Latrunculin B or ATP Depletion Induces Cofilin-dependent Translocation of Actin into Nuclei of Mast Cells*

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EXPERIMENTAL PROCEDURES

The existence of nuclear actin has long been questioned, but recent years have brought several lines of strong evidence indicating the regulated nuclear import and export of actin (2). Actin has been shown to contain two functional leucine-rich nuclear export sequences, and their disruption leads to nuclear actin accumulation and consequently to a decrease in cell proliferation (3). However, actin itself does not possess any nuclear localization sequence. The actin-binding protein, cofilin, contains a classical bipartite SV40-type nuclear localization sequence (4) and translocates into nuclei together with actin after a heat shock (4, 5) or dimethyl sulfoxide (Me2SO) treatment (6).

Very little is known about the physiological function of nuclear actin. Monomeric actin is a well known inhibitor of DNase I (7), and the actin-DNase I complex is stabilized by cofilin (8), but the relevance of these interactions is still unknown. Actin may constitute a part of the nuclear matrix, the non-chromatin fraction of the nucleus, enabling compartmentalization of functions or reorganization of chromatin (2). Recently, a novel protein, EAST, has been characterized as a component of the nuclear matrix, localized exclusively to the extrachromosomal nuclear domain (9). An increase in the levels of EAST (after a heat shock or due to its overexpression) led to an expansion of this domain and to accumulation of actin within it. Relevant to our study is the finding that cellular levels of G-actin control activation of a transcription factor, serum response factor. An increase in G-actin level, induced by an actin-destabilizing drug, latrunculin B, or by manipulation of some actin regulatory proteins, suppresses specific serum response factor target genes such as srf and vinculin (10). In another study, latrunculin B was found to delay nuclear division in yeast; latrunculin B-induced disruption of actin filaments led to an incorrect spindle position, which in turn seems to promote stress-activated mitogen-activated protein kinase pathway (11).

Here we report that latrunculin B-induced disassembly of F-actin in mast cells promotes an entry of actin and cofilin into the nuclei. The competence to translocate actin is retained after cell permeabilization despite leakage of cytosolic proteins. Using the permeabilized cell system, we have shown that accumulation of nuclear actin is blocked by addition of an anti-cofilin antibody. Depletion of ATP from either intact or permeabilized cells also promoted an increase of nuclear actin and cofilin. In this case, the nuclear translocation was associated with an increase in F-actin content.

Increasing cellular G-actin, using latrunculin B, in either intact or permeabilized rat peritoneal mast cells, caused translocation of both actin and an actin regulatory protein, cofilin, into the nuclei. The effect was not associated with an increase in the proportion of apoptotic cells. The major part of the nuclear actin was not stained by rhodamine-phalloidin but could be visualized with an actin antibody, indicating its monomeric or a conformationally altered state, e.g. cofilin-decorated filaments. Introduction of anti-cofilin into permeabilized cells inhibited nuclear actin accumulation, implying that an active, cofilin-dependent, import exists in this system. Nuclear actin was localized outside the ethidium bromide-stained region, in the extrachromosomal nuclear domain. In permeabilized cells, the appearance of nuclear actin and cofilin was not significantly affected by increasing [Ca2+] and/or adding guanosine 5′-O-(3-thiotriphosphate), but was greatly promoted when ATP was withdrawn. Similarly, ATP depletion in intact cells also induced nuclear actin accumulation. In contrast to the effects of latrunculin B, ATP depletion was associated with an increase in cortical F-actin. Our results suggest that the presence of actin in the nucleus may be required for certain stress-induced responses and that cofilin is essential for the nuclear import of actin.

We have previously used latrunculin B (LB) to explore the relationship between actin cytoskeleton and secretion in rat peritoneal mast cells (RPMC) (1). During these studies, we have consistently noticed accumulation of actin in the nuclei of LB-treated cells. Nuclear actin was visible even in cells that, as a consequence of the LB treatment, have lost all other structures recognizable by phalloidin. Here we address the mechanisms involved in and conditions necessary for this translocation.

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The abbreviations used are: LB, latrunculin B; ABA, anti-β-actin; CB, chloride buffer; EB, ethidium bromide; FA, fluorescein isothiocyanate-annexin; GTP-S, guanosine 5′-O-(3-thiotriphosphate); GB, glutamate buffer; PI, propidium iodide; RPMC, rat peritoneal mast cells; RP, rhodamine-phalloidin; SL-O, streptolysin-O; PIPES, piperazine-N,N′-bis(2-ethanesulfonic acid).
Cells—RPMC were prepared as described previously (12). The cells were resuspended in a solution containing 137 mM NaCl, 2.7 mM KCl, 1.0 mM CaCl₂, 2 mM MgCl₂, 5.6 mM glucose, 1 mg/ml bovine serum albumin, and 20 mM NaPipes, pH 7.2 (chloride buffer, CB). For confocal microscopy, cells in CB were allowed to attach to glass slides for 1 h at room temperature (~25,000 cells/well). Suspended cells were used for flow cytometry.

Permeabilization and Cell Treatments—Glass-attached cells were washed with 137 mM potassium glutamate, 2 mM MgCl₂, 1 mg/ml bovine serum albumin, 20 mM NaPipes, pH 6.8 (glutamate buffer, GB) and then exposed for 8 min at room temperature to SL-O at 0.4 IU/ml in GB, 3 mM EGTA. After permeabilization, cells were washed with GB free of soluble components and excess SL-O. Where indicated, cells were exposed to 200 µg LB/ml CB for 1 h before permeabilization and/or to 20 µM LB/ml GB, 100 µM EGTA for 10 min after permeabilization, both at room temperature. After the pretreatments, permeabilized cells were further incubated for 20 min at 30 °C with 3 mM EGTA, 3 mM ATP, GB (in some experiments, ATP was omitted). LB, if present, was diluted by half to 10 µg/ml. Where indicated, free Ca²⁺ concentration was buffered by 3 mM Ca²⁺ EGTA buffer system, pH 6.8, calculated using appropriate dissociation constants as given by Martell and Smith (13). Pretreatment of permeabilized cells with antibodies, anti-cofilin or anti-vinculin (both at 1:10 dilution), was performed before the addition of latrunculin B. To deplete ATP in intact cells, metabolic inhibitors, 6 mM deoxyglucose and 10 µM antimycin in CB, were applied at 30 °C for the indicated times.

Staining—Cells were fixed for 20 min at room temperature with 3% paraformaldehyde, 4% polyethylene glycol, 3 mM EGTA, GB; washed with 50 mM glycine, 100 µM EGTA, GB; and then exposed to a mild detergent, lysophosphatidylcholine (80 µg/ml), 100 µM EGTA, GB. Finally, cells were stained with either 0.3 µM rhodamine phalloidin (RP) (20 min) or with anti-β-actin (ABA, 1 h) or with anti-cofilin (1 h) all in 1 mM EGTA, GB (GBE). Blocking with 5% goat serum in GBE (20 min) and washing with GBE were performed before each step of the immunostaining. ABA was an affinity-purified monoclonal antibody (clone AC-15) from Sigma (used at 1/200 dilution), and it recognizes an N-terminal epitope. Using enzyme-linked immunosorbent assay, we have found that AC-15 antibody to be truly β-actin-specific. AC-15 recognizes β-F-actin equally well as β-G-actin, and its binding to actin is unaffected either by cross-linking or by the interaction of actin with cofilin. Anti-human cofilin antibody (“9771”) was raised in rabbits against complexes of recombinant human cofilin (14) and rabbit skeletal muscle. The complexes were prepared from F-actin mixed (1:1) with cofilin at pH 8.0 and centrifuged to remove residual F-actin. The cofilin antibodies were then affinity purified on Sepharose-cofilin and used at 1/50 dilution. The affinity of cofilin antibody for cofilin was unaffected by the interaction of cofilin with actin. The antibody binds equally well to cofilin and cofilin-actin complexes. Results of our antibody tests are available as Supplemental Material. The secondary antibodies (both at 1/10 dilution in GBE) were goat-anti-mouse IgG-biotin and goat-anti-rabbit IgG-biotin, respectively, both from Sigma. Cy2-streptavidin (1/50, from Amersham Biosciences) was the tertiary layer. In double-label experiments, RP or ethidium bromide (EB, 0.5 µM) was added with the secondary antibody. This is particularly important for ABA + RP staining, since phalloidin was found to interfere with the ABA staining, while ABA did not seem to affect the staining with phalloidin.

Confocal Microscopy—Stained cells were observed using an IX-70 inverted microscope (Olympus), fitted with an Ultraview confocal imaging system (PerkinElmer Life Sciences, Cambridge, UK). This comprises a dual wavelength argon/krypton laser (Omnicrome) and a CSU-10 confocal scanning unit (Yokogawa, Japan). This system utilizes Nikon objectives to allow real-time confocal imaging. Digital images (mostly equatorial slices) were collected with no pixel binning, using a cooled 12-bit digital interline UltraPix FKI 1000 camera (G2) with 1024 x 1024 pixels (PerkinElmer Life Sciences). Read-out speed was 0.5 MHz and sensitivity 0.72 electrons/gray level. Image capture was controlled by the software package “Ultraview” (“Spatial Module” configuration). For the detection of Cy2 fluorescence, excitation was at 488 nm, and emission was collected with a multiband-pass filter (transmitting between 500 and 540 nm). For the detection of RP or EB fluorescence, excitation was at 488 nm, and emission was collected between 500 and 540 nm. Olympus U Plan Apochromat 100× objective was used; images of equatorial slices that section nuclei are shown.

Western Blotting/Immunoblotting—Samples of cell fractions were dissolved in Laemmli sample buffer (15) and analyzed by electrophoresis in 10% polyacrylamide vertical slab gels. Proteins were transferred onto nitrocellulose. The membranes were probed with an ABA (1/2000) followed by goat anti-mouse antibody (1/2000) as described (16) and finally developed using ECL reagents (Amersham Biosciences). All figures shown are representative of at least three separate experiments.

**RESULTS**

**Latrunculin B Causes Translocation of Actin into Nuclei of Intact Cells**—Resting RPMC, stained for filamentous actin with RP, exhibited only the prominent rings of cortical actin with no internal structures visible (Fig. 1, top left panel). Staining with a monoclonal ABA gave somewhat different results; in addition to the cortex, punctate internal foci were visible together with low levels of perinuclear, sometimes nuclear, staining (Fig. 1, top right panel, and Fig. 7, top left panel). No staining was observed in the absence of the primary antibody.
Nuclear Actin in Mast Cells

TABLE I

|                         | Healthy cells | Early apoptotic | Mid-late apoptotic | Broken cells |
|-------------------------|---------------|-----------------|--------------------|--------------|
| Fluorescence intensity  |               |                 |                    |              |
| %                       | %             | %               | %                  | %            |
| Low FA-low PI           | 80.7          | 5.5             | 10.2               | 3.8          |
| Hi FA-low PI            | 90.2          | 3.8             | 5.1                | 0.9          |
| Hi FA-hi PI             |               |                 |                    |              |
| Low FA-hi PI            |               |                 |                    |              |

Proportion of apoptotic cells after LB treatment

Suspended intact cells were incubated for 2 h at 37 °C with control buffer (0.25% Me₂SO, CB), LB (20 μg/ml), or, as a positive control, staurosporine (Stauro) (2 μM) + cycloheximide (CHI) (10 μg/ml). Cells were then washed and incubated with FA (1:100), PI (2 μg/ml), 5 mM CaCl₂, CB for 5 min at room temperature in the dark. Cell fluorescence was quantitated using flow cytometry. Histogram partitioned into four domains according to FA and PI fluorescence intensity. Duplicate samples of 10,000–15,000 cells were analyzed per condition. Results are representative of three independent experiments. No increase in percent of apoptotic cells is apparent after 2 h of treatment with LB, and the same was true for longer treatments (4 and 6 h, not shown). The top row shows distribution of cells during different stages of apoptosis: early stages exhibit high annexin V but low propidium iodide staining, while in later stages staining by both markers is intense.

(not shown). RPMC contain about 2.6 ng total actin per 10⁶ cells (as determined by densitometry of Coomassie Blue-stained gels (17); densitometry of Western blots after ABA staining produced a value of 2.3 ng/10⁶ cells (not shown). The close agreement indicates that most of the actin in mast cells is β-actin. The cortical staining disappeared within 10 min of LB treatment (Fig. 1); ABA-stained cells showed stronger diffuse staining throughout the cell due to the increase in the monomer concentration, and in some cells, intense nuclear staining was already apparent. The proportion of cells with nuclear actin increased with the time of treatment and this was also true for RP-stained cells, although with a discernable delay. After 60 min with LB, most cells exhibited nuclear actin, stained by both RP and ABA.

After 1-h treatment of intact cells with 20 μg of LB/ml, RP staining decreases by 60–70%; secretion from these cells is inhibited by about 40%, and the effect is fully reversible (1). The plasma membrane of these cells remains intact as indicated by Bodipy-sphingomyelin staining (18). Consequently it is unlikely that nuclear actin is associated with apoptotic cells. To confirm that actin changes were not linked to apoptosis, LB-treated cells were stained with FA and PI and examined using flow cytometry (Table I). Annexin V marks the early stage of apoptosis (hi FA-low PI), it binds with high affinity to phosphatidyl serine, which appears on the outer leaflet of the plasma membrane during the early stages of programmed cell death. During the mid-late stages of apoptosis, membrane integrity is lost allowing propidium iodide to associate with DNA (hi FA-hi PI). Treating cells with staurosporine and cycloheximide, agents known to induce apoptosis (19), provided a positive control. After 2 h of LB treatment, there was no increase in the proportion of early or mid-apoptotic cells.

Latrunculin B Causes Translocation of Actin into Nuclei of Permeabilized Cells—SL-O, a bacterial exotoxin, binds to membrane cholesterol and then oligomerizes to form pores with a diameter up to 30 nm, allowing passage of proteins of molecular weight up to 400 kDa (Ref. 20 and references within). Permeabilized mast cells retain integrity of their cellular architecture and respond to stimulation by calcium and/or GTPγS (12) as well as to the activation of cell surface receptors (21). After permeabilization, cytosolic components gradually leak out of the cells. Using ABA and Western blotting, we have found that about 60% of cellular actin leaks out within 15 min. This is in good agreement with our previous determination of actin leakage by densitometry of Coomassie Blue-stained gels (17). The leakage was increased to ~95% in the presence of LB, and no such increase was seen in the presence of cytochalasin (not shown).

Mast cells, permeabilized under control conditions (8 min with streptolysin-O, washed with GB followed by a 20-min exposure to 3 mM EGTA, 3 mM ATP, GB) showed either none or very weak nuclear staining with RP or ABA, respectively (Fig. 2A, Control). Cortical staining was clearly visible, but occasionally (see Fig. 2A, top left panel), a few spontaneously degranulating cells could be seen with filamentous actin around secretory granules. When intact cells were pretreated for 1 h with LB and then permeabilized in its absence, many cells retained nuclear actin, stained by both RP and ABA (Fig. 2A, LB → SL-O). It is noteworthy that actin translocation into nuclei occurred also when LB was applied after the permeabilization (Fig. 2A, SL-O → LB); in this case most of the nuclear actin was not stained by phalloidin. LB treatment both before and after permeabilization resulted in the appearance of nuclear actin in all cells (Fig. 2A, LB → SL-O → LB), but only a proportion was recognized by phalloidin (Fig. 2B). Distinct dot-like nuclear actin structures were apparent, particularly when the LB protocol was used (Fig. 2B), and they could be seen on all z-axis confocal slices throughout the nuclei (taken 0.2 μm apart along the z axis; not shown). Differential interference contrast microscopy showed that these dot structures lie close to and within the nuclear envelope, concentrically with the structure seen in the middle of the nucleus, presumably a nucleolus (Fig. 3A). The nuclear structures could be visualized with a DNA/RNA stain, EB, and actin was excluded from these EB-stained domains (Fig. 3B).

Localization of Cofilin in Intact and Permeabilized Mast Cells; Effect of Latrunculin B—Cofilin, a small actin-binding protein, has previously been reported to enter nuclei together with actin after a heat shock (4, 5). To establish whether F-actin disassembly induces nuclear entry of cofilin concomitantly with that of actin, the presence of this protein in control and LB-treated cells was studied using an affinity-purified anti-cofilin. Intact cells exhibited diffuse staining throughout the cell with some cells showing low levels of nuclear staining (Fig. 4A, bottom left panel). No staining was observed in the absence of the primary antibody except for the few cells, most probably those that have degranulated spontaneously (not shown); such cells with artificial high staining are also visible in Fig. 4. There was a significant increase in anti-cofilin staining of the nuclei of intact cells after LB treatment (Fig. 4A, bottom right panel). In parallel, actin presence in the nuclei of LB-treated cells was revealed by ABA (Fig. 4A, top panels).

Cofilin entry into nuclei could also be induced in permeabilized cells. Cells, that were treated with LB after their permeabilization (protocol SL-O → LB), exhibited stronger nuclear staining with both anti-cofilin (Fig. 4B, bottom panels) and with ABA (Fig. 4B, top panels) relative to control cells.

Effect of Anti-cofilin on the Nuclear Accumulation of Actin—The permeabilized cell system, with its capacity to support nuclear entry of both actin and cofilin, provides a good opportunity to examine whether cofilin is required for latrunculin B-induced nuclear accumulation of actin. The 9771 cofilin antibody is entirely specific for cofilin and is able to bind cofilin both free and when complexed to actin (see Supplemental Ma-
The antibody was introduced into permeabilized cells prior to latrunculin B treatment and anti-vinculin was used as a control. As expected, control cells exhibited actin cortex and very low levels of nuclear/perinuclear staining (Fig. 5A). In the absence of any antibody (Fig. 5B) or in the presence of the control antibody, anti-vinculin (Fig. 5C), LB caused cortical actin disassembly and an increase in nuclear actin. In the presence of anti-cofilin, LB induced cortical actin disassembly but failed to induce nuclear accumulation actin (Fig. 5D).

Absence of ATP Promotes Accumulation of Actin in the Nuclei of Permeabilized and Intact Cells—A small proportion of permeabilized cells exhibit nuclear or perinuclear staining with ABA even under control conditions (Fig. 6A, top right panel and see also Figs. 2A, 4B, and 5A). This staining increased considerably when cells were maintained in the absence of ATP (Fig. 6A, bottom right panel). Phalloidin did not recognize this nu-
clear actin, but RP staining of the cortex was somewhat increased in the absence of ATP (Fig. 6A, bottom left panel). Flow cytometric assay of RP fluorescence showed the relative F-actin level of cells maintained in the absence of ATP to increase by 5–10% relative to those with ATP (not shown). This is also reflected by a stronger cortical staining by ABA of cells without ATP (compare the two right panels in Fig. 6, A and B).

Immunofluorescence with anti-cofilin of cells exposed to EGTA in the presence and absence of ATP revealed that ATP in permeabilized cells was also crucial for preventing accumulation of nuclear cofilin (Fig. 6B, left panels). Calcium (pCa 5) and GTPγS, two agents capable of promoting secretion from permeabilized mast cells, did not seem to have any significant effect on cofilin localization (not shown). A small decrease in nuclear staining was occasionally apparent in GTPγS-treated cells, but the effect was marginal. Likewise, nuclear staining of actin with ABA was somewhat reduced in the presence of GTPγS (not shown). The main determinant of cofilin nuclear localization was absence of ATP, and the same was true for actin. Excepting the nuclei, the staining with anti-cofilin was dispersed throughout the cell, and no increase in the cortical staining was apparent in the absence of ATP, unlike that with ABA (Fig. 6B).

Treatment of intact mast cells with inhibitors of glycolysis (6 mM deoxyglucose) and oxidative phosphorylation (10 μM antimycin) causes depletion of ATP. Concentrations of ATP are reduced to <5% and <1% of the original level (~2.3 mM) within 10 and 20 min at 30 °C, respectively (22). Fig. 7 shows that these inhibitors induced translocation of actin into nuclei. This was already apparent after 20-min treatment and became very prominent with increasing time. Nuclear actin was not recognized by phalloidin (not shown). Again, cortical actin staining increased progressively with the time of metabolic inhibition.

Internal Foci of Monomeric Actin—Permeabilization did not eliminate the presence of the punctate internal foci stained by ABA. In fact, they were visible more clearly in permeabilized than in intact cells, and this must be due to the leakage of soluble actin and therefore to a higher signal/noise ratio. At higher magnification, these foci could be seen throughout the cell, as if delineating cellular organelles/structures (Fig. 6C, right panel; see also Fig. 2A, control cells, bottom left panel). Examination of a stack of confocal slices (taken 0.2 μm apart along the z axis) has shown the foci of actin to be present at all planes (not shown). After latrunculin B treatment, these foci are less prominent, especially when LB was applied both before and after permeabilization (Fig. 2A, bottom right panel).

DISCUSSION

This paper demonstrates cofilin-dependent translocation of actin into nuclei of mast cells exposed to two different stress-related conditions: 1) latrunculin B-induced disassembly of cortical F-actin and 2) ATP depletion, associated with an increase in F-actin.

Latrunculins destabilize actin filaments by binding to G-actin at the site adjacent to the nucleotide-binding cleft (23–25). This prevents the re-incorporation of actin into filaments and depletes cellular F-actin over a period of time; the rate of this process depends on the rate of filament turnover (26, 27). In contrast, cytochalasins, the most frequently used actin-de-
stabilizing drugs, bind to the barbed ends of filaments and prevent elongation and shortening from these ends. Cytochalasins inhibit polymerization of actin in response to cell activation but usually do not deplete cellular F-actin. Indeed, we have not seen any reduction in RP staining of resting mast cells, intact or permeabilized, in response to cytochalasin (not shown). It is remarkable that LB could induce accumulation of nuclear actin in permeabilized cells, indicating that the components required for the translocation are retained although a large proportion of cytosolic proteins have leaked out.

The response to LB was very fast; nuclear actin appeared within 10 min, suggesting an active process rather than passive diffusion, particularly with regard to the large size of actin (43 kDa). Initially, this actin was not accessible to phalloidin; RP staining of nuclei was delayed relative to that by ABA (Fig. 1). This indicates that actin enters as a monomer or that it forms oligomers complexed with cofilin. Cofilin binding to the filaments changes their conformation (twist), which prevents RP binding (28). Indeed, we have observed cofilin translocation into nuclei in both intact and permeabilized cells after LB treatment (Fig. 4). At the later stages of LB treatment, RP staining of nuclei became progressively more apparent although the major part of nuclear actin was still not accessible to RP (Fig. 1). Since not all actin translocated to nuclei will be complexed with LB, it means that after the dissociation of cofilin from actin monomers the LB-free actin could form RP-accessible filaments. This dissociation from cofilin may be promoted by the presence of LIM kinase (LIMK) in the nucleus, which would phosphorylate and therefore inactivate cofilin (29, 30).

Cofilin antibody inhibited accumulation of nuclear actin in LB-treated permeabilized cells (Fig. 5). The antibody is entirely specific for cofilin and able to bind cofilin both free and complexed to actin (Supplemental Material). The nuclear localization sequence is on the opposite side of ADF/cofilin than its actin-binding face (28, 31). We speculate that the antibody binds nearer the nuclear localization sequence and prevents access to it and therefore nuclear translocation. Our results support the existence of an active, cofilin-dependent nuclear import of actin in this system. Cofilin binds away from the LB-binding site, similar to but distinct from the gelsolin-binding site (31). This is consistent with cofilin-dependent increase in nuclear translocation of actin after LB treatment; more actin monomer is available to the cofilin to bind to and subsequently to translocate into the nuclei. Accumulation of actin in the nuclei of latrunculin B-treated maize root cells was reported recently (32). The authors suggest that this may be due to the changed conformation of G-actin after latrunculin binding that may interfere with the nuclear export of G-actin. This is an interesting possibility, but it would not explain the appearance of nuclear actin under other conditions such as ATP depletion, heat shock, or Me6SO treatment.

Nuclear actin was localized outside the region of high DNA/RNA density (Fig. 3), similar to the reported presence of actin (after a heat shock or after overexpression of EAST protein) in the extrachromosomal nuclear domain (9). Nuclear actin in mast cells was often present in the form of distinct dots, apparent on all confocal slices throughout the nuclei. This suggests a formation of either vertical actin rods or very concentrated actin grains. The protocol, including LB treatment both before and after the permeabilization (LB → SL-O → LB), resulted in the sharpest images of such dots. This could be a consequence of an aggregation of nuclear actin under these conditions together with leakage of any non-aggregated actin (thus increasing the signal to noise ratio). The existence of nuclear actin in dot-like structures was previously revealed in differentiated myogenic cells by immunofluorescence with an actin antibody, 2G2. This antibody (but not phalloidin) seems to recognize a specific actin conformation, present in the nuclei (but not in the cytoplasm) of these cells (33). Again, the staining did not co-localize with the DNA-specific Hoechst stain.

Latrunculin B did not increase the proportion of apoptotic (FA-positive) cells (Table I). Nuclear accumulation of actin seems to be controlled differently from the induction of apoptosis. For example in hepatocytes, inhibitors of protein synthesis were found to induce both apoptosis and an increase in nuclear G-actin staining, but only apoptosis could be prevented by a pretreatment with caspase inhibitors (34). It seems that accumulation of nuclear actin forms a part of a general response to stress that is not causally related to apoptosis, but it may precede it. We have, however, not detected any increase in FA-positive cells even after 6 h of LB treatment (not shown). Stress-induced sequesteration of actin into the nucleus may be important for preventing formation of inappropriate cytosolic structures. An increase in monomeric actin level activates autoregulatory feedback mechanisms at both the transcriptional and the post-transcriptional level, which lead to a decreased synthesis of actin and some actin-binding proteins (35–37). Specific serum response factor target genes are suppressed (10) and nuclear division delayed (11). Our results imply that some of these responses may involve regulatory mechanisms dependent on the presence of actin in the nucleus.

Nuclear entry of actin could also be induced by ATP depletion of both intact or permeabilized mast cells, but in this case, levels of F-actin in the cortex actually increased (Figs. 6 and 7). Nuclear actin was not recognizable by phalloidin. As confirmed by immunostaining with cofilin antibody, ATP depletion has also caused cofilin to enter the nuclei of mast cells, and this was independent of calcium (not shown). The effect of GTP7S was marginal and needs to be further investigated. The co-localization of cofilin and actin in the nuclei again suggests the existence of actin-cofilin complexes that cannot bind phalloidin. ATP depletion will promote dephosphorylation, and therefore active state, of cofilin (38). It has been shown that cofilin is capable of nuclear translocation in this dephosphorylated state (39, 40). Moreover, ATP depletion will also increase levels of ADP-G-actin, which binds to cofilin more strongly than ATP-G-actin (41, 42), promoting its nuclear accumulation. In cultured neurons, ATP depletion caused formation of cytoplasmic actin-ADF/cofilin rods, and this was associated with an increase in the level of dephosphorylated ADF/cofilin (38). No rods, however, were visible in the nuclei of these ATP-depleted neurons.
Increase in F-actin level has been previously reported in ischemic proximal tubule kidney cells; this was coincident with a collapse of microvilli and appearance of F-actin in the perinuclear region (43, 44). This rather paradoxical observation (in vitro, polymerization of actin is promoted by ATP) may be due to the presence of a range of cytosolic actin-regulatory proteins (45) as well as to the rigor state of the actomyosin in the cortex. We have reported previously that myosin II-based contraction of the membrane cytoskeleton is a prerequisite for its disassembly; the latter is induced by addition of ATP together with calcium to permeabilized mast cells (46). Addition of ATP alone causes a small but reproducible decrease in cortical F-actin level (5–10% decrease in RP fluorescence), while withdrawal of ATP causes an F-actin increase. Although RP and ABA cortical staining of cells without ATP was stronger than that of cells with ATP, no such increase was visible for cortical staining with anti-cofilin. In most cells, cofilin concentration is much smaller than that of actin (47). In ATP-depleted mast cells, actin moved into the nuclei accompanied by cofilin, but actin was not labeled with anti-cofilin. In most cells, cofilin concentration is much smaller than that of actin (47). In ATP-depleted mast cells, actin moved into the nuclei accompanied by cofilin, but actin was not labeled with anti-cofilin.

Finally, where does the nuclear actin come from? This question is pertinent considering that nuclear translocation of actin occurs in permeabilized cells that have lost ~60% of their total actin by leakage. We have previously postulated the existence of an actin monomer pool that is bound to intracellular structures (Fig. 6C). Similar ABA-stained foci were seen in cultured fibroblasts. Observation of fibroblasts after the microinjection of fluorescently labeled actin revealed that these foci form near the leading edge and move centripetally toward the nucleus (50). Such actin foci could be the source/storage sites for both nuclear and newly polymerized actin.

In conclusion, it can be expected that cofilin-dependent entry of actin into nuclei forms a part of cellular response to stress in general. Permeabilized mast cells, which retain their capacity for both nuclear translocation and de novo polymerization of actin, should provide an excellent system for further investigation.

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REFERENCES
1. Pendleton, A., and Koffer, A. (2001) Cell Motil. Cytoskeleton 48, 37–51
2. Rando, O. J., Zhao, K., and Crabtree, G. R. (2000) Trends Cell Biol. 10, 92–97
3. Wada, A., Fukuda, M., Mishima, M., and Nishida, E. (1998) EMBO J. 17, 1635–1644
4. Iida, K., Matsumoto, S., and Yahara, I. (1992) Cell Struct. Funct. 17, 39–46
5. Nishida, E., Iida, K., Yonezawa, N., Koyasu, S., Yahara, I., and Sakai, H. (1987) Proc. Nat. Acad. Sci. U.S.A. 84, 5262–5266
6. Abe, H., Nagaoka, R., and Ohnita, T. (1983) Exp. Cell Res. 206, 1–10
7. Blikstad, I., Markey, F., Carlson, L., Persson, T., and Lindberg, U. (1978) Cell 15, 935–943
8. Nosworthy, N. J., Kekic, M., and dos-Remedios, C. G. (2001) Proteomics 1, 1513–1518
9. Wasser, M., and Chia, W. (2000) Nat. Cell Biol. 2, 268–275
10. Sotiropoulos, A., Ginestis, D., Copeland, J., and Treisman, R. (1999) Cell 98, 159–169
11. Gachet, Y., Tournois, S., Millar, J. B., and Hyams, J. S. (2001) Nature 412, 352–355
12. Howell, T. W., and Gomperts, B. D. (1987) Biochim. Biophys. Acta 927, 177–183
13. Martell, A. E., and Smith, R. M. (1974) Cell 10, 1–15
14. Wada, A., Fukuda, M., Mishima, M., and Nishida, E. (1998) EMBO J. 17, 1635–1644
15. Watanabe, Y. (1991) J. Cell Biol. 113, 191–206
16. Iida, K., Matsumoto, S., and Yahara, I. (1992) Cell Struct. Funct. 17, 376–378
17. Yamanuka, E. G., Somasundaram, T., Boring, T. A., Spector, I., and Bubb, M. R. (2000) J. Biol. Chem. 275, 28120–28127
18. Wada, A., Fukuda, M., Mishima, M., and Nishida, E. (1998) EMBO J. 17, 1635–1644
19. Nosworthy, N. J., Kekic, M., and dos-Remedios, C. G. (2001) Proteomics 1, 1513–1518
20. Wasser, M., and Chia, W. (2000) Nat. Cell Biol. 2, 268–275
21. Sotiropoulos, A., Ginestis, D., Copeland, J., and Treisman, R. (1999) Cell 98, 159–169
22. Gachet, Y., Tournois, S., Millar, J. B., and Hyams, J. S. (2001) Nature 412, 352–355
23. Howell, T. W., and Gomperts, B. D. (1987) Biochim. Biophys. Acta 927, 177–183
24. Martell, A. E., and Smith, R. M. (1974) Cell 10, 1–15
25. Watanabe, Y. (1991) J. Cell Biol. 113, 191–206
26. Iida, K., Matsumoto, S., and Yahara, I. (1992) Cell Struct. Funct. 17, 376–378
27. Yamanuka, E. G., Somasundaram, T., Boring, T. A., Spector, I., and Bubb, M. R. (2000) J. Biol. Chem. 275, 28120–28127
28. Ayscough, K. R., Stryker, J., Pokala, N., Sanders, M., Crews, P., and Drubin, D. G. (1997) J. Cell Biol. 137, 399–411
29. McGough, A., Pope, B., Chiu, W., and Weeds, A. (1997) J. Cell Biol. 138, 771–781
30. Stanyon, C. A., and Bernard, O. (1999) Int. J. Biochem. Cell Biol. 31, 389–394
31. Lappalainen, P., Fedorov, E. V., Fedorov, A. A., Almo, S. C., and Drubin, D. G. (1997) EMBO J. 16, 5520–5530
32. Baluska, F., Jasik, J., Edelmann, H. G., Salajova, T., and Volkmann, D. (2000) Proc. Nat. Acad. Sci. U.S.A. 97, 8305–8309
33. Gonsior, S. M., Platz, S., Buchmeier, S., Scheer, U., Jockusch, B. M., and Smiley, N. M., and Mizuno, K. (1999) Biochem. J. 338, 793–798
34. Baluska, F., Jasik, J., Edelmann, H. G., Salajova, T., and Volkmann, D. (2000) Proc. Nat. Acad. Sci. U.S.A. 97, 8305–8309
35. Gonsior, S. M., Platz, S., Buchmeier, S., Scheer, U., Jockusch, B. M., and Smiley, N. M., and Mizuno, K. (1999) Biochem. J. 338, 793–798
36. Reuner, K. H., Wiederhold, M., Dunker, P., Just, I., Bohle, R. M., Kroger, M., and Katz, N. (1995) Eur. J. Biochem. 230, 32–37
37. Nosworthy, N. J., Kekic, M., and dos-Remedios, C. G. (2001) Mol. Biol. Cell 12, 155–170
38. Nosworthy, N. J., Kekic, M., and dos-Remedios, C. G. (2001) Proteomics 1, 1513–1518
39. Wasser, M., and Chia, W. (2000) Nat. Cell Biol. 2, 268–275
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