Both DNA content analysis by propidium iodide staining followed by flow cytometry and DNA fragmentation analysis by agarose gel electrophoresis confirmed that jasplakinolide enhanced apoptosis upon IL-2 deprivation (data not shown); polymerization of actin thus appeared to affect either the apoptotic signal or the apoptotic process.

Stabilization of Actin Accelerated the Appearance of Apoptotic CTLL-20 Cells after IL-2 Withdrawal—Treatment with jasplakinolide accelerated the appearance of apoptotic cells (Fig. 3A) after withdrawal of IL-2, although the total proportion of cells (90–95%, Fig. 3A and data not shown) that eventually completed apoptosis remained unchanged. Condensed nuclear morphology indicative of apoptosis was not observed in the Me2SO-treated cell population until 12 h after deprivation of IL-2, and most cells had undergone apoptosis by 24 h (Fig. 3A), consistent with time courses previously reported (22, 23). Stabilization of actin accelerated the apoptotic process: cells treated with jasplakinolide, unlike Me2SO-treated cells, exhibited condensed nuclei at 8 h, and over 80% were dead by 18 h. The most dramatic difference was observed approximately 12 h after withdrawal from IL-2, when jasplakinolide treatment resulted in apoptosis of 50–60% of cells, whereas only 10–30% of Me2SO-treated cells were apoptotic (Fig. 3A and data not shown). CTLL-20 cells incubated with jasplakinolide in the presence of rhIL-2 (100 units/ml) did not undergo apoptosis during the time course of these experiments.

A dose-response curve of jasplakinolide demonstrated that concentrations as low as 50 nM were sufficient to enhance apoptosis, whereas concentrations of 25 nM and below had no effect on the apoptotic process (Fig. 3B). The IC50 for this enhancement was calculated as approximately 35 nM, a concentration similar to the reported IC50 of 34 nM for inhibition of proliferation of a prostate carcinoma cell line (11). At the concentrations used in these experiments, the addition of Me2SO (0.02–0.1%) did not increase the number of cells undergoing apoptosis after IL-2 deprivation compared with cells incubated in cRPMI-10% alone (data not shown).

Stabilization of Actin Altered the Time Course of Commitment to Apoptosis—To address whether actin stabilization altered the time course of commitment to apoptosis, CTLL-20 cells treated with jasplakinolide or Me2SO were deprived of IL-2 for varying lengths of time, after which 100 units/ml rhIL-2 was added back to the cultures. All samples were incubated for a total of 26 h and then fixed for quantification of apoptotic cells (Fig. 4). All treatment groups could be rescued by readdition of rhIL-2 within 8 h of deprivation, whereas neither could be rescued 14.5 h after withdrawal. Notably, readdition of rhIL-2 within 12 h of withdrawal was sufficient to rescue the Me2SO-treated cells, but not those treated with jasplakinolide, suggesting that actin stabilization altered the time course of commitment to apoptosis.

The Effect of the Stabilization of Actin on Apoptosis Occurred Upstream of Caspase Activation—Because the stabilization of actin by jasplakinolide altered the time of commitment to apoptosis, we predicted that caspase activity would be detected at an earlier time point in cells that had been treated with jasplakinolide. To test this prediction, we investigated the time

![Fig. 1. Jasplakinolide dramatically alters the morphology of the actin cytoskeleton in CTLL-20 cells. CTLL-20 cells were incubated with 100 nM jasplakinolide or 0.02% Me2SO (DMSO) in the absence of rhIL-2 for 8 h. Actin morphology was visualized by staining with an anti-actin monoclonal antibody and a Texas Red-conjugated secondary anti-mouse-IgM antibody. Nuclei were stained with Hoechst 33258. Images are magnified x 1600. Photomicrographs shown are representative of three independent experiments.](http://www.jbc.org/)

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course of induction of caspase-3-like activity. It has been previously demonstrated that caspase-3 (CPP32), but not caspase-1 (ICE), is activated in CTLL-20 cells undergoing apoptosis after IL-2 deprivation (24). CTLL-20 cells were incubated either with jasplakinolide (100 nM) or Me$_2$SO (0.02%) in the absence or presence (100 units/ml) of rhIL-2 for 8 or 12 h (Fig. 5 and data not shown). Cells were harvested after incubation for 12 h and fixed. Percentage of apoptotic cells was determined by Hoechst staining and counting condensed nuclei. Data shown are percentage of apoptotic cells ± 2 S.D., calculated as described under “Materials and Methods” and are representative of two independent experiments. Inset, chemical structures of jasplakinolide and its analog, jasplakinolide B, reproduced from Ref. 11 with permission. B, CTLL-20 cells were treated with 100 nM jasplakinolide or 0.02% Me$_2$SO and either withdrawn from (solid line) or maintained in (dotted line) 100 units/ml rhIL-2 and incubated for 15 h. Cells were then fixed in 1% paraformaldehyde for TUNEL staining. FL1-H indicates the degree of FITC labeling of the cell populations. FITC-bright populations were determined by comparison to the background levels of staining within each sample. Data are representative of two independent experiments.

FIG. 2. Stabilization of actin by jasplakinolide increased the number of CTLL-20 cells that have undergone apoptosis. A, CTLL-20 cells were treated with 0.1% Me$_2$SO (DMSO), 100 nM jasplakinolide, or 100 nM or 1 μM jasplakinolide B (Jas B) upon withdrawal from IL-2 (solid columns). Cell populations similarly treated with drug or vehicle were maintained in 100 units/ml rhIL-2 (open columns). All cells were harvested after incubation for 12 h and fixed. Percentage of apoptotic cells was determined by Hoechst staining and counting condensed nuclei. Data are shown for apoptotic cells ± 2 S.D., calculated as described under “Materials and Methods” and are representative of two independent experiments. Inset, chemical structures of jasplakinolide and its analog, jasplakinolide B, reproduced from Ref. 11 with permission. B, CTLL-20 cells were treated with 100 nM jasplakinolide or 0.02% Me$_2$SO and either withdrawn from (solid line) or maintained in (dotted line) 100 units/ml rhIL-2 and incubated for 15 h. Cells were then fixed in 1% paraformaldehyde for TUNEL staining. FL1-H indicates the degree of FITC labeling of the cell populations. FITC-bright populations were determined by comparison to the background levels of staining within each sample. Data are representative of two independent experiments.
again suggesting that actin stabilization affected the transduction of an apoptotic signal and not just the downstream effector phase of the apoptotic process.

The Overexpression of the Anti-apoptotic Protein Bcl-xL Attenuated the Effect of Actin Stabilization on Apoptosis—Bcl-xL, a member of the Bcl-2 family, has been shown to protect cells from apoptosis induced by cytokine withdrawal (15). To test whether Bcl-xL inhibited the enhancement of apoptosis by this actin-modulating agent, CTLL-2 cells overexpressing Bcl-xL were treated with jasplakinolide (100 nM) or Me2SO (0.02%), deprived of IL-2 for the indicated times, and fixed for Hoechst staining and counting. Cells were harvested at the indicated times, and the percentage of apoptotic cells was determined by Hoechst staining and counting. Data are shown as percentage of apoptotic cells ± 2 S.D. and are representative of two independent experiments. Bcl-xL overexpression appeared to attenuate, if not ablate, the effects of actin stabilization.

**DISCUSSION**

Jasplakinolide is a cyclodepsipeptide isolated from the marine sponge, *Jaspis johnstoni*, originally found to be an effective antifungal agent at micromolar concentrations (25) and later found to have antiproliferative activity in the nanomolar range in acute myeloid leukemia and prostate carcinoma cells (11, 13). Jasplakinolide has been shown to induce actin polymerization (10, 26, 27) and to bind F-actin competitively with phal-loidin with a dissociation constant ($K_d$) of approximately 15 nM (10); this agent has been used to investigate the functions of actin microfilaments in a variety of cellular processes, including ion transport, endocytosis, and adhesion (28–31). Here we demonstrated that jasplakinolide effectively modified the actin microfilament network and altered the time course of commitment to apoptosis.
cytoskeleton in T lymphocytes (Fig. 1), a property that allowed us to use this compound to investigate a potential role for actin in the transduction of an apoptotic signal in these suspension cells.

Stabilization of actin microfilaments by jasplakinolide accelerated commitment to apoptosis of CTLL-20 cells deprived of IL-2. Jasplakinolide did not induce apoptosis in the presence of IL-2 and was thus not toxic to the cells. The enhancement of apoptosis was not specific to CTLL-20 cells nor to IL-2 dependence, as treatment with jasplakinolide also enhanced apoptosis of the pre-B, IL-3-dependent cell line Ba/F3 upon growth factor deprivation. Analysis of DNA content by flow cytometry, DNA fragmentation, and TUNEL confirmed that the nuclear morphology observed by fluorescence microscopy in jasplakinolide- and Me2SO-treated, IL-2-deprived CTLL-20 cells was secondary to the induction of apoptosis, not necrosis (Fig. 2B and data not shown). The ability of jasplakinolide to enhance apoptosis correlated with its ability to bind to and stabilize F-actin. In addition, stabilization of actin appeared to sensitize the cells to cytokine deprivation; treatment with jasplakinolide increased the percentage of cells that underwent apoptosis at low concentrations of IL-2 (data not shown). Finally, the stabilization of actin appeared to affect the transduction of the apoptotic signal, as the enhancement occurred upstream of commitment to apoptosis and induction of caspase-3-like activity and could be attenuated by the overexpression of the anti-apoptotic protein Bcl-xL.

The process of apoptosis has been divided into two phases, the initiation/commitment phase and the downstream effector phase (32, 33). Actin has long been postulated to play a role in the effector phase of morphologic changes associated with apoptosis (34, 35). Recent reports indicate that actin itself is cleaved during apoptosis (35–39); cleavage of actin has been suggested to be both an effector of the morphological changes associated with apoptosis as well as a mechanism of DNase I activation (35, 36, 40, 41). Changes in the levels of total cellular actin and of F-actin have also been associated with the apoptotic process (38, 42). We offer evidence to suggest that actin also plays a role in the initial phase of commitment to apoptosis, independent of a requirement for adhesion.

The mechanism by which modulation of actin altered the apoptotic signal and/or commitment is unclear. One possibility is that actin stabilization by jasplakinolide has been demonstrated to affect ion transport across the cell membrane in a variety of cell systems (28, 29). As apoptosis of CTLL-20 cells in response to IL-2 deprivation is associated with intracellular acidification (43), it could be postulated that an alteration in ion flux could alter the rate of cell death. However, acidification has been shown not to be required for progression to apoptosis (43), and treatment with calcium ionophore did not prevent the effect of jasplakinolide (data not shown); we thus consider this possibility unlikely.

An alternative explanation for the modification of the apoptotic process by jasplakinolide stems from the observation that the effective concentrations of jasplakinolide are far lower than the molar content of actin in the cell and that the $K_d$ of jasplakinolide binding to F-actin ($-15$ nM) (see Ref. 10) is consistent with the IC$_{50}$ of $34$ nM for inhibition of proliferation (11) and for the enhancement of apoptosis ($-35$ nM) observed here. The discrepancy in effective drug versus purported target concentrations would suggest either that binding between jasplakinolide and F-actin is positively cooperative (10) or that the effect of jasplakinolide results from drug competition with an actin-binding protein present at much lower concentrations in the cell. In this regard, gelsolin has also been shown to displace phalloidin from actin filaments (44); whether gelsolin and jasplakinolide compete for the same binding site(s) on actin has not yet been investigated. Gelsolin is a well conserved actin-regulatory protein that can bind actin monomers and sever actin filaments (45), and gelsolin has recently been shown to be a substrate of caspase-3 (46). In addition, overexpression of gelsolin has been shown to inhibit apoptosis upstream of the caspase cascade (47). We are currently investigating a role for gelsolin in this model system of apoptosis.

The actin cytoskeletal architecture, by virtue of its ability to organize the cytoplasm by compartmentalizing and localizing proteins, can act as a scaffolding element for signaling intermediates. The enzymatic activities of a few actin-associated proteins, such as casein kinase II (48) and GRK5 (49), have been reported to be modified by binding directly to actin. Changes in actin polymerization that altered cytoskeletal architecture could therefore affect either enzymatic activity or substrate availability (see also Ref. 49). A number of plausible candidate downstream effectors could thus be influenced by stabilization of the actin cytoskeleton. The Rho family of GTPases has been shown to be involved not only in the regulation of gene transcription, cell cycle progression, and programmed cell death but also in the regulation of actin rearrangement (50–52). In addition, signaling intermediates including phosphatidylinositol 3-kinase (phosphatidylinositol 3-kinase), protein kinase C-ζ, the Wiskott-Aldrich syndrome protein, coflin, and the proto-oncogenes VAV and c-ABL have all been shown to influence cell survival, cell cycle control, and/or the cell death program and have been directly or indirectly implicated in the regulation of the actin cytoskeleton. Pretreatment of CTLL-20 cells with wortmannin, an inhibitor of phosphatidylinositol 3-kinase, did not affect apoptosis in response to IL-2 withdrawal or the effect of jasplakinolide on this process. Whether modification of actin by jasplakinolide impacts upon signaling pathways other than that of phosphatidylinositol 3-kinase is the subject of further investigation.

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