F-actin modulates swelling-activated chloride current in cultured chick cardiac myocytes

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Zhang, Jianping, Terje H. Larsen, and Melvyn Lieberman. F-actin modulates swelling-activated chloride current in cultured chick cardiac myocytes. Am. J. Physiol. 273 (Cell Physiol. 42): C1215–C1224, 1997.—The integrity of F-actin and its association with the activation of a Cl− current (Icl) in cultured chick cardiac myocytes subjected to hyposmotic challenge were monitored by whole cell patch clamp and fluorescence confocal microscopy. Disruption of F-actin by 25 µM cytochalasin B augmented hyposmotic cell swelling by 51% (from a relative volume of 1.54 ± 0.10 in control to 2.33 ± 0.21), whereas stabilization of F-actin by 20 µM phallolidin attenuated swelling by 15% (relative volume of 1.31 ± 0.05). Trace fluorochrome-labeled (fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate) phallolidin revealed an intact F-actin conformation in control cells under hyposmotic conditions despite the considerable changes in cell volume. Sarcoplasmic F-actin was very disorganized and occurred only randomly beneath the sarcolemma in cells treated with cytochalasin B, whereas no changes in F-actin distribution occurred under either isosmotic or hyposmotic conditions in cells treated with phallolidin. Swelling-activated Icl (68.0 ± 6.0 pA/pF at +60 mV) was suppressed by both cytochalasin B (22.7 ± 5.1 pA/pF) and phallolidin (22.5 ± 3.5 pA/pF). On the basis of these results, we suggest that swelling of cardiac myocytes initiates dynamic changes in the cytoarchitecture of F-actin, which may be involved in the volume transduction processes associated with activation of Icl.

cell volume; cytoskeleton; cytochalasin B; phallolidin; whole cell patch clamp; confocal microscopy

cARDiAC CELLS, like many other cell types, volume regulate when challenged by a reduction of extracellular osmolarity (30). Regulatory volume decrease (RVD) activated under hyposmotic conditions is commonly accomplished by a net loss of intracellular inorganic and organic osmolytes along with osmotically obliged water (14, 30, 31). Activation of a Cl−-selective conductance has been associated with volume regulatory processes in response to cardiac cell swelling (33, 34, 40). Little information is currently available regarding the signaling mechanisms responsible for the activation of these Cl− channels. A number of second messenger systems are known to activate membrane transporters involved in cell volume regulation (1, 7, 14, 31). Our previous studies also indicate that intracellular Ca2+ and adenosine 3’,5’-cyclic monophosphate (cAMP) levels determine the activation of the swelling-induced Cl− current (Icl) in cultured chick cardiac myocytes (10, 38). However, mechanisms that sense changes in cell volume and initiate intracellular second messengers are still largely unknown.

Several lines of evidence have implicated the cytoskeleton in cell volume regulation. Cell swelling is associated with changes in F-actin conformation in a variety of cell types (27). Disruption of F-actin with cytochalasin B, an inhibitor of actin polymerization, has been shown to abolish RVD (5, 8, 9, 22), and, therefore, an intact F-actin network was considered to be essential for a normal volume regulatory response. Identification of possible cytoskeletal determinants of volume regulation has been complicated by the observation that stabilization of F-actin with phallolidin, a compound that prevents F-actin depolymerization, also inhibits RVD (32). In addition, F-actin conformation in many cell types appeared to undergo dynamic changes with a transient disappearance of F-actin at the onset of cell swelling followed by a gradual reorganization coincident with RVD (4, 12, 26, 41). F-actin is also known to modulate a number of membrane transporters activated by cell swelling (27). However, diverse results were reported from different cell types. In P12 pheochromocytoma cells (5) and shark rectal gland cells (26), disruption of F-actin under isosmotic conditions activated Cl− channels similar to those identified with hyposmotic swelling, whereas a volume-regulated Icl in myeloma cells was only enhanced by F-actin disruption under mild hyposmotic conditions (19). By contrast, the swelling-activated Icl in human endothelial cells was not affected by F-actin disruption (29). In cardiac myocytes, we have reported an early transient current (Iswel) associated with hyposmotic swelling and volume regulation (38). Cells treated with cytochalasin B displayed an absence of Iswel (11). The functional relationship between F-actin modulation and the swelling-activated Icl is yet to be established.

The present study was conducted to explore the role of F-actin in the volume regulatory processes associated with activation of Icl during cardiac cell swelling. F-actin was modulated using either depolymerizing or stabilizing reagents, and the swelling-induced changes in membrane conductance and F-actin architecture were monitored by whole cell patch-clamp and fluorescence confocal microscopy, respectively. Our results demonstrate that swelling of cultured chick cardiac myocytes initiates signal transduction mechanisms that involve F-actin reorganization. The dynamic disassembly and reassembly of F-actin in response to cell swelling appear to be a component of the volume transduction processes that regulate the activation of Icl.

MATERIALS AND METHODS

Cell preparation. Single myocytes were isolated from 11-day-old embryonic chick hearts by enzymatic dissociation as described previously (15). The resultant myocyte-enriched supernatant was seeded at a density of ~0.5 × 10⁶ cells on 35-mm untreated culture dishes (Corning 25050) or on 12-mm
Solutions. The control external solution was a N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered salt solution of the following composition (in mM): 142 NaCl, 5.4 KCl, 0.8 NaH₂PO₄, 0.8 MgSO₄, 2.0 CaCl₂, 5.6 dextrose, and 10 HEPES, adjusted to pH 7.4 with NaOH and with an osmolality of 290 mosmol/l. Solutions were rendered hyposmotic by reduction of the NaCl concentration. Hyposmotic solutions used in fluorescence studies contained 25% of total NaCl concentration and had an osmolality of ~100 mosmol/l, whereas hyposmotic solutions used for cell volume and electrophysiological studies contained 75% of total NaCl concentration with an osmolality of ~230 mosmol/l. In electrophysiological experiments, N-methyl-d-glucamine (NMDG) and l-aspartic acid were used as partial substitutes for NaCl in isosmotic solution, such that NaCl concentration remained constant between changes in external osmolality. The solution used to block K⁺ currents was prepared by adding 1 mM BaCl₂ and replacing NaH₂PO₄ and MgSO₄ with equimolar concentrations of NaCl and MgCl₂, respectively. Cl⁻ free solution was prepared by replacing Cl⁻ with aspartate salts. The pipette solution for whole cell patch-clamp studies contained (in mM) 95 l-aspartic acid, 100 NMDG, 2 MgCl₂, 0.5 CaCl₂, 1.0 ethylene glycol-bis(β-aminoethylether)-N,N,N’,N’-tetraacetic acid, 30 tetraethylammonium chloride, 10 HEPES, and 5.0 KATP, adjusted to pH 7.2 with NMDG. The osmolality of all solutions was measured with a vapor pressure osmometer (model 5500; Wescor, Logan, UT). The Cl⁻ channel blocker 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB; Research Biochemicals, Natick, MA) and cytochalasin B (Sigma, St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO) as stock solutions and were used for F-actin staining and subsequent microscopic studies.

Solutions. The control external solution was a N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered salt solution of the following composition (in mM): 142 NaCl, 5.4 KCl, 0.8 NaH₂PO₄, 0.8 MgSO₄, 2.0 CaCl₂, 5.6 dextrose, and 10 HEPES, adjusted to pH 7.4 with NaOH and with an osmolality of 290 mosmol/l. Solutions were rendered hyposmotic by reduction of the NaCl concentration. Hyposmotic solutions used in fluorescence studies contained 25% of total NaCl concentration and had an osmolality of ~100 mosmol/l, whereas hyposmotic solutions used for cell volume and electrophysiological studies contained 75% of total NaCl concentration with an osmolality of ~230 mosmol/l. In electrophysiological experiments, N-methyl-d-glucamine (NMDG) and l-aspartic acid were used as partial substitutes for NaCl in isosmotic solution, such that NaCl concentration remained constant between changes in external osmolality. The solution used to block K⁺ currents was prepared by adding 1 mM BaCl₂ and replacing NaH₂PO₄ and MgSO₄ with equimolar concentrations of NaCl and MgCl₂, respectively. Cl⁻ free solution was prepared by replacing Cl⁻ with aspartate salts. The pipette solution for whole cell patch-clamp studies contained (in mM) 95 l-aspartic acid, 100 NMDG, 2 MgCl₂, 0.5 CaCl₂, 1.0 ethylene glycol-bis(β-aminoethylether)-N,N,N’,N’-tetraacetic acid, 30 tetraethylammonium chloride, 10 HEPES, and 5.0 KATP, adjusted to pH 7.2 with NMDG. The osmolality of all solutions was measured with a vapor pressure osmometer (model 5500; Wescor, Logan, UT). The Cl⁻ channel blocker 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB; Research Biochemicals, Natick, MA) and cytochalasin B (Sigma, St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO) as stock solutions and were used for F-actin staining and subsequent microscopic studies.

Electrophysiological recording. Membrane currents were recorded using the patch-clamp technique in the whole cell configuration (13). Patch pipettes were fabricated from boro-silicate glass capillary tubing (7052; Garner Glass, Claremont, CA) and were fire polished just before use. The pipette resistance was 3–5 MΩ when filled with pipette solution. Current recordings were obtained using an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, CA). Currents were low-pass filtered at 2 kHz by a four-pole Butterworth filter and were acquired by a Gateway 2000 486DX computer using a Digidata 1200 data acquisition system (Axon Instruments). pCLAMP-6 software (Axon Instruments) was used to generate voltage protocols and to digitize and analyze the whole cell currents.

Whole cell currents were elicited by voltage ramps every 20 s, over the voltage range from −90 to +60 mV at a rate of ±0.5 V/s, from a holding potential of −40 mV. The distributed capacitance was compensated immediately after the formation of a gigaohm seal. Cell membrane capacitance was estimated by integrating the transient current response to a 5-mV hyperpolarizing step and dividing by that voltage step. Cell capacitance and series resistance were not compensated during the experiments. All currents were normalized to cell membrane capacitance (pA/pF).

Data analysis and statistics. All data are presented as digitized recordings or, in the case of a series of measurements, as means ± SE; n represents the number of experiments. Statistical analysis was made by Student’s t-test for paired or unpaired data, and a significant difference was assumed at P < 0.05.

RESULTS

Effect of F-actin modulators on cell volume. Conformational changes induced in the cytoskeletal network by cell swelling (3, 41) prompted us to examine the relationship between cytoskeletal integrity and cardiac cell volume control. Cells pretreated with cytochalasin B (25 µM) for ~16 h were superfused in isosmotic bath
solution containing the same concentration of cytochalasin B. After 1 day of incubation with cytochalasin B, most cells remained spherical but demonstrated an increase in volume \([3.2 \pm 0.6 \times 10^3 \mu m^3 \text{ (n = 30)} \text{ to } 3.9 \pm 0.3 \times 10^3 \mu m^3 \text{ (n = 18); P < 0.01}]\). This increase in cell size coincided with an increase in cell membrane capacitance \([7.9 \pm 0.3 \text{ pF (n = 30)} \text{ to } 11.5 \pm 0.6 \text{ pF (n = 18); P < 0.01}]\), consistent with the growth of embryonic cardiac myocytes during prolonged incubation in culture medium. After establishment of the whole cell patch-clamp configuration whereby the cytoplasm was dialyzed by the pipette solution, the cells were exposed to a hyposmotic solution \((230 \text{ mosmol/l})\). The effect of cytochalasin B on cell volume is illustrated in Fig. 1. When challenged by external hyposmolarity, cells treated by cytochalasin B started to swell at the rate similar to that of the control group. After \(~10\text{ min}\), the volume of control cells already reached a plateau of \(1.54 \pm 0.10\) \((n = 5)\) times the volume in isosmotic solution, whereas cells treated with cytochalasin B continued to swell for \(~20\text{ min}\) in hyposmotic solution and approached a plateau of \(2.33 \pm 0.21\) \((n = 6; P < 0.01)\) times the volume in isosmotic solution. The absence of cell volume regulation in whole cell patch-clamp experiments, as observed in many cell types \((19, 34, 40)\), is likely due to dialysis of the cell interior with an infinite pool of the pipette solution, which prevents changes in intracellular osmolarity during hyposmotic challenge \((40)\).

Potentiation of the cell volume increase by cytochalasin B suggests that F-actin integrity is critical in cell volume homeostasis, and dissociation of the cell membrane from the cytoskeleton may contribute a further increase in cell volume when hyposmotically challenged. To test this conclusion, experiments were performed with phalloidin, which stabilizes F-actin. Cells were treated with phalloidin \((20 \mu M)\) for \(~20\text{ h}\), and the same concentration of phalloidin was included in the pipette solution during experiments. The rate of cell swelling among phalloidin-treated cells was much slower than control or cytochalasin B-treated cells (Fig. 1). After \(~10\text{ min}\) of superfusion in hyposmotic solution, cell volume reached a plateau of \(1.31 \pm 0.05\) \((n = 6; P < 0.05)\) times the volume in isosmotic solution (Fig. 1).

F-actin distribution during hyposmotic challenge. To determine how F-actin distribution changes during hyposmotic cell swelling, similar experiments were carried out by visualizing F-actin with TRITC-labeled phalloidin. After 1 day in culture under normal conditions, a considerable proportion of chick cardiac myocytes was symmetrically spherical and contained a centrally localized nucleus. As shown in Fig. 2, F-actin
staining revealed a characteristic sarcomere-like pattern in the central areas of the sarcoplasm surrounding the nucleus, i.e., bands of F-actin were organized in a repetitive fashion consistent with the cross-striation of myofibrillar sarcomeres localized in the perinuclear areas of cardiac myocytes. F-actin was also located in the periphery of the sarcoplasm as a thin layer in close apposition to the sarcolemma. In some instances, finely stained filaments bridged the central and peripheral sites of F-actin. Considerable changes in cell morphology and F-actin staining were observed when cells were treated with cytochalasin B. Compared with DMSO controls (Fig. 3A), cytochalasin B-treated cells were binucleated and asymmetrically spherical with an irregular surface appearance and an increase in cell volume. F-actin was randomly distributed in the perinuclear space, and subsarcolemmal F-actin staining was either significantly reduced or completely absent (Fig. 3D). In contrast, cells treated with phalloidin showed a normal differentiation in cell morphology, and F-actin distribution in these cells was comparable to the control cells (Fig. 3G).

Changes in F-actin staining were studied further under hyposmotic conditions. When chick cardiac myocytes were exposed to hyposmotic solution, a peak level of swelling was attained within ~3 min followed by a RVD (30, 40). Therefore, we examined F-actin distribution after 3 min (Fig. 3, B, E, and H) and 20 min (Fig. 3, C, F, and I) of exposure to hyposmotic solution (100 mosmol/l). During the first 3 min of cell swelling, the cell volume increase was mainly localized in the sarcoplasm between centrally localized sarcomeres and the sarcolemma (Fig. 3, B, E, and H). Fluorescence staining revealed that F-actin remained in perinuclear and subsarcolemmal areas in DMSO (Fig. 3B)- and phalloidin (Fig. 3H)-treated cells, and sarcomere-like organization of staining was observed. In contrast, F-actin in cytochalasin B-treated cells was randomly organized in the perinuclear areas (Fig. 3E). After 20 min of exposure to hyposmotic solution, cell swelling in cytochalasin B-treated cells was pronounced (Fig. 3F). No further changes in cell volume or morphology were observed in DMSO (Fig. 3C)- and phalloidin (Fig. 3I)-treated cells; F-actin organization appeared nor-

Fig. 3. Laser scanning confocal microscopy of F-actin staining in cardiac myocytes subjected to hyposmotic swelling. Cells treated with dimethyl sulfoxide (DMSO) alone (A-C) were used as controls for the swelling experiments. Cell preparations were fixed before (A, D, and G) or after 3 (B, E, and H) and 20 (C, F, and I) min of exposure to the hyposmotic (Hypo) solution. DMSO-treated cells under isosmotic (Iso) conditions (A) reveal F-actin staining similar to that observed in untreated cells (Fig. 2). F-actin distribution in the central and cortical areas appears to be unchanged both during cell swelling (B) and the subsequent volume regulatory decrease (C). Note the presence of fine strands of F-actin staining that connects the central and peripheral localized F-actin (B, double arrowheads). F-actin staining of cytochalasin B-treated cells (D-F) was disorganized in the perinuclear areas and was absent in the periphery (double arrow) of the sarcoplasm. Cells treated with fluorescein isothiocyanate-conjugated phalloidin (G-I) display normal F-actin staining. Sarcomere-like pattern of F-actin (arrow) can be seen in the central area of the sarcoplasm, and F-actin is also present in the subsarcolemmal region (G). F-actin integrity remains intact under hyposmotic conditions (H and I), and F-actin staining that spans the cell periphery and perinuclear areas is also apparent (H). Asterisks indicate swelling-induced increase in sarcoplasmic space. N, nucleus; S, sarcoplasm. Magnification = ×2,200.
F-actin modulation and the swelling-activated $I_{\text{Cl}}$. Hyposmotic swelling of cultured chick cardiac myocytes is known to activate an outwardly rectifying $I_{\text{Cl}}$ (40). To determine the role of F-actin in $I_{\text{Cl}}$ activation, we studied the effect of F-actin disruption and stabilization on $I_{\text{Cl}}$ during cell swelling. Cells were pretreated with either 25 µM cytochalasin B or 20 µM phalloidin as described above. Under control conditions, swelling of chick cardiac myocytes in response to a hyposmotic challenge activated (≤ 3 min) a time-independent $I_{\text{Cl}}$-selective current (Fig. 5). The current-voltage relationship, as plotted in Fig. 5C, displayed characteristics similar to those demonstrated in mammalian cardiac myocytes (33, 34). The peak current amplitude measured at the voltage of +60 mV was 68.0 ± 6.0 pA/pF ($n = 8$).

The background whole cell current did not change in cytochalasin B-treated cells under isosmotic conditions. However, during hyposmotic superfusion, cytochalasin B suppressed the swelling-activated $I_{\text{Cl}}$ (Fig. 6A). The peak current amplitude measured at +60 mV only reached 22.7 ± 5.1 pA/pF ($n = 8; P < 0.01$) after 10-min superfusion with hyposmotic solution (Fig. 6A). The whole cell current did not increase until cytochalasin B was washed from the hyposmotic bath solution, at which time a rapid increase in whole cell current was observed (Fig. 6A). The quick reversal of the whole cell current was consistent with the observed rapid recovery of F-actin conformation after removal of cytochalasin B (see Fig. 4).

The nature of the swelling-activated current in the presence of cytochalasin B was further evaluated to compare with the whole cell current activated under the control conditions. As shown in Fig. 7, rapid removal of external Cl$^-$ almost completely abolished the outward current at +60 mV, with a slight increase in the inward holding current at −40 mV ($n = 4$). The current-voltage relationship (Fig. 7B) also displayed a positive shift of the reversal potential in the absence of external Cl$^-$, consistent with a current carried by anions. The swelling-activated current observed in the presence of cytochalasin B was also inhibited by a Cl$^-$.
channel blocker (NPPB; 100 µM) without a shift of reversal potential of the current-voltage relationship (Fig. 7B). The extent of the current inhibition was 62.7 ± 5.3% (n = 4), which is comparable to the effect of NPPB under the control conditions (39).

Cytochalasin B inhibition of the swelling-activated I_{Cl} implies that F-actin must be intact to enable transduction of the volume-induced signal that activates I_{Cl}. To further explore this role of F-actin, cells were treated with phalloidin (20 µM) to stabilize F-actin. The swelling-activated I_{Cl} in the presence of phalloidin was similar to the response obtained from cells treated with cytochalasin B (Fig. 6B). After ~10 min of swelling in hyposmotic solution, the peak current amplitude measured at +60 mV was only 22.5 ± 3.5 pA/pF (n = 8), significantly below the current level measured in the control group under the same conditions (68.0 ± 6.0 pA/pF, n = 8; P < 0.01). No further increase in whole cell current was observed when cells were superfused in the hyposmotic solution for >10 min.

An attenuation of the swelling-activated current as well as a positive shift of its reversal potential upon removal of external Cl^- was also observed in cells treated with phalloidin, as shown in Fig. 8. Application of the Cl^- channel blocker NPPB (100 µM) largely inhibited the swelling-activated current (Fig. 8) to the degree of 60.6 ± 4.7% (n = 3), indicating a Cl^- selective nature of this swelling-activated current.

Figure 9 summarizes the effect of F-actin modulation on the swelling-activated I_{Cl}. Both F-actin disruption and stabilization significantly suppressed I_{Cl} at all voltages tested. Cytochalasin B and phalloidin seemed to inhibit I_{Cl} without altering the characteristics of the current-voltage relationship (Figs. 7 and 8).

**DISCUSSION**

Swelling-induced conformational changes in the cytoskeletal network have been documented for many cell types (for review, see Ref. 27). Disruption of F-actin is associated with a loss of RVD during cell swelling (5,
The dependence of cell volume regulation on F-actin integrity suggests a critical role for the cytoskeleton in the signal transduction process that initiates volume regulation. This study investigates whether activation of a swelling-induced $I_{\text{Cl}}$ in cultured chick cardiac myocytes is associated with a signal transduction pathway that involves structural changes in F-actin. Our data indicate that an intact linkage between the F-actin and the cell membrane is important for activation of $I_{\text{Cl}}$. Disruption of the F-actin network is associated with an alteration of steady-state cell volume and excessive cell swelling under hyposmotic conditions. Our results also demonstrate that hyposmolality induces dynamic changes in the F-actin cytoarchitecture of cardiac myocytes along with an increase in cell volume. Such reorganization is important to maintain a normal actin-membrane connection during cell swelling and may contribute to the signaling mechanisms that sense changes in cell volume and initiate the regulatory cellular response. Maneuvers that either disrupt or stabilize F-actin impede the dynamic changes of F-actin and suppress the swelling-activated $I_{\text{Cl}}$.

Effect of cytochalasin B and phalloidin on F-actin in cardiac myocytes. Cytochalasin B and phalloidin were used to modulate the F-actin architecture in this study. Cytochalasin B is a fungal toxin that permeates the cell membrane and inhibits actin polymerization (2). Early studies demonstrated that cardiac myocytes exposed to cytochalasin B experienced myofibril disruption and loss of spontaneous contractility (20, 23, 36). Unlike other cell types (3, 4, 19), a pronounced effect of cytochalasin B on cardiac myocytes was only observed after prolonged treatment (20, 23). In addition to the proposed decrease in sensitivity to cytochalasin B during myocardial development (23), myofibrillar-associated F-actin and the dense layer of the submembranous F-actin network in these cells may also contribute to the prolonged period of preincubation. Although cytochalasin D is a more specific disrupter of F-actin cytoskeleton, our previous studies of cytochalasin B in cultured embryonic chick cardiac myocytes (20) and the parallel use of fluorescence confocal microscopy in this study were able to assure the proper disruption of F-actin structure with cytochalasin B and to provide appropriate physiological correlates. In our experiments, incubation of cells with cytochalasin B for 16 h almost completely eliminated the submembranous F-actin network and caused extensive changes in cell morphology. These results are in good agreement with previous observations in cardiac myocytes and other cell types (3, 6, 19, 29). Phalloidin, on the other hand, is a fungal toxin from poisonous mushrooms that stabilizes F-actin by strengthening monomer-monomer interactions (2). Although phalloidin is effectively taken up by pinocytosis in certain cell types (2, 19, 24), a similar mechanism for phalloidin uptake has never been reported in cardiac myocytes. Our results indicate that,
after incubation with FITC-phalloidin for ~20 h, F-actin staining with phalloidin was observed in the morphologically intact cardiac myocytes. These structural observations provided the rationale for us to study the relationship between F-actin modulation and the swelling-activated $I_{\text{Cl}}$.

During hyposmotic swelling, the submembranous F-actin was still present, indicative of a dynamic process that maintains the structural connection between F-actin and the sarcolemmal membrane. This observation is unlike early reports obtained in myeloma cells (19), in which changes in the membrane-associated F-actin ring did not accompany the increase in cell volume. These results implied a swelling-induced dissociation of the cortical F-actin network from the plasma membrane. However, hyposmotic swelling in shark rectal gland cells was accompanied by the transient disappearance of F-actin fluorescence, followed by a gradual reconstitution of F-actin in parallel with a RVD (41). Such reorganization of the F-actin architecture has been implicated in the volume regulatory processes of shark rectal gland cells. Lack of dissociation between F-actin and the cell membrane, as observed when cultured chick cardiac myocytes undergo a volume change, could be attributed to the rapid changes in polymerization/depolymerization of the submembranous F-actin as well as the bridge-like strands of F-actin in cardiac myocytes.

Volume response to F-actin modulation. Our cell volume measurement was undertaken from the whole cell patch-clamped cells of which the intracellular environment was effectively buffered by the pipette solution. As water enters the cell by external hypotonicity, dilution of the cell contents is compensated by the ionic composition of the pipette solution, which maintains the gradient for water influx and allows the cell to swell beyond the theoretically expected volume change. Such a configuration could contribute, in part, to the lack of cell volume regulation in the whole cell patch-clamped cells (40). Although dilution of the cell contents by external hypotonicity may also stimulate solute entry through membrane transport pathways, this does not seem to occur in cultured chick cardiac myocytes, since a loss of intracellular Na$^+$, K$^+$, and amino acid contents was always observed in the same cell type during hyposmotic swelling (30). Cultured chick cardiac myocytes treated with cytochalasin B undergo a larger than normal increase in cell volume when challenged by external hypotonicity. Insertion of new membrane components into sarcolemma appeared to be unlikely because no significant changes in membrane capacitance were observed during hyposmotic swelling. Cardiac myocytes are known to possess a small volume-to-surface area ratio due to considerable invaginations of sarcolemma (21). Such invaginations have been shown in yeast cells to be surrounded by densely stained F-actin (28). Actin binding proteins tightly connect the F-actin and sarcolemmal invaginations to provide an interface for cytoskeleton-membrane linkage, which may play a unique role in cell volume regulation. Disruption of F-actin by cytochalasin B could remove the mechanical restraint on the sarcolemma and allow the cells to swell without the insertion of new membrane components. On the other hand, cells treated with phalloidin became rigid due to the stabilization of the F-actin network. When phalloidin-treated myocardial cells were subject to a hyposmotic challenge, the tightened cytoskeleton-membrane linkage could have constrained the mechanical distension of the sarcolemma to attenuate cell swelling.

Response of swelling-activated $I_{\text{Cl}}$ to F-actin modulation. Although cytochalasin B and phalloidin have opposite effects on the F-actin, our data indicate that both compounds inhibit the swelling-activated $I_{\text{Cl}}$. These results are distinct from reports in some cell types that disruption of F-actin by cytochalasin B increased the sensitivity of ion channels to cell swelling or membrane stretch (17, 19, 32). As a typical example, the volume-regulated $I_{\text{Cl}}$ in myeloma cells was enhanced by cytochalasin B and was inhibited by phalloidin (19). No direct evidence, however, has indicated that these channels were involved in the extrusion of intracellular osmolytes during cell volume regulation. By contrast, observations from many cell types have indicated that cytochalasin B inhibits the RVD in response to hyposmotic cell swelling (4, 5, 8, 22). Our results indicate that maneuvers either disrupting or stabilizing the F-actin cytoskeleton suppress the swelling-activated $I_{\text{Cl}}$ without altering the characteristics of the Cl$^-$ channel, as demonstrated by the effect of external Cl$^-$ removal and the Cl$^-$ channel blocker NPPB. These data suggest that swelling of cardiac myocytes is associated with a functionally dynamic change in F-actin that may involve depolymerization and repolymerization of the F-actin network. As a consequence, the linkage between the cytoskeleton and the sarcolemma would be maintained to enable cell volume regulation. Disruption or stabilization of F-actin accompanies the suppression of the swelling-activated $I_{\text{Cl}}$ and concomitantly attenuates cell volume regulation.

Functional consequences of swelling-induced changes in F-actin. The association of F-actin dynamics and the activation of $I_{\text{Cl}}$ during cell swelling suggest the involvement of F-actin in the signal transduction mechanism(s) that may directly or indirectly modulate membrane transport (25). For example, disruption of F-actin by cytochalasin B increased the cAMP content of S49 mouse lymphoma cells (35), a result consistent with our previous observation that elevation of intracellular cAMP concentration inhibits the swelling-activated $I_{\text{Cl}}$ in cultured chick cardiac myocytes (10). On the basis of these findings, we suggest a regulatory role of F-actin in volume-sensitive membrane transport through cAMP-dependent pathways. Equilibrium of F-actin assembly and disassembly can also be modulated by a series of intracellular second messenger systems, such as calcium and phosphoinositides (16). Studies with Ehrlich ascites tumor cells have shown that calcium is required for the reorganization of F-actin during cell swelling, and calmodulin has been suggested to be
involved in the process of F-actin reorganization (4). Pertinent to these observations, we have reported that the swelling-induced activation of I_{K1} is dependent on intracellular calcium (38).

In conclusion, the structural dynamics of F-actin appear to function in conjunction with intracellular second messenger systems to regulate cell volume. F-actin reorganization induced by cell swelling may transduce a signal either directly to regulatory transporters associated with the membrane cytoskeleton or indirectly through intracellular second messenger systems. The organization of F-actin, in turn, may also be regulated such that several signaling pathways could interact to achieve the integrated response to cell volume perturbation.

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