Journal of Cellular Biochemistry 65:469–478 (1997)

Autoregulation of Actin Synthesis Responds to Monomeric Actin Levels

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Abstract Regulation of the assembly and expression of actin is of major importance in diverse cellular functions such as motility and adhesion and in defining cellular and tissue architecture. These biological processes are controlled by changing the balance between polymerized (F) and soluble (G) actin. Previous studies have indicated the existence of an autoregulatory pathway that links the state of assembly and expression of actin, resulting in the reduction of actin synthesis after actin filaments are depolymerized. We have employed the marine toxins swinholide A and latrunculin A, both disrupting the organization of the actin-cytoskeleton, to determine whether this autoregulatory response is activated by a decrease in the level of polymerized actin or by an increase in monomeric actin concentrations in the cell. We showed that in cells treated with swinholide A the level of filamentous actin is decreased, and using a reversible cross-linking reagent, we found that actin dimers are formed. Latrunculin A also disassembled actin filaments, but produced monomeric actin, followed by a reduction in actin and vinculin expression, while swinholide A treatment elevated the synthesis of these proteins. In cells treated with both latrunculin A and swinholide A, dimeric actin was formed, and actin and vinculin synthesis were higher than in control cells. These results suggest that the substrate that confers an autoregulated reduction in actin expression is monomeric actin, and when its level is decreased by dimeric actin formation, actin synthesis is increased. J. Cell. Biochem. 65:469–478.

Key words: actin autoregulation; swinholide A; dimeric actin

Actin is the major protein of all eukaryotic cells and constitutes the building block of microfilaments. Modulations in the organization of the microfilaments are necessary during important cellular processes, including cell division, motility, wound healing and tissue morphogenesis [Cooper, 1991; Bray, 1992]. The changes in the organization of the actin-cytoskeleton are controlled by the assembly or disassembly of actin filaments that is often driven by external stimuli, such as adhesion to the extracellular matrix or to neighboring cells, or by growth factors and cytokines. The rapid alterations in microfilament structure in response to such stimulations are possible owing to a large pool of unpolymerized actin that coexists in the cell with polymerized actin [for reviews, see Carlier and Pantaloni, 1994; Theriot, 1994]. The state of actin assembly is most probably monitored by sensitive regulatory mechanisms that enable compensation for changes in the pool of actin subunits during changes in the organization of the cytoskeleton. One such mechanism could be the existence of an autoregulatory pathway that directly links the level of actin assembly with that of its synthesis. Previous studies have indicated that the treatment of cells with drugs that can either increase the level of monomeric (G) actin or stabilize polymeric (F) actin has a profound effect on the synthesis of actin, and could be accounted for by a change in actin mRNA level [Serpinskaya et al., 1990, 1991; Bershadsky et al., 1995; Reuner et al., 1991, 1995a,b].

While these studies suggested that there is an inverse correlation between unpolymerized (soluble) actin and the rate of actin synthesis, it is unclear whether this autoregulatory pathway is controlled by the level of G- or F-actin. The drugs used to alter the state of actin polymerization in the above experiments could increase the concentration of G-actin only by de-

Contract grant sponsors: USA–Israel Binational Foundation, Forcheimer Center for Molecular Genetics, Pasteur–Weizmann Research Program, Israeli Ministry of Science.

Dedicated to Ursula Glück, in memorium.

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Received 9 December 1996; accepted 3 February 1997

creasing the content of filamentous (F) -actin, and vice versa.

In this study, we have used a new compound, swinholide A [Carmeli and Kashman, 1985], a macrolide derived from marine sponges, that is highly potent in severing actin filaments, and has the unique ability to stabilize actin in a dimeric form in vitro [Bubb et al., 1995]. This compound should allow to decrease the level of F-actin without significantly increasing the concentration of G-actin, by sequestering actin monomers in a dimeric form. We show here that swinholide A can be used to distinguish between the effects induced by increasing intracellular G-actin, from those induced by a reduction in F-actin and disruption of the actin-cytoskeleton. The results suggest that the autoregulatory mechanism that links the state of actin assembly with that of actin synthesis is sensitive to the intracellular concentration of monomeric actin.

MATERIALS AND METHODS

Cell Culture and Radioactive Labeling of Cellular Proteins

Balb/C-3T3 clone A31 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum (Gibco Laboratories, Grand Island, NY) at 37°C in a humidified atmosphere with 7.5% CO_2. Cells were also incubated in DMEM supplemented with 10% serum in the presence of latrunculin A (10 μM) and/or swinholide A (100 nM). Latrunculin A and swinholide A, isolated as previously described [Spector et al., 1983, 1989; Carmeli and Kashman, 1985], were obtained from Dr. I. Spector (SUNY, Stonybrook, NY). Following treatment with these drugs, the cells were pulse labeled with 200 μCi/ml ^{35}S-methionine for 30 min in methionine-free DMEM in the presence of the various drugs, and the newly synthesized proteins analyzed by two-dimensional (2-D) gel electrophoresis.

Cell Fractionation

Cells cultured on 35-mm dishes (5×10^5 cells/dish) were labeled overnight with ^{35}S-methionine (50 μCi/ml), washed with phosphate-buffered saline (PBS) at room temperature, and extracted with 0.5 ml of cytoskeleton buffer (50 mM MES pH 6.8, 1 mM EGTA, 50 mM KCl, 1 mM $MgCl_2$, 1 mM PMSF, 0.1 mM aprotinin, 1 mM pepstatin, 0.1 mM leupeptin, 0.5% Triton X-100) per dish, at room temperature. After 1 min, the Triton X-100-soluble fraction was removed. The Triton X-100 insoluble fraction was scraped off the dishes into the same buffer. Both fractions were concentrated by ethanol precipitation and resuspended in 100 μl of O'Farrell's lysis buffer A [O'Farrell, 1975], and equal volumes (20 μl) of this protein lysate were separated by 2-D gel electrophoresis.

Gel Electrophoresis of Proteins and Computerized Quantitation of 2-D Gels

Protein extracts were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli [1970] or by 2-D isoelectric focusing (IEF) followed by SDS–PAGE as described [Ben-Ze'ev, 1990]. Equal amounts of TCA precipitable radioactive proteins were analyzed using 8% polyacrylamide gels. The protein levels were determined in the autoradiograms of 2-D gels using a laser densitometer and computerized analysis of the images with the PDquest software, as described [Garrels, 1989]. All protein spots of the 2-D autoradiogram of each gel were matched, and 50–100 protein spots were used for normalizing the different gels. Quantitation of levels of synthesis for individual proteins was performed with such normalized data obtained from 2-D gels [Glück et al., 1992].

Chemical Cross-linking and Immunoblotting

Control cell cultures (5×10^5 cells), and cells treated with either latrunculin A or swinholide A for 4 hr were extracted in 100 μl of 1% Triton X-100, 100 mM NaCl, 20 mM Hepes, pH 7.4, 2 mM EDTA, 1 mM PMSF, at 4°C, and the Triton X-100 insoluble fraction was precipitated by centrifugation at 14,000 rpm; 20 μl of the soluble extracts was incubated with or without 1 mM dithiobis-succunimidyl-propionate (DSP) (Pierce Chemical Co., Rockford, IL) dissolved in dimethyl formamide. The final concentration of dimethyl formamide was less than 5%. The cell lysates were incubated on ice with DSP for 45 min, as described [Berryman et al., 1995]. The reaction was stopped by boiling the samples in Laemmli's sample buffer lacking β-mercaptoethanol (nonreducing conditions). Proteins were separated by SDS–PAGE and electrophoretically transferred from gels to nitrocellulose [Towbin et al., 1979]. Actin was visualized on the blots with a monoclonal anti actin antibody

(AC-15, Sigma Immunochemicals), followed by secondary antibody using enhanced chemiluminescence (Amersham).

RNA Isolation and Hybridization With cDNA

Total RNA was isolated by the acid-guanidinium method of Chomczynski and Sacchi [1987], and hybridization conditions with [^{32}P]-dCTP-labeled cDNA were as described by Ben-Ze'ev et al [1990]. The following cDNA probes were used: mouse β-actin, [Farmer et al., 1983], γ-actin 3′ UTR [Erba et al., 1986], and GAPDH [Dani, 1984].

Immunofluorescence

Cells cultured on glass coverslips were permeabilized with 0.5% Triton X-100 in 50 mM MES pH 6.1, 2.5 mM EGTA, 2.5 mM $MgCl_2$ for 1 min and then fixed with 3% paraformaldehyde in PBS. Actin filaments were stained with tetramethyl rhodamine isothiocyanate (TRITC)-phalloidin (Sigma, St. Louis, MO). The cells were examined by epifluorescence with a Zeiss Axiophot microscope using a ×100/1.3 Plan Neofluar lens and photographed using Kodak T-max ASA 3200 film.

RESULTS

Organization and Assembly of Actin in Swinholide A-Treated Cells

To study the organization and expression of actin in cells treated with swinholide A, 3T3 cells were incubated with this drug at 100 nM and compared to untreated cells, and to cells treated with another macrolide, latrunculin A (10 μM), that affects actin assembly [Spector et al., 1989; Bershadsky et al., 1995]. Within 1 hr, both components induced a retraction of the cell body, leaving behind long cellular processes, and stress fibers were not apparent [Fig. 1B; cf. Fig. 1A], in agreement with previous observations [Bubb et al., 1995; Bershadsky et al., 1995].

We have analyzed whether this treatment with swinholide A resulted in depolymerization of the actin-cytoskeleton and release of actin into a Triton X-100-soluble fraction. The distribution of ^{35}S-methionine-labeled actin between a Triton X-100-soluble and an -insoluble fraction showed that while in control 3T3 cells the majority of actin (77%) was Triton-insoluble [Fig. 2A, Table I], both swinholide A and latrunculin A induced a significant shift of cellular

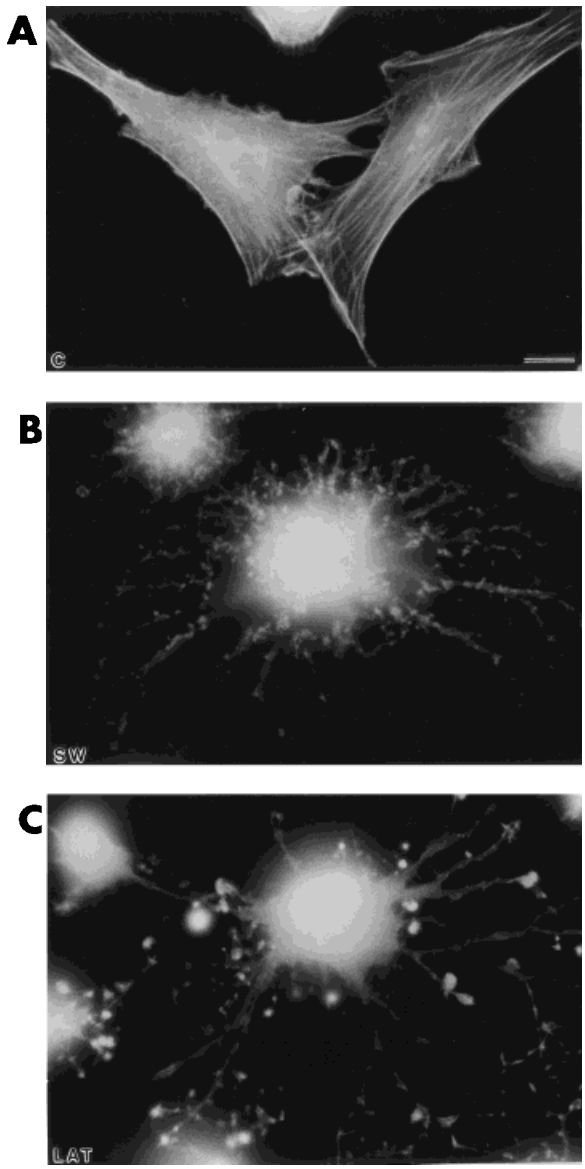

Fig. 1. The effect of swinholide A and latrunculin A on the organization of actin in 3T3 cells. Untreated cells **(A)** and cells treated for 8 hr with either 100 nM of swinholide A **(B)** or 10 μM latrunculin A **(C)** were fixed and stained with rhodamine-phalloidin to visualize polymerized actin. C, control; SW, swinholide A; LAT, latrunculin A. Bar = 10 μm.

actin into the Triton X-100-soluble fraction that contained the majority of actin in such cells [Fig. 2B,C, Table I]. This indicated that most of the total cellular actin was dissociated from the Triton-cytoskeleton in swinholide A- and latrunculin A-treated cells.

Actin Synthesis and RNA Content in Swinholide A-Treated Cells

We have previously shown that latrunculin A inhibits the expression of actin and vinculin

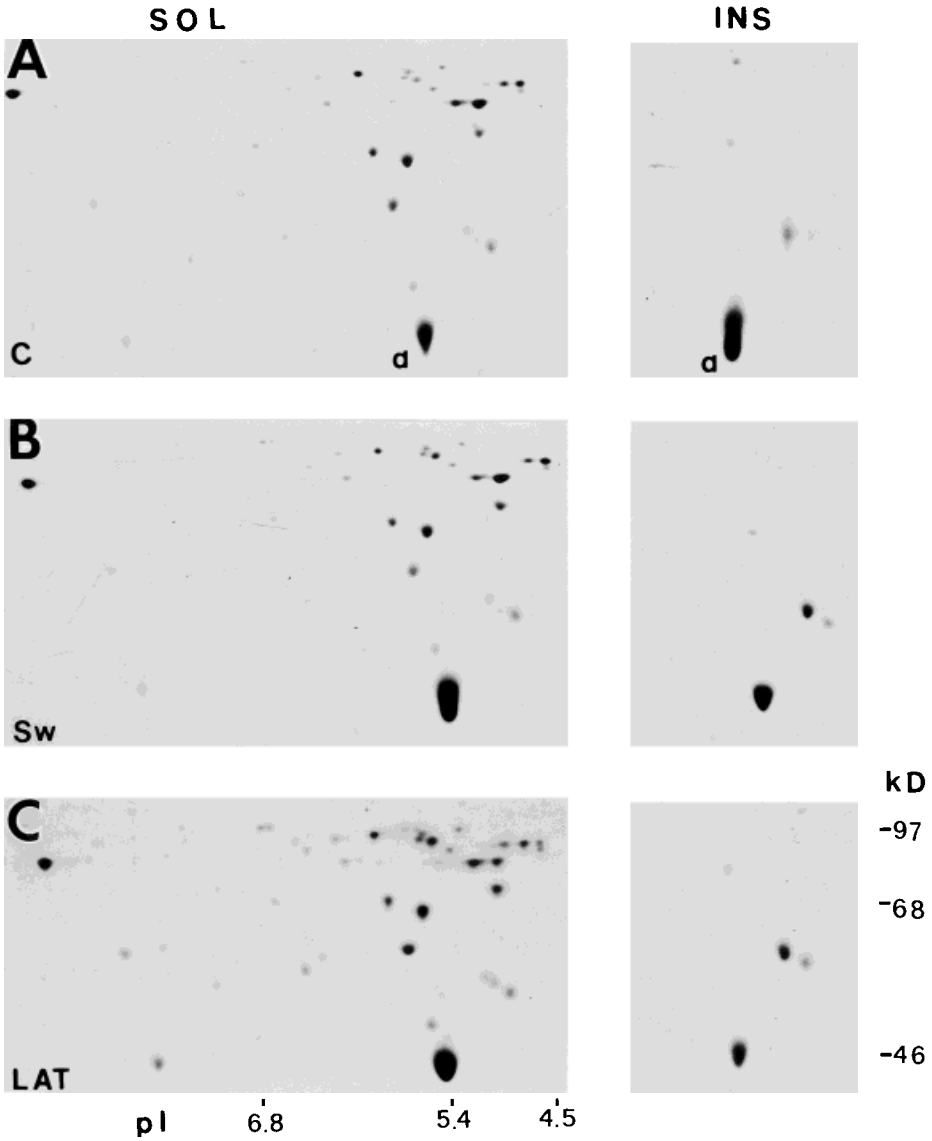

Fig. 2. Distribution of actin between Triton X-100-soluble and insoluble fractions of swinholide A treated cells. 3T3 cells were labeled overnight with ^{35}S-methionine, and either left untreated **(A)** or incubated for 8 hr with swinholide A **(B)** or latrunculin A **(C)**. Equal volumes from the Triton X-100-soluble (SOL) and -insoluble (INS) fractions were analyzed by 2-D gel electrophoresis. a, actin.

[Bershadsky et al., 1995]. Since the effects of swinholide A and latrunculin A on cell morphology, on the organization of the actin cytoskeleton (Fig. 1), and on the ratio between the amount of soluble and cytoskeletal actin were similar [Fig. 2, Table I], we examined the effect of these compounds on cytoskeletal protein synthesis.

Cells were incubated with latrunculin A or swinholide A for 8 hr and then pulse-labeled for 30 min with ^{35}S-methionine. Equal amounts of radioactive proteins were analyzed by two dimensional gel electrophoresis. The results presented in Figure 3 show that while latrunculin A treatment-induced a decrease in new β- and γ-actin synthesis [Fig. 3C; cf. Fig. 3A], in swinholide A-treated cells actin synthesis was significantly elevated [Fig. 3C; Table I]. Furthermore, in cells treated with both swinholide A and latrunculin A, the inhibition conferred by latrunculin A on actin synthesis was reversed, and the level of actin synthesis in such cells was higher than in control 3T3 cells [Fig. 3D; cf. Fig. 3A].

The synthesis of vinculin was reduced in latrunculin A-treated cells [Bershadsky et al., 1995] (Fig. 3B; cf. Fig. 3A) but was elevated in

TABLE I. Effects of Latrunculin A and Swinholide A on Actin Assembly and Synthesis*

Drug treatment	Actin synthesis (%)	% Triton X-100-soluble actin
Control	100	23 ± 3
Latrunculin A	35 ± 12	72 ± 10
Swinholide A	175 ± 18	57 ± 8
Swinholide A + latrunculin A	132 ± 12	ND

*The levels of actin synthesis and the percentage of total cellular actin that is Triton X-100 soluble were determined by automatic computerized analysis of the 2-D gels shown in Fig. 2, after normalizing the actin spot to all the spots matched on the 2-D gels, as described in Materials and Methods.
ND, not determined.

cells treated with swinholide A either in the absence [Fig. 3C] or in the presence of latrunculin A (Fig. 3D). These results indicate that the regulation of actin and vinculin synthesis is coupled to the state of actin assembly. By contrast, the synthesis of the α and β-tubulin isoforms in these cells was not affected similarly by these drugs (Fig. 3), suggesting that tubulin synthesis is not coupled to the state of organization of the microfilament system. In addition to these major changes in actin and vinculin synthesis in cells treated with these toxins, other minor changes in several other proteins were also observed (Fig. 3).

To determine whether the effect on actin synthesis by swinholide A results from changes in actin RNA levels, Northern blots were hybridized with cDNA probes specific for β- and γ-actin. The levels of both β- and γ-actin were reduced in latrunculin A-treated cells (Fig. 4A,B, lane 2), while in swinholide A-treated cells there was a moderate increase in the level of both β- and γ-actin RNA amounts (Fig. 4A,B, lane 1).

Formation of Actin Dimers in Swinholide A-Treated Cells

The results presented above indicated that polymeric actin is disrupted and released from an association with the Triton X-100-insoluble cytoskeleton in swinholide A-treated cells and suggested that the Triton-soluble actin complexes formed in these cells had a different effect on actin synthesis than in latrunculin-treated cells where G-actin levels are increased. Since our previous studies implied that the level of monomeric actin could be the critical parameter in this autoregulation [Bershadsky et al., 1995], the ability of swinholide A to sequester actin in a dimeric form in vitro [Bubb et al., 1995], was postulated to be responsible for the induction in actin synthesis of swinholide A-treated cells.

To determine whether swinholide A treatment resulted in actin dimer formation in cells, Triton X-100-soluble lysates from control, and cells incubated with either latrunculin A or swinholide A or both latrunculin and swinholide A, were cross-linked with DSP, a reversible cross-linker that forms homobifunctional thiol-cleavable cross-links. The cross-linked proteins were analyzed by SDS–PAGE under nonreducing conditions, and Western blots of such gels were reacted with anti actin antibody to detect higher molecular weight actin complexes. The results summarized in Figure 5 demonstrate that in swinholide A-treated cells, actin could be detected in addition to a monomeric form, also in a higher molecular weight complex of ~115 kDa (Fig. 5, lane 3), while in either latrunculin A (Fig. 5, lane 2), or control 3T3 cell lysates (Fig. 5, lane 1), such complexes were not detected. In cells treated simultaneously with both swinholide A and latrunculin A, actin dimers were also formed (Fig. 5, lane 4). Moreover, in cells first treated with latrunculin A for 2 hr, followed by 2 hr with swinholide A, the presence of actin dimers was detected at a similar extent to that in cells treated with swinholide A alone (Fig. 5, lane 5; cf. lane 3). Thus, swinholide A could override the effect of latrunculin A. Latrunculin A, when added for 2 hr after 2 hr swinholide A treatment, could not eliminate actin dimer formation (Fig. 5, lane 6).

The analysis of Western blots from such cross-linked cell lysates by nonreducing SDS–PAGE with antibodies to nonmuscle tropomyosin isoforms, α-actinin and vinculin, did not detect these proteins in the cross-linked complex (data not shown), suggesting that the higher molecular weight actin complex is composed of actin molecules alone. When Western blots of cross-linked proteins were analyzed by SDS–PAGE under reducing conditions, the higher molecular weight dimeric actin was not detected (data not shown). These results suggest that swinholide A can sequester actin in a dimeric form in 3T3 cells in the presence or absence of latrunculin A.

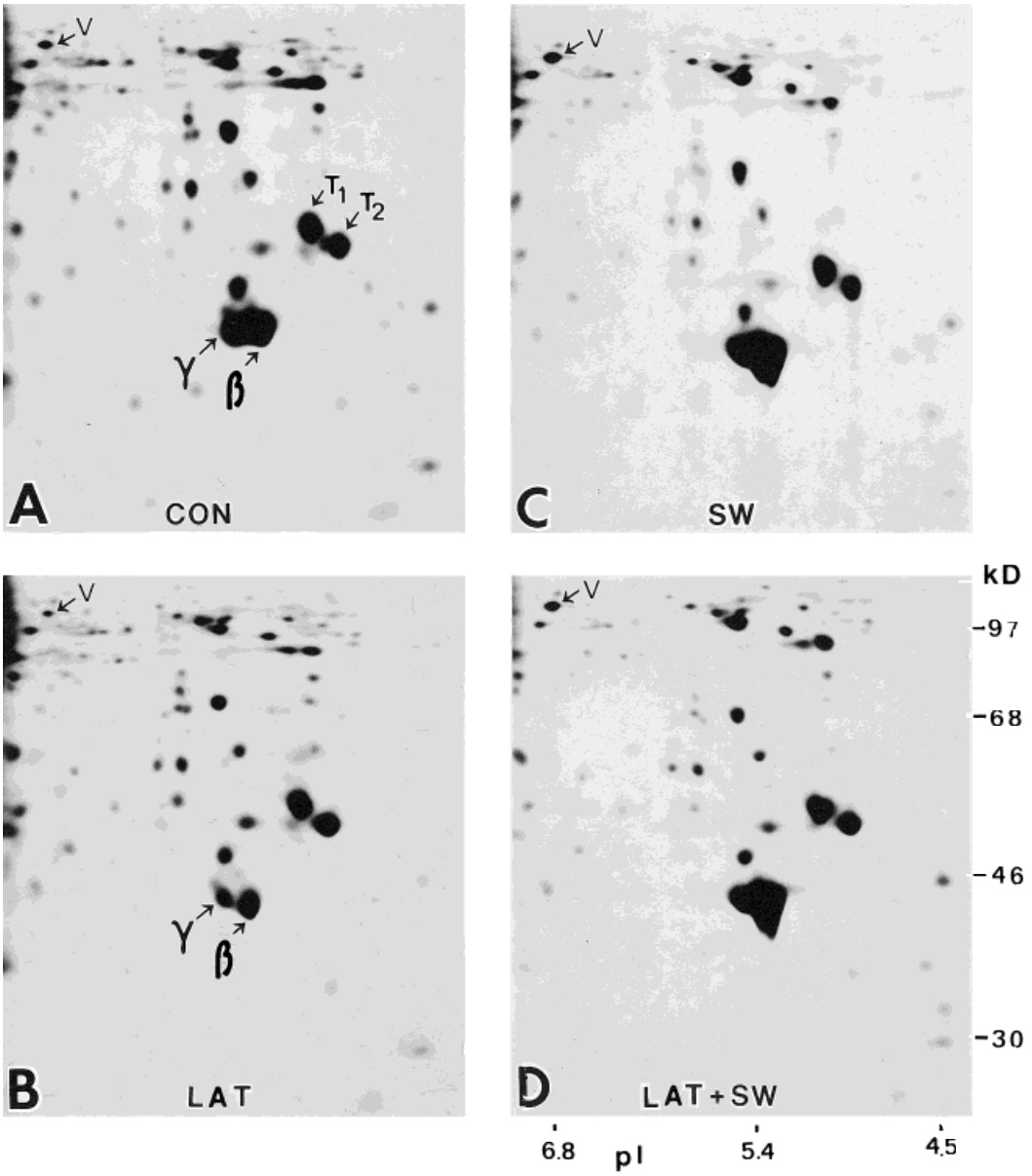

Fig. 3. Pattern of newly synthesized proteins in swinholide A- and latrunculin A-treated cells. Control (CON) 3T3 cultures (A) and cells incubated for 8 hr with either (B) latrunculin A (LAT), (C) swinholide A (SW), or (D) a mixture of latrunculin- and swinholide A (LAT+ SW), were pulse labeled for 30 min with ^{35}S-methionine and equal amounts of radioactive proteins analyzed by 2-D gel electrophoresis. pI, isoelectric point; v, vinculin; T_1, α-tubulin; T_2, β-tubulin; β,γ,β- and γ-actin isoforms.

DISCUSSION

In this study we have employed swinholide A [Bubb et al., 1995] and latrunculin A [Spector et al., 1983, 1989], two marine compounds that can specifically interact with actin, and compared their effects on the organization of the actin-cytoskeleton, on the ratio between soluble and insoluble (cytoskeletal) actin, and the synthesis of actin and vinculin. Both drugs disrupted the actin-cytoskeleton and similarly affected cell morphology. In addition, they caused a marked decrease in the amount of cytoskeletal (polymerized) actin and an increase in soluble actin (Table I). The effects of these toxins on actin synthesis, however, differed: latrunculin A inhibited the synthesis of actin, while swinholide A elevated its expression.

What, then, is the difference between the mechanism(s) of action of swinholide A and latrunculin A that is responsible for the different effects of these compounds on actin expression? In vitro studies have demonstrated that

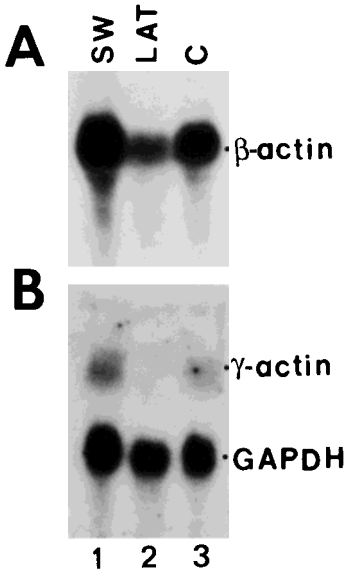

Fig. 4. Expression of actin RNA in cells treated with swinholide A. Total RNA was extracted from cells incubated with swinholide A *(lane 1)*, latrunculin A *(lanes 2)*, and control 3T3 cells *(lane 3)*. Northern blots containing equal amounts of RNA, per lane, were hybridized with cDNAs to β-actin **(A)**, γ-actin **(B)**, followed by rehybridization with GAPDH cDNA **(B)**.

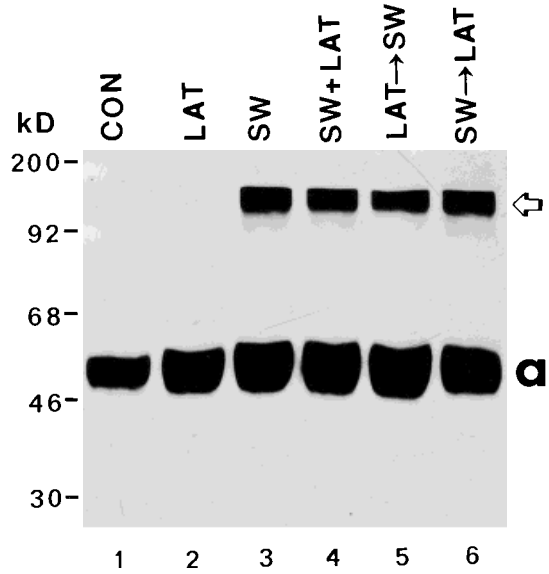

Fig. 5. Analysis of cross-linked actin in lysates of swinholide A-treated cells. Triton X-100-soluble cell extracts from control cells *(lane 1)* and cells treated with either latrunculin A for 4 hr *(lane 2)*; swinholide A for 8 hr *(lane 3)*; latrunculin plus swinholide A for 4 hr *(lane 4)*; latrunculin A for 2 hr followed by swinholide A for 2 hr *(lane 5)*; or swinholide A for 2 hr followed by latrunculin A for 2 hr *(lane 6)* were cross-linked with DSP, separated under non reducing conditions by SDS–PAGE, and Western blots incubated with anti-actin antibody were visualized by the ECL method. *Arrowhead*, cross-linked dimeric actin. a, monomeric actin. Abbreviations as in Fig. 3.

swinholide A has an unique capability to promote the formation of actin dimers [Bubb et al., 1995]. In the present study, using a reversible cross-linking reagent, we have demonstrated that swinholide A induces the formation of actin dimers in cells, while latrunculin A does not. Furthermore, in cells treated with both swinholide A and latrunculin A, actin dimers were formed, suggesting that swinholide A can override the effect of latrunculin A (Fig. 5).

The molecular weight of the actin dimers detected in cell lysates from swinholide A-treated cells was higher (~115 kDa) than that reported by Bubb et al. (1995) in their in vitro studies with purified actin. In vitro, swinholide A was shown to induce the formation of "lower"-actin dimers with an apparent molecular mass of 86 kDa by SDS–PAGE [Bubb et al., 1995], corresponding to antiparallel orientation of the actin monomers linked via Cys-374 residues, as previously reported [Millonig et al., 1988; Hesterkamp et al., 1993]. In the present study, we observed formation of only "upper" dimers with an apparent molecular mass of ~115 kDa that most probably represent adjacent actin subunits linked along the short pitch of the F-actin helix by the bridge formed between Cys-374 and Lys-191 [Elzinga and Phelan, 1984]. During the cross-linking procedure in cell lysate from swinholide A-treated cells no exogenous swinholide A was added. This may have led to the conversion of antiparallel "lower" actin dimers, that were formed by swinholide A in live cells, into the "upper" conformation. In addition, the cross-linking reaction in cell lysates was most probably only partially efficient, and relatively low levels of actin could be detected as dimers in the Triton-soluble fraction of swinholide A-treated cells (Fig. 5). The different cross-linking reagents used in this study and in that of Bubb et al., [1995] (DSP, versus PBM) may also account for the difference in the molecular mass of the actin dimers.

Irrespective of the different molecular mass of actin dimers, the cross-linking studies have shown that, in latrunculin A-treated cells, the Triton X-100-soluble actin is in a monomeric form, while in cells treated with swinholide A in the presence or the absence of latrunculin A, a significant part of soluble actin was sequestered in dimers.

Why, then, is actin synthesis not inhibited in swinholide A-treated cells, but elevated? A possible explanation could be that dimerization of soluble actin decreases the concentration of the actin form that is active in autoregulation. The

actin dimers formed from soluble actin could still be active in inducing the autoregulatory response; the effective concentration, however, of the active form of actin is reduced by dimerization. In the extreme case, when all the soluble actin is sequestered in dimers, this concentration is decreased by twofold, as compared to soluble actin that is completely monomeric. Since swinholide A was capable of both forming actin dimers and reversing the effect on actin synthesis in the presence of latrunculin A, it is conceivable that the level of monomeric actin was reduced below the concentration in control cells, and this could lead to an increase in actin synthesis (Fig. 6). This result is in agreement with those obtained with phalloidin, that stabilizes polymeric actin and reduces the level of monomeric actin, and enhances actin synthesis [Serpinskaya et al., 1990; Bershadsky et al., 1995; Reuner et al., 1991, 1995b].

A strong effect of the swinholide A-induced actin dimers is also predicted if, in contrast to monomeric actin, dimeric actin cannot participate in the autoregulation of actin synthesis, owing to the altered affinity of actin dimers toward target "factor(s)." Such factors may be important in the autoregulation process. This view is supported by studies demonstrating that some actin-binding proteins have different affinities towards monomeric and dimeric actin [Bubb et al., 1994].

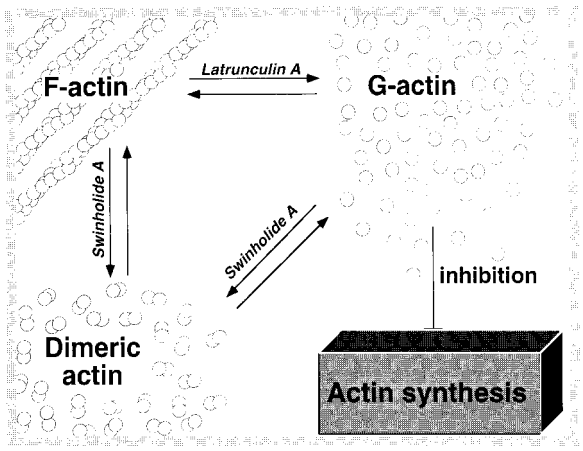

Fig. 6. Summary scheme for the effects of swinholide A and latrunculin A on the state of actin organization and synthesis. Both latrunculin A and swinholide A disrupt the actin-cytoskeleton and similarly decrease the level of F-actin. Latrunculin A treatment leads to an increase in G-actin level, while swinholide A alone, or in combination with latrunculin A, reduces the level of G-actin. Inhibition of actin synthesis was observed only upon treatment with latrunculin A, while swinholide A, with or without latrunculin A, enhanced actin synthesis. Therefore, disruption of F-actin, by itself, does not inhibit actin synthesis, and the elevation in G-actin is probably involved in the specific reduction of actin synthesis.

Taken together, the results comparing the effects of swinholide A and latrunculin A on actin synthesis strongly suggest that a major parameter determining the autoregulatory inhibition of actin expression is the level of monomeric, G-actin, and not the level of cytoskeletal actin, or the changes in cell morphology per se. This notion is also supported by studies using other compounds that either decrease the level of polymerized actin, such as *Clostridium botulinum* C2 toxin, or increase its level, like phalloidin [Serpinskaya et al., 1990, 1991; Reuner et al., 1991; Bershadsky et al., 1995], or manipulate G-actin levels with hypotonic treatment [Reuner et al, 1995b], or overexpress ectopically introduced actin [Lloyd et al., 1992]. In contrast, cytochalasins that are known to disrupt actin filaments, but do not change the level of F-actin, or even elevate it in certain cells [Morris and Tannenbaum, 1980; Rao et al., 1982], do not affect, or may even enhance, actin synthesis [Tannenbaum and Goodman, 1983].

Interestingly, while microfilament bundles are disrupted and cell shape and adhesion are dramatically changed in swinholide A-treated cells, the expression of vinculin was higher in these cells, in contrast to the effect on vinculin expression by latrunculin A [Bershadsky et al., 1995]. This may indicate that changes in vinculin assembly and expression are linked to those of actin, and supports the view that coordinate changes in the assembly of the cytoskeleton in the cell can regulate the expression of cytoarchitectural genes [Ben-Ze'ev, 1986, 1991].

While this actin autoregulation may include a transcriptional regulation of the actin gene, as suggested for hepatocytes treated with phalloidin [Reuner et al., 1995a], in fibroblasts, actin autoregulation shares many common characteristics with tubulin autoregulation [Ben-Ze'ev et al., 1979; Cleveland et al., 1981; Cleveland 1988] that operates by a cytoplasmic mechanism which remains functional in enucleated cells [Bershadsky et al., 1995]. This mechanism may consist of a co-translational degradation of the actin mRNA that depends on the nascent polypeptide and the level of unpolymerized actin, as demonstrated for tubulin autoregulation [Yen et al., 1988]. Other possibilities could include an association of G-actin with factors that may enhance actin mRNA degrada-

tion from the 5′ or 3′ untranslated region of the actin mRNA, or with the translational elongation factor 1α [Liu et al., 1996].

The nonrandom distribution of actin mRNA in the cell that includes its mobilization to the leading edge of endothelial cells during cellular injury [Hoock et al., 1991] or wound healing [Brock et al., 1996], or in response to stimulation with PDGF [Latham et al., 1994] suggests that local changes in the level of actin polymerization may regulate actin mRNA stability and actin synthesis, to spatially regulate actin expression in response to changes in the cellular environment. These different mechanisms together with the autoregulatory response that links actin synthesis to its mode of assembly conceivably serve in the spatiotemporal regulation of actin expression in response to stimulation for growth, differentiation and morphogenesis.

Current studies employing mutated actin constructs are in progress in our laboratory to determine the sequences in actin mRNA that can confer the autoregulatory response in actin synthesis linked to the level of G-actin.

ACKNOWLEDGMENTS

We thank Benny Geiger for useful suggestions during this study and Dr. I. Spector (SUNY, Stonybrook, NY) for reagents. These studies were supported in part by grants from the USA–Israel Binational Foundation, the Forchheimer Center for Molecular Genetics, the Pasteur-Weizmann Research Program (to A.B-Z.) and the Israeli Ministry of Science (to A.D.B.).

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