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Actin filament organization regulates the induction of lens cell differentiation and survival

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Abstract

The actin cytoskeleton has the unique capability of integrating signaling and structural elements to regulate cell function. We have examined the ability of actin stress fiber disassembly to induce lens cell differentiation and the role of actin filaments in promoting lens cell survival. Three-dimensional mapping of basal actin filaments in the intact lens revealed that stress fibers were disassembled just as lens epithelial cells initiated their differentiation in vivo. Experimental disassembly of actin stress fibers in cultured lens epithelial cells with either the ROCK inhibitor Y-27632, which destabilizes stress fibers, or the actin depolymerizing drug cytochalasin D induced expression of lens cell differentiation markers. Significantly, short-term disassembly of actin stress fibers in lens epithelial cells by cytochalasin D was sufficient to signal lens cell differentiation. As differentiation proceeds, lens fiber cells assemble actin into cortical filaments. Both the actin stress fibers in lens epithelial cells and the cortical actin filaments in lens fiber cells were found to be necessary for cell survival. Sustained cytochalasin D treatment of undifferentiated lens epithelial cells suppressed Bcl-2 expression and the cells ultimately succumbed to apoptotic cell death. Inhibition of Rac-dependent cortical actin organization induced apoptosis of differentiating lens fiber cells. Our results demonstrate that disassembly of actin stress fibers induced lens cell differentiation, and that actin filaments provide an essential survival signal to both lens epithelial cells and differentiating lens fiber cells.

Keywords: Lens; Actin; Cytoskeleton; Differentiation; Apoptosis; Survival; Bcl-2

Introduction

Various signaling pathways, including FGF-2, IGF-1, TGF-β, N-cadherin, and α6 integrin, have been shown to be involved in the induction of lens cell differentiation (Beebe et al., 1987; Ferreira-Cornwell et al., 2000; Le and Musil, 2001; Lovicu and McAvoy, 2001; Richiert and Ireland, 1999; Walker et al., 2002a,b). Despite the identification of these differentiation initiating signals, the molecular mechanisms underlying the induction of lens cell differentiation are not well understood. Interestingly, most of the proteins that have been demonstrated to induce lens cell differentiation have profound effects on the organization of the actin cytoskeleton (Ferreira-Cornwell et al., 2000; Maddala et al., 2003). One mechanism by which these signaling pathways regulate actin cytoskeletal structures is through the activation of the small RhoGTPases Rho and Rac (Hotchin and Hall, 1995; Miyamoto et al., 1995). While the formation and maintenance of actin stress fibers is signaled by RhoA and its downstream effector ROCK (Kawabata et al., 2004; Riento and Ridley, 2003), cortical actin fibers are induced by Rac (Nobes and Hall, 1995). Growth factors, integrins, and cadherins all activate RhoGTPases to control actin organization. FGF-2 and TGF-β stimulate the RhoA-dependent formation of actin stress fibers (Koyasu et al., 1988; Lee and Gotlieb, 2002), and α6 integrin, cadherins, IGF-1, and FGF-2 activate Rac and cortical fiber formation (Johnston et al., 1995; Kadowaki et al., 1986; Mainiero et al., 1997; Noren et al., 2001; Shaw et al., 1997). In undifferentiated lens epithelial cells in culture, activation of the RhoA/ROCK pathway by factors such as FGF-2, TGF-β, and PDGF induces formation of actin stress fibers (Maddala et al., 2003). Rac activity is more selectively

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stimulated by EGF and FGF-2 (Maddala et al., 2003). Regulation of actin cytoskeletal organization may have a crucial role in signaling lens cell differentiation. In a previous study, we showed that lens epithelial cell differentiation is induced by suppression of Src kinase signaling (Walker et al., 2002a). One of the hallmarks of this inductive differentiation signal is the rapid loss of actin stress fibers and the reorganization of actin as cortical fibers concomitant with the assembly of N-cadherin cell–cell junctions. This suggested the possibility that the disassembly of actin stress fibers provides an initiating signal for lens cell differentiation.

Lens cells are an ideal cell type for studying the role of the actin cytoskeleton in the regulation of differentiation. In vivo, undifferentiated cells are present as an epithelial monolayer that lines the anterior surface of both the embryonic and the mature lens. As these epithelial cells enter the region of the lens equator, they pass through a zone of proliferation, following which they withdraw from the cell cycle and initiate their differentiation, a process that involves dramatic changes in both gene expression and cell shape (Bassnett, 2005; Menko, 2002). These changes are temporally correlated with the induction of the canonical mitochondrial cell death pathway and the activation of caspase 3-like proteases, which play an essential role in the initiation of lens cell differentiation (Weber and Menko, 2005). In vitro, we have shown that this differentiation transition involves molecular remodeling of the cytoskeleton, cell–cell adherens junctions (Ferreira-Cornwell et al., 2000), and cell–matrix adhesions (Walker and Menko, 1999; Walker et al., 2002b). In the cortical region of the embryonic lens, the dramatic process of lens fiber cell elongation takes place coincident with the accumulation of lens differentiation-specific proteins such as the crystallins. After the morphogenetic changes associated with elongation have occurred, the maturing lens fiber cells lose their organelles and nuclei (Bassnett, 1995; Bassnett and Beebe, 1992; Kuwabara, 1975; Kuwabara and Imaizumi, 1974; Modak and Perdue, 1970). This process involves a subset of apoptotic-related proteases distinct from those that signal lens differentiation initiation including the caspases (Foley et al., 2004; Wride et al., 1999). Although apoptotic-signaling molecules are activated for both the initiation of lens cell differentiation and lens cell maturation, the differentiating lens cells survive. The survival signals necessary to protect lens cells from apoptosis during differentiation have not yet been examined.

The literature provides compelling evidence that the actin cytoskeleton supports cell survival, and disruption of the actin cytoskeleton induces apoptotic pathways. For example, in mammary epithelial cells, the inhibition of actin polymerization is sufficient to activate apoptosis (Martin and Leder, 2001). Apoptosis of these cells can be prevented by overexpression of the cell survival protein Bcl-2 prior to the depolymerization of actin filaments. In capillary endothelial cells, drug induced loss of actin stress fibers induces cell rounding and apoptotic cell death and is correlated with suppression of Akt and activation of caspases (Flusberg et al., 2001). Anoikis, apoptosis caused by loss of integrin/matrix interactions, is directly related to the disorganization and disassembly of actin filaments (Frisch and Screaton, 2001). Anoikis can be prevented if cortical actin filaments are formed independent of cell/matrix adhesion through the expression of constitutively active Rac (Cheng et al., 2004; Coniglio et al., 2001). The role of actin filaments in lens cell differentiation and survival is not known.

In this study, we show that actin filaments provide a cell survival signal in both lens epithelial and fiber cells and that the disassembly of actin stress fibers in lens epithelial cells provides an initiating signal for lens cell differentiation.

Materials and methods

Preparation and treatment of primary lens cultures

Differentiating primary lens cell cultures were prepared as previously described (Menko et al., 1984). Briefly, lens cells were isolated from embryonic day 10 quail lenses by trypsinization and agitation. Cells were plated on laminin and cultured in M199 with 10% FBS. For the depolymerization of actin filaments, cultures were treated with 1 μM cytochalasin D (Biomol, Plymouth Meeting, PA). Jasplakinolide (100 nM, Molecular Probes, Eugene, OR) was used to stabilize actin filaments. To induce apoptotic-related pathways, cultures were treated with staurosporine (250 nM, Biomol), a broad-spectrum kinase inhibitor. The ROCK inhibitor Y-27632 (10 μM, Biomol) was used to examine the role of cellular signals that regulate actin stress fiber organization. The role of cortical actin fiber organization in lens cells was examined by treating lens cell cultures with the specific Rac inhibitor NSC23766 (100 μM, Biomol). Note that NSC23766 selectively inhibits GEF interaction with Rac at the Trio/Tiam1 binding site and therefore does not block all Rac pathways (Gao et al., 2004). Control cultures were exposed to DMSO (Sigma, St. Louis, MO), the solvent for the pharmacological agents used in these studies.

Protein extraction and immunoblotting

Culture samples were extracted in Triton/OG buffer (44.4 mM *n*-Octyl β-D glucopyranoside, 1% Triton X-100, 100 mM NaCl, 1 mM $MgCl_2$, 5 mM EDTA, 10 mM imidazole, pH 7.4) containing 1 mM sodium vanadate, 0.2 mM H_2O_2, and Protease Inhibitor Cocktail (Sigma). Protein concentrations were quantified using the BCA assay (Pierce, Rockford, IL).

For all studies, 15 μg of protein extracts was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) on precast 8–16% Tris–glycine gels (Novex, San Diego, CA). Proteins were electrophoretically transferred onto Immobilon-P membranes (Millipore, Bedford, MA). Bcl-2 (N-19), Bcl-$X_{L/S}$, c-IAP1/2 (ch-IAP1), and survivin (FL-142) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to β-actin and PCNA were obtained from Sigma. hILP/XIAP (IAP3) antibody was purchased from BD Biosciences (San Diego, CA), and antibody to phospho-GSK3 α/β (Ser 21/9) was obtained from Cell Signaling Technology (Beverly, MA). Antibodies to filensin and CP49 were generous gifts from Dr. Paul FitzGerald (University of California, Davis, CA). Aquaporin-0 (MP28) antibody was prepared as described previously (Menko et al., 1984). Antibody to δ-crystallin was kindly provided by Dr. Joram Piatigorsky (NEI, Bethesda, MD). Vimentin antibody (AMF-17b) was obtained from Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Secondary antibodies conjugated to horseradish peroxidase (Jackson Laboratories, West Grove, PA) were detected using ECL reagent from Amersham Biosciences (Piscataway, NJ). Immunoblots were scanned, and densitometric analysis was performed using Kodak 1D software (Eastman Kodak Company, Rochester, NY).

Caspase 3 assay

CaspACE Assay System, Colorimetric from Promega Labs (Madison, WI) was used to measure caspase 3-like activity. Cells were extracted, and caspase

activity from equal amounts of total protein was determined as specified by the manufacturer. Protein concentrations were determined using a modified Bradford assay (BioRad, Hercules, CA).

TUNEL assay

Cultures were rinsed with PBS twice then fixed for 15 min in 3.7% formaldehyde diluted in PBS. In situ Cell Death Detection Kit, TMR Red from Roche Molecular Biochemicals (Indianapolis, IN) was used to label the fragmented DNA of apoptotic cells. Nuclei of living and apoptotic cells were counterstained with DAPI (Molecular Probes).

Annexin V

Vybrant Apoptosis Assay Kit #2 was purchased from Molecular Probes. This kit uses AlexaFluor 488-conjugated annexin V to visualize phosphatidylserine exposed on the outer leaflet of the plasma membrane, a hallmark of apoptosis. For the quantification of annexin V labeling, 20 images were acquired per condition, and the average intensity of annexin V labeling in the image field was determined using Metamorph version 6.2 software package (Universal Imaging Corporation, Downingtown, PA).

Fluorescent labeling of actin and plasma membranes

Whole lenses and lens cell cultures were fixed with 3.7% formaldehyde (Polysciences, Warrington, PA) and permeabilized with 0.25% Triton X-100 (Sigma). Actin was labeled by incubating fixed lenses and cultures with AlexaFluor 488-conjugated phalloidin (Molecular Probes). To label cell plasma membranes, whole lenses freshly isolated from embryonic chicks were incubated with 2 μg/ml SP-DiOC$_{18}$ (Molecular Probes) for 1 h at 37°C then 4 h at 4°C. Lenses were then fixed, permeabilized, and co-stained with phalloidin to label actin as detailed above.

Image analysis

Standard fluorescence and phase microscopy were performed using either Nikon Eclipse 80i or Nikon Diaphot 300 microscopes (Optical Apparatus, Ardmore, PA), and images were acquired using Metamorph version 6.2 (Universal Imaging Corporation, Downingtown, PA). Confocal microscopy was performed using the Zeiss LSM510 META confocal microscope. Z-stacks of phalloidin and SP-DiOC$_{18}$ labeled lenses were collected, and three-dimensional images of actin filaments in the intact lens were prepared using Voxx2 software (Indiana University, Indianapolis, IN). Single optical planes (1.16 μm) at basal and lateral aspects of the cells were selected using the LSM5 Image Browser.

Results

Disassembly of actin stress fibers occurs as lens differentiation is initiated in vivo

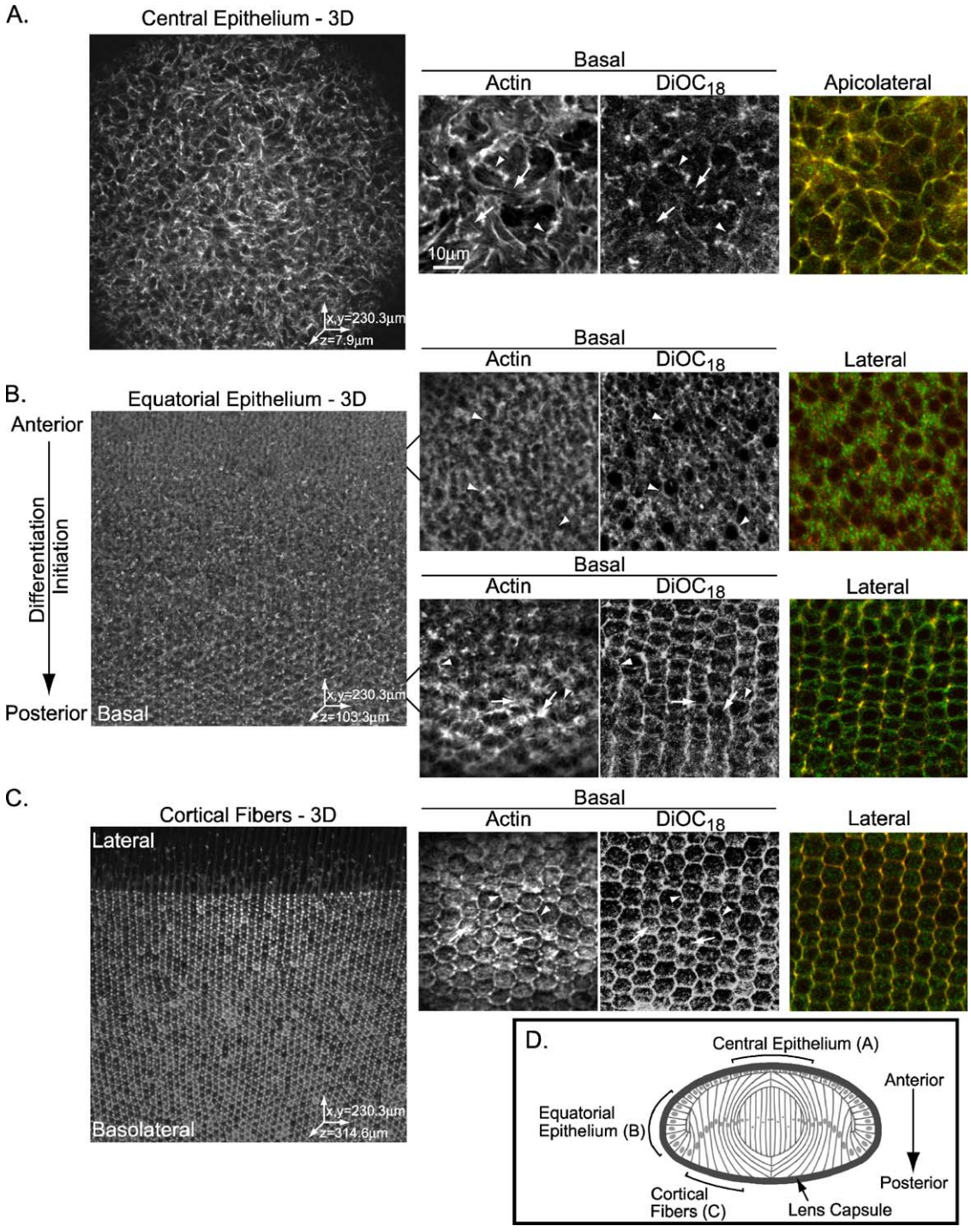
We began our studies by examining the state of organization of the actin cytoskeleton as lens epithelial cells initiate their differentiation in vivo. Using confocal imaging combined with computer analysis, we have created a three-dimensional (3D) model of the actin cytoskeleton in the intact embryonic lens. Special emphasis was placed on the examination of actin filament architecture in the basal and lateral domains of cells in the central and equatorial lens epithelia, a task not possible in previous studies using mid-sagittal sections of the lens (Bassnett et al., 1999; Beebe et al., 2001; Fischer et al., 2000; Straub et al., 2003; Zhou and Menko, 2002). In Fig. 1, we present images of the actin cytoskeleton through three critical regions of the developing lens: the undifferentiated central epithelium in the anterior region of the lens (Fig. 1A), the equatorial zone where differentiation is initiated (Fig. 1B), and the cortical region where lens fiber cell elongation occurs (Fig. 1C). These studies were performed at embryonic day (E)10, the developmental stage diagrammed in Fig. 1D. Each region is viewed from the basal aspects of the lens looking inward. Note that the lens cell basal surfaces are linked to the lens capsule, a thick basement membrane structure that surrounds the lens (Fig. 1D). The apical domains of these lens cells always face the center of the lens, where they contact the apical surfaces of opposing cells. In addition to the 3D perspective, images are also provided of the actin cytoskeleton in optical sections corresponding to basal, lateral, or apicolateral aspects of the cells in which the plasma membrane is counterstained with SP-DiOC$_{18}$ (Fig. 1).

3D imaging revealed that actin stress fibers were the primary actin filament structures of undifferentiated lens epithelial cells in vivo (Fig. 1A), as previously reported in studies of lens epithelial whole mounts in situ (Liou and Rafferty, 1988). These actin stress fibers were located at the cells' basal surfaces (Fig. 1A, arrows) where they are most likely linked to extracellular matrix components of the lens capsule through their integrin

Fig. 1. Actin filaments are reorganized from stress fibers to cortical fibers as lens cells differentiate in vivo. Actin filaments in whole E10 lenses were labeled with fluorescent-conjugated phalloidin (red in color images), and the plasma membrane was labeled with SP-DiOC$_{18}$ (green in color images) for spatial orientation. Lenses were viewed by confocal microscopy. Z-stacks were collected and imported into Voxx software to render 3D models that are viewed en face, from the aspect of the lens basement membrane capsule looking into the lens (left panels, actin only). Images from single optical planes (1.16 μm thick; right panels) were chosen to illustrate actin filament organization at basal and apicolateral or lateral regions of the cells (right panels). Arrow and arrowhead markings are labeled in parallel on DiOC$_{18}$ and actin images. (A) In the undifferentiated lens cells of the central epithelium in the anterior of the lens, stress fibers were the predominant actin filament structure. Actin stress fibers were organized along the cells' basal surfaces where they contact the lens capsule (arrows). Lamellipodial extensions were frequently observed with strong actin labeling at the cell edges (arrowheads). Actin filaments in the apicolateral aspects of these cells in the region of tight junctions had a cortical arrangement typical of polarized epithelia. (B) The 3D image of the equatorial epithelium of the lens, where lens cells initiate their differentiation, is viewed from its anterior (top) to posterior (bottom) aspects (see Supplemental Movie 1). Actin stress fibers were no longer present. In the anterior-most region of the equatorial epithelium, phalloidin staining in the cells' basolateral aspects was amorphous and diffuse (arrowheads). Few actin filaments were detected at the cells' lateral borders. A smooth transition in actin filament organization was observed as the cells moved through the equatorial zone. At the equator, actin filament staining was diffuse and disorganized. As the cells moved to the posterior-most aspects of the equatorial epithelium, actin filaments in the cells' basal aspects localized to cell–cell interfaces (arrowheads) and dense clusters that radiated out to the cell periphery (arrows) in a distinct pattern reflective of the increasing structural organization of these cells. Imaging of a single optical plane revealed that this actin organization continued along the cells' lateral surfaces. (C) In the cortical fiber cell region of the embryo lens, actin filaments were present at the basal tips of the lens fiber cells in a pattern typical of lens BMCs (arrows). Prominent actin staining was also present basally and basolaterally along all sides of the hexagonally packed cells but missing from the vertices (arrowheads, also see Supplemental Movie 2). Throughout the lateral aspects of these fiber cells, cortical actin localization extended around the entire perimeter of the cells (lateral plane) and along the fiber cell–cell borders (3D view). Panel (D) shows a diagram of the E10 chick lens noting the three regions of differentiation where actin was imaged.

receptors (Schoenwaelder and Burridge, 1999). In addition, we observed lamellipodial-like cell protrusions with prominent labeling of actin coinciding with $DiOC_{18}$ labeling of cell edges (Fig. 1A, arrowheads). The observation of dynamic actin structures, including stress fibers and filaments that produce lamellipodia, offers a probable explanation for the spreading of the basal surface of lens epithelial cells along the capsule (Bassnett, 2005). In many cell types in culture, actin stress fibers maintain the undifferentiated state (McBeath et al., 2004; Woods et al., 2005), and we hypothesize that actin stress fibers may function similarly in the lens epithelium. At the apicolateral domain of these undifferentiated lens epithelial cells, in the region where tight junctions form (Zampighi et al., 2000) and the cells maintain a polygonal morphology (Bassnett, 2005), actin filaments were organized as cortical fibers (Fig. 1A, right panel).

Important to our studies was the discovery that actin stress fibers were disassembled as the lens epithelial cells entered the



anterior-most region of the equatorial zone (Fig. 1B, top). Here, only amorphous and diffuse actin staining was detected along the cells' basal surfaces. This loss of actin stress fibers was coincident with the initiation of lens cell differentiation. The actin filaments in the apical domain of these cells remained cortical (data not shown), and surprisingly, few actin filaments were present at lateral cell–cell interfaces (Fig. 1B, right panel). Note that cells in the equatorial zone had a greatly reduced diameter when compared to the undifferentiated cells of the anterior epithelium. Although cell diameter was reduced, the cells increase lengthwise with no net change of volume (Bassnett, 2005). This decreased cell diameter suggested that significant cell compaction had taken place, a phenomenon we have previously shown is linked to lens differentiation initiation in culture (Ferreira-Cornwell et al., 2000; Walker et al., 2002a).

The basal actin cytoskeleton continued to change as the cells moved through the equatorial zone (Fig. 1B, viewed top to bottom; Supplemental Movie 1). At the cells' basal surfaces, diffuse actin staining was gradually replaced by dense condensations of actin filaments in the center of the cell that radiated out to the cell borders (Fig. 1B, arrows). Some limited cortical actin filaments were also seen in the basal region of posterior equatorial cells (Fig. 1B, arrowheads). In the posterior-most region of the equatorial zone, cortical actin fibers extended along the cells' lateral borders, consistent with their function in the assembly of stable N-cadherin cell–cell junctions (Ferreira-Cornwell et al., 2000; Leong et al., 2000). The formation of cortical fibers in the equatorial zone represents a dynamic change in actin filament structures that we speculate has important function beyond structure.

Much of fiber cell differentiation takes place in the cortical fiber region of the embryonic lens (Fig. 1D) where the cells undergo extensive elongation and acquire a distinctive hexagonal packed morphology. Here, an extensive cortical actin network became organized at lateral cell–cell borders in the region juxtaposed to the cells' basal surfaces (Fig. 1C, basolateral; Supplemental Movie 2). Interestingly, actin fibers were excluded from the cell vertices in the basal region but were present at fiber cell vertices in the lateral regions of the cells. Cortical actin fibers extended along the length of these elongating fiber cells (Fig. 1C, right panel) and were much more organized than at earlier stages of differentiation. These actin fibers are important for stabilization of lens fiber morphology (Fischer et al., 2000; Lee et al., 2000) and may also play a signaling role in lens differentiation. At the posterior tips of these differentiating fiber cells where they contact the lens basement membrane capsule, actin was organized in a dense meshwork pattern (Fig. 1C, arrows). This actin structure is likely to be involved in attachment of the lens fiber cells to the capsule. The basal actin structures observed in the cortical lens fiber cells by this new technique are likely the same as the basement membrane complexes previously described by Bassnett et al. (1999). Our 3D actin filament map provides new insights into actin filament reorganization as likely determinants of the lens cell differentiation state.

Actin reorganization in primary quail lens cell cultures mimics differentiation in vivo

When cells are isolated from the embryonic lens and placed in primary culture on a laminin substratum, they form colonies of well-spread epithelial cells (Menko et al., 1984). Within approximately 4 days in culture, the lens cells begin to differentiate; they withdraw from the cell cycle, express differentiation-specific markers, assemble stable N-cadherin cell–cell junctions, and compact (Ferreira-Cornwell et al., 2000; Menko et al., 1984, 1987; Walker et al., 2002a). Since these features parallel those exhibited by lens cell differentiation in vivo, this primary lens culture system has provided many investigators with an ideal model with which to examine mechanisms of differentiation (Chandrasekher and Sailaja, 2003; Fischer et al., 2000; Le and Musil, 2001; Wride and Sanders, 1998). In this study, we utilize this primary, differentiating lens culture system to investigate the function of actin stress fibers and cortical filaments as a survival signal and test the hypothesis that disassembly of actin stress fibers is an initiating signal for lens cell differentiation. As in vivo, the primary actin filament structures of lens epithelial cells in culture are actin stress fibers (Fig. 2A, left panel). The transition to a differentiated lens cell type was accompanied by the disassembly of actin stress fibers and the subsequent reorganization of actin as cortical fibers (Fig. 2A, right panel). This reorganization of actin filaments as lens differentiation was initiated paralleled that which we have described in vivo (Fig. 1).

The reorganization of actin filaments was temporally coincident with the induction of many lens differentiation-specific proteins including δ-crystallin, filensin, and CP49 (Fig. 2B). A number of proteases are activated during lens cell differentiation, including caspases and calpains (Roy et al., 1983; Weber and Menko, 2005). Temporal activation of caspase 3-like proteases, which play a role in the induction of lens differentiation (Weber and Menko, 2005), paralleled that of lens differentiation in vivo; low in undifferentiated epithelial cells, increased three-fold as differentiation was initiated, and then returned to basal levels (Fig. 2B). However, even though actin is a known caspase target (Chen et al., 1996; Mashima et al., 1997), we found no evidence of actin cleavage during this stage of lens differentiation in culture (Fig. 2C), a measure of the importance of actin protection during lens differentiation initiation. In contrast, other proteins such as the intermediate filament proteins vimentin and CP49 and the lens water channel protein aquaporin-0 were cleaved, likely by caspases or calpains.

Actin stress fibers protect lens epithelial cells from apoptosis

We posited that actin stress fibers provide a survival signal that prevents lens epithelial cells from activating apoptotic pathways. To investigate this hypothesis, undifferentiated lens epithelial cells in culture were exposed to cytochalasin D (CD), an efficient inhibitor of actin polymerization. Within 10 min after the addition of CD, stress fiber disassembly was extensive

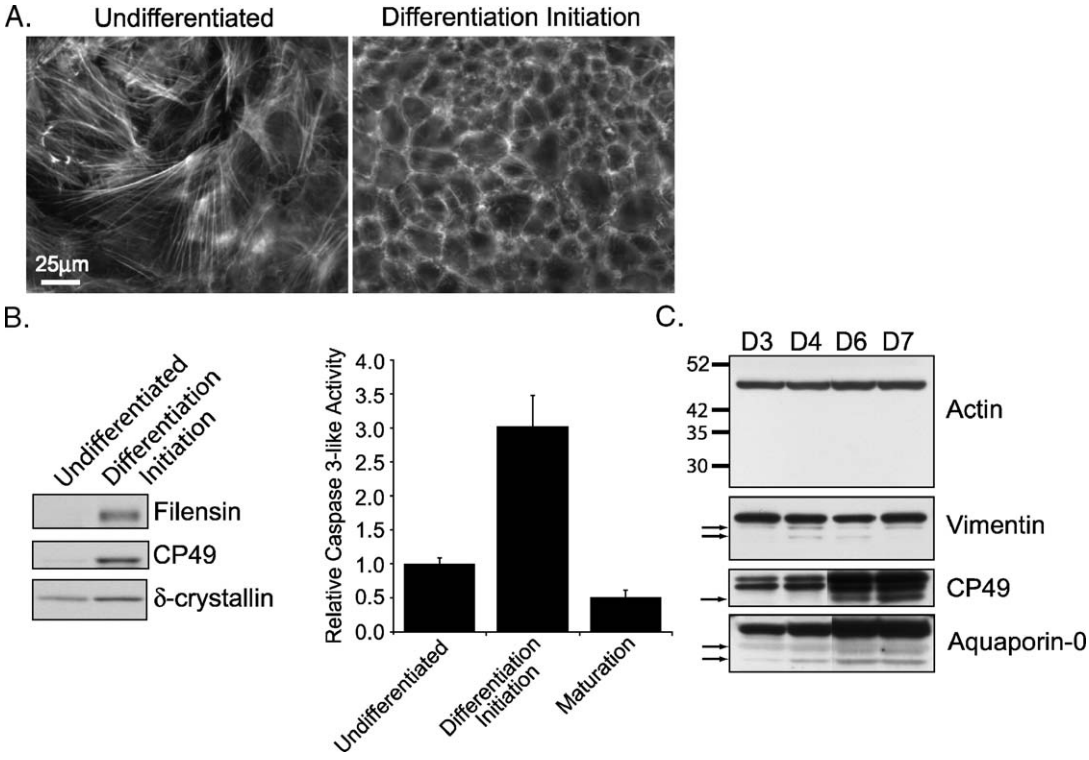


Fig. 2. Stress fibers are disassembled and actin is reorganized into cortical fibers in differentiating lens cells in culture. (A) Actin filament organization in primary lens epithelial cells at day 2 (undifferentiated) and day 4 (differentiation initiation) in culture was determined by labeling with fluorescent-conjugated phalloidin. (B) Protein extracts isolated from primary lens cell cultures as undifferentiated cells and during early stages of differentiation were immunoblotted for the lens differentiation-specific proteins δ-crystallin, filensin, and CP49. Caspase 3-like activity was assayed at different stages of differentiation in primary lens cell culture, as undifferentiated lens epithelial cells, at early stages of lens differentiation (differentiation initiation) and as differentiated lens structures began to form (maturation). (C) Protein extracts isolated from primary lens cell cultures at day 3, day 4, day 6, and day 7 were immunoblotted for actin to examine proteolytic cleavage following activation of caspases for ABC differentiation. These studies show that the primary lens culture model closely parallels lens differentiation in vivo. Actin stress fibers, predominant in undifferentiated lens cells, were reorganized into cortical fibers during the early stages of differentiation initiation. This was accompanied by increased expression of lens differentiation-specific markers and induction of caspase 3-like proteases. While actin was not targeted for cleavage, increased cleavage products (arrows) were observed for three control proteins, vimentin, CP49 and aquaporin-0. Immunoblots were overexposed for better visualization of proteolytic fragments. All studies presented in this figure were repeated at least 3 times with similar results.

(Fig. 3A). By 24 h of CD treatment, no actin filaments could be detected, only diffuse staining remained (Fig. 3A). The depolymerization of actin filaments induced by CD was sufficient to activate caspase 3-like proteases (Fig. 3B) and induce flipping of phosphatidylserine to the outer leaflet of the plasma membrane (Fig. 3C, annexin V) within 24 h. Both are early events in the apoptotic process (Goldstein et al., 2000), and activation of caspase 3-like proteases is associated with initiation of lens cell differentiation (Goldstein et al., 2000; Weber and Menko, 2005). At this time in culture, the CD-treated cells had not fully executed the apoptotic program. Staining with the TUNEL assay, which labels fragmented DNA and is a marker for late stage apoptosis was detected in only 4.5% of the cells (Fig. 3D). In addition, there were very few condensed nuclei (Fig. 3D, DAPI), another late stage apoptosis marker. However, when lens cultures were maintained in the presence of CD for 48 h, there were many condensed nuclei, and the cells had highly refractile membrane blebs (Fig. 3E), both consistent with an advanced stage of apoptotic cell death. These results demonstrate that the loss of actin stress fibers in lens epithelial cells induced an apoptotic pathway resulting in cell death.

Staurosporine has been shown to cause disassembly of the actin cytoskeleton (Hedberg et al., 1990). This broad spectrum kinase inhibitor also causes apoptosis in a wide range of cell types through the activation of the intrinsic mitochondrial death pathway (Bertrand et al., 1994; Yang et al., 1997). In the lens, activation of apoptotic-related pathways following short-term exposure to staurosporine induces differentiation (Weber and Menko, 2005), while prolonged exposure of lens cells to staurosporine leads to apoptosis (Andersson et al., 2000; Ishizaki et al., 1993, 1998). Here, we investigated whether staurosporine induction of apoptotic pathways in lens epithelial cells was related to the loss of actin stress fibers. When undifferentiated lens epithelial cells, whose actin cytoskeleton was organized as stress fibers, were exposed to 250 nM staurosporine for 2 h, there was a significant loss of their actin stress fibers (Fig. 4A). Some short actin filaments did persist as evidenced by a residual diffuse actin staining. The disassembly of actin stress fibers by staurosporine resulted in contraction of lens epithelial cells (Fig. 4B). To explore whether there was a direct connection between the staurosporine-induced disassembly of actin stress fibers and staurosporine induction of an apoptotic pathway in lens epithelial cells, lens cultures were

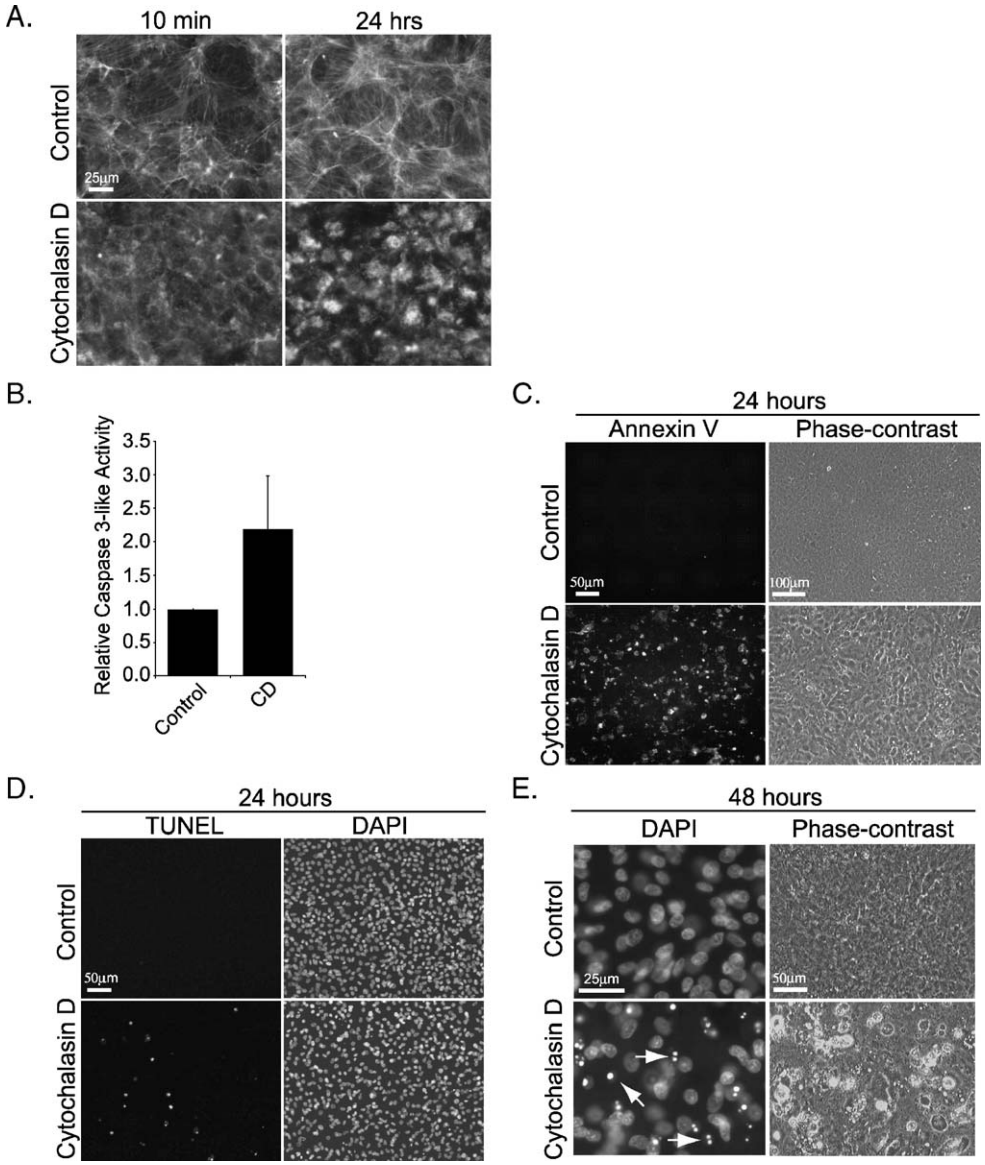


Fig. 3. Actin filaments provide protection from apoptosis in lens epithelial cells. (A) Primary lens epithelial cell cultures were treated with 1 μM cytochalasin D (CD), and actin was labeled with fluorescent-conjugated phalloidin to confirm the depolymerization of actin filaments. After only 10 min, actin filaments were markedly disorganized and by 24 h no discernable filamentous structures remained. Lens epithelial cell cultures were treated for 24 h with 1 μM cytochalasin D (CD) and examined for markers of apoptosis. (B) Exposure to CD activated caspase 3-like activity approximately 2.25-fold over controls. (C) Annexin V labeling indicated that early stages of apoptosis had been induced by CD. Note that annexin V and phase images are of different fields. (D) Labeling of cleaved DNA by the TUNEL assay showed that depolymerization of actin by CD induced only low levels of apoptosis in lens epithelial cells within 24 h. In contrast, after 48 h of treatment with CD (E), lens epithelial cell cultures exhibited extensive membrane blebbing and cell rounding, indicative of late stage apoptosis. DAPI labeling showed that the nuclei of these cells had a pyknotic morphology (arrows, E), also characteristic of advanced stage apoptosis. DAPI and phase images are of different fields.

treated with 100 nM jasplakinolide, an actin stabilizing drug, for 24 h prior to challenging them with staurosporine. Exposure to jasplakinolide effectively stabilized actin stress fibers and prevented their depolymerization by staurosporine (Fig. 4A). Actin stress fibers in cultures treated with jasplakinolide alone were similar to controls. The stabilization of actin stress fibers by jasplakinolide maintained the well-spread morphology of lens epithelial cells and prevented their contraction upon exposure to staurosporine (Fig. 4B). Furthermore, stabilization of actin filaments with jasplakinolide protected lens epithelial cells from cell death, reducing staurosporine-induced apoptosis after 4 h by 44% (Fig. 4C). These studies demonstrated that the stabilization of actin stress fibers protects lens epithelial cells against apoptosis and that staurosporine induction of apoptotic pathways in lens cells involves disassembly of actin stress fibers.

Disassembly of actin stress fibers signals initiation of lens cell differentiation

Depolymerization of actin with CD was sufficient to activate apoptotic pathways, an inductive signal we have previously shown to be important to lens cell differentiation initiation (Weber and Menko, 2005). We hypothesized that the

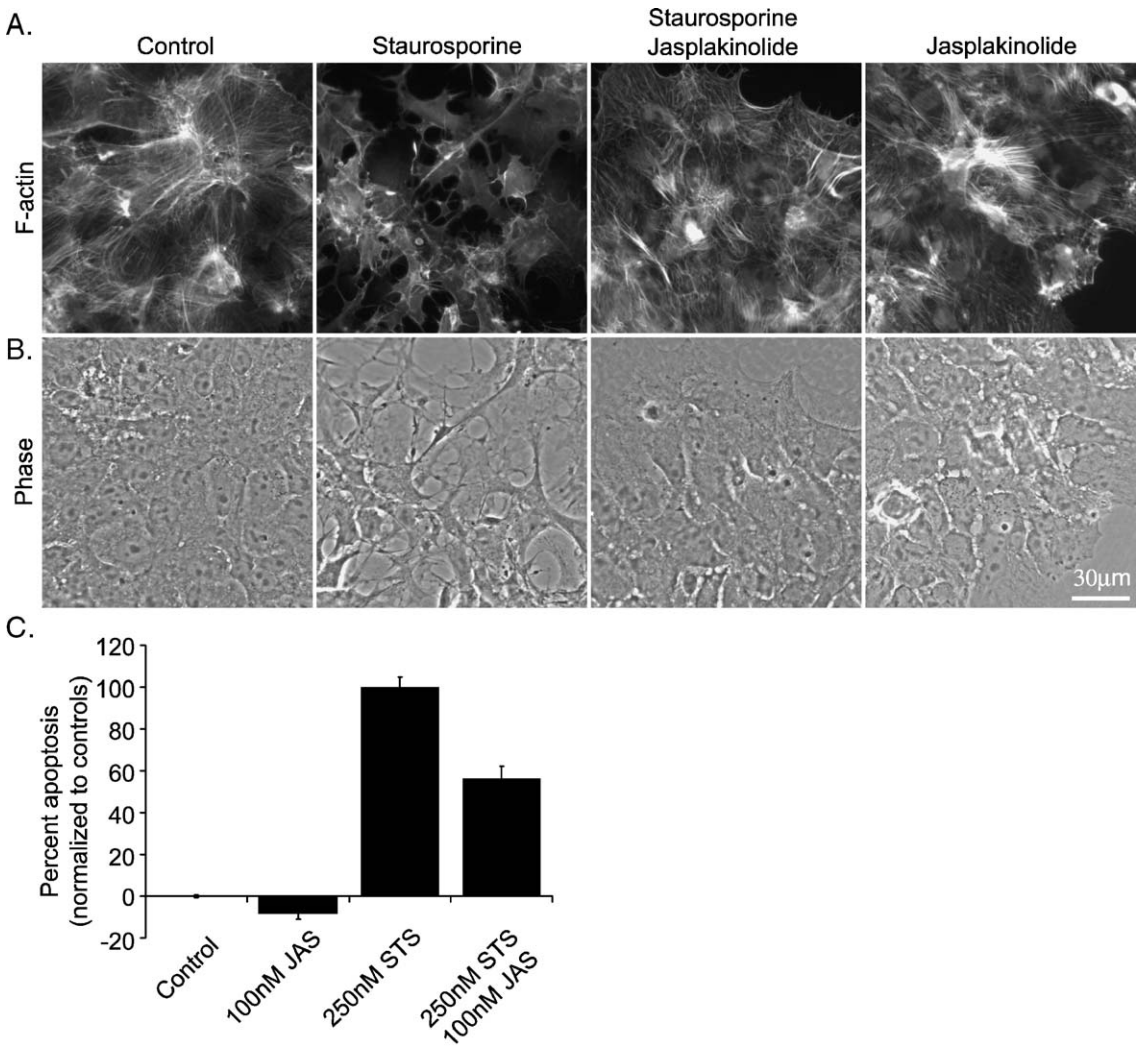


Fig. 4. Staurosporine induces apoptotic pathways in lens epithelial cells through the disassembly of actin stress fibers. Primary embryonic quail lens epithelial cell cultures were exposed to 250 nM staurosporine in the presence and absence of 100 nM jasplakinolide, an actin stabilizing drug, for 2 h. Cultures were observed (A) following labeling with fluorescent-tagged phalloidin, which binds filamentous actin or (B) by phase contrast microscopy. Phalloidin and phase images are of the same field. Exposure to staurosporine caused disassembly of actin stress fibers. The staurosporine-treated cells had a contracted morphology typical of the early stages of apoptosis. Jasplakinolide stabilized actin stress fibers and prevented their disassembly by staurosporine. These cells did not contract and remained well spread similar to controls. Cell morphology and actin stress fibers in cells treated with jasplakinolide alone were similar to controls. (C) To test whether staurosporine induction of apoptotic pathways required actin filament disassembly, lens epithelial cells were pretreated with 100 nM jasplakinolide (JAS) for 24 h to stabilize actin filaments prior to exposure to 250 nM staurosporine for 4 h (STS). Induction of apoptosis was assessed by annexin V labeling. Stabilization of actin filaments with jasplakinolide decreased annexin V labeling following exposure to staurosporine. These results demonstrate that staurosporine induction of apoptotic pathways requires disassembly of actin stress fibers, and that actin filaments provide a cell survival signal. All studies presented in this figure were repeated at least 3 times with similar results.

disassembly of actin stress fibers as occurs in vivo was alone sufficient to induce lens cell differentiation. Using the primary lens culture system, we found that the depolymerization of actin stress fibers by CD was a potent inducer of lens differentiation (Fig. 5A). Within 24 h of exposure to CD, there was a significant induction of the lens differentiation-specific markers δ-crystallin, filensin, CP49, and aquaporin-0. The level of expression of these differentiation markers continued to increase over the next 24 h. Since initiation of lens differentiation also requires withdrawal from the cell cycle (Zhang et al., 1998), we examined whether CD treatment affected lens cell proliferation, using PCNA expression as a marker (Fig. 5B). The exposure to CD caused significant suppression of PCNA and suggested that the loss of actin stress fibers induced cell cycle withdrawal. To confirm that disassembly of the actin cytoskeleton was indeed an inductive signal for lens cell differentiation and that our observations were not due to secondary consequences following actin depolymerization, we treated primary lens epithelial cell cultures for only 6 h with CD, removed the CD-containing media, and then cultured the treated cells for an additional 24 h in normal media. Following the removal of CD, cells rapidly reformed their actin filaments and apoptosis did not ensue (Fig. 5C). Lens cell differentiation markers were strongly induced following this short-term disassembly of actin filaments (Fig. 5D). The ability of short-term CD treatment to induce lens cell

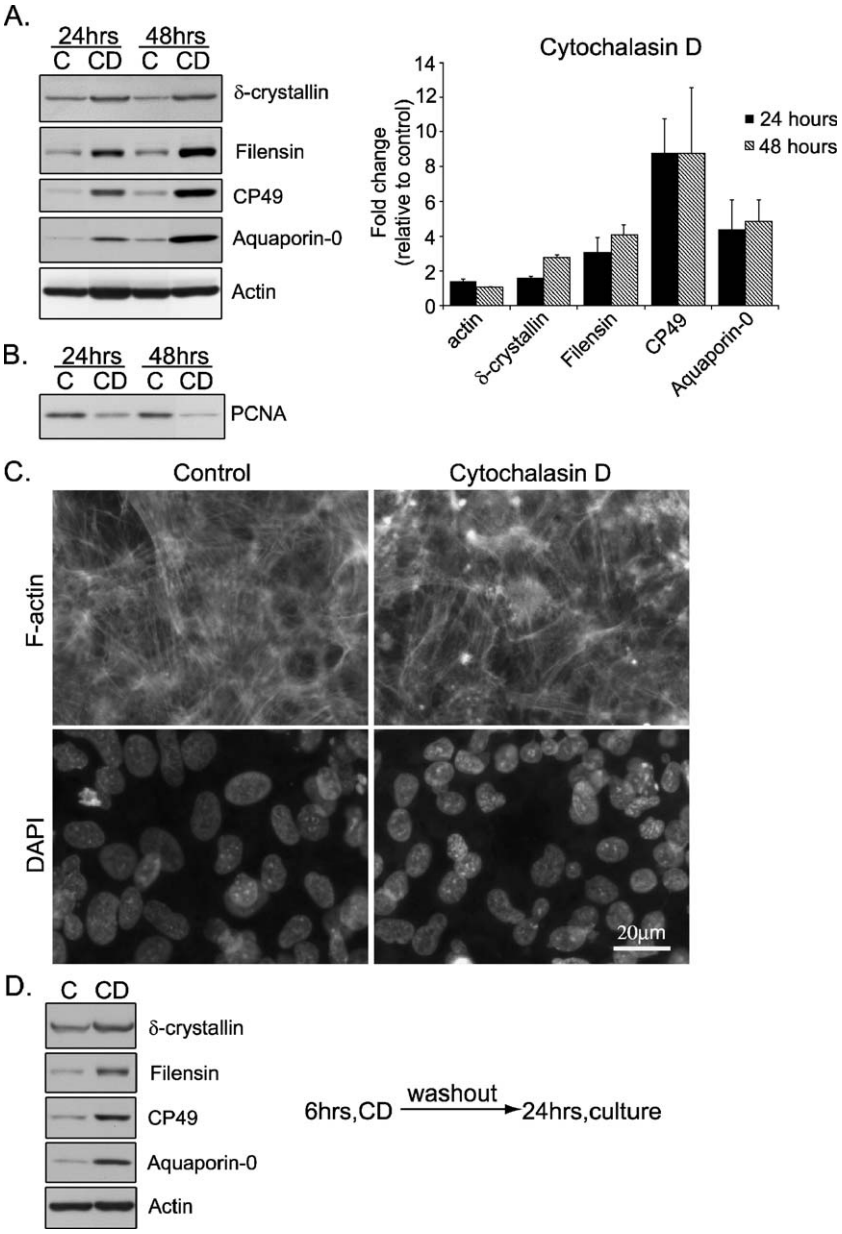


Fig. 5. Depolymerization of actin induces lens cell differentiation. Lens epithelial cell cultures were treated for 24–48 h with 1 μM cytochalasin D (CD). Protein extracts of CD treated cultures were immunoblotted for (A) δ-crystallin, filensin, CP49, and aquaporin-0 to examine the induction of lens cell differentiation and (B) PCNA to examine proliferation. Exposure to CD for 24–48 h induced expression of lens differentiation markers. (C) To examine the ability of transient actin filament disassembly to signal lens cell differentiation, primary lens epithelial cell cultures were exposed to 1 μM CD for 6 h then washed and cultured in normal media. Within 2 h after the removal of CD, actin filaments had reformed. Nuclear morphology was normal indicating that apoptosis was not induced by this short-term exposure to CD. (D) Protein was extracted from cells treated with CD for 6 h then cultured in normal media for 24 h and the expression of lens cell differentiation markers examined by immunoblot analysis. Short-term exposure of lens epithelial cells to CD induced expression of differentiation markers. All studies presented in this figure were repeated at least 3 times with similar results.

differentiation is supportive of a direct role for the disassembly of actin stress fibers in signaling differentiation initiation. Together, these results demonstrated that the disassembly of actin filaments is sufficient to signal the induction of lens cell differentiation.

In order to verify the connection between actin stress fiber disassembly and the induction of lens differentiation, we treated lens epithelial cells with the ROCK inhibitor Y-27632. This inhibitor blocks the function of ROCK, the primary downstream effector of RhoA, and many of the morphological features associated with RhoA activation, including stress fiber formation and maintenance (Uehata et al., 1997). Y-27632 does not interfere with the formation of actin filament structures induced through other RhoGTPases (Hirose et al., 1998; Salhia et al., 2005). Exposure of lens epithelial cells to Y-27632 caused loss of actin stress fibers, with subsequent reorganization as cortical actin fibers (Fig. 6A) as occurs in vivo (see Fig. 1). The disassembly of actin stress fibers with the ROCK inhibitor was sufficient to induce lens cell differentiation as evidenced by increased expression of lens differentiation-specific proteins

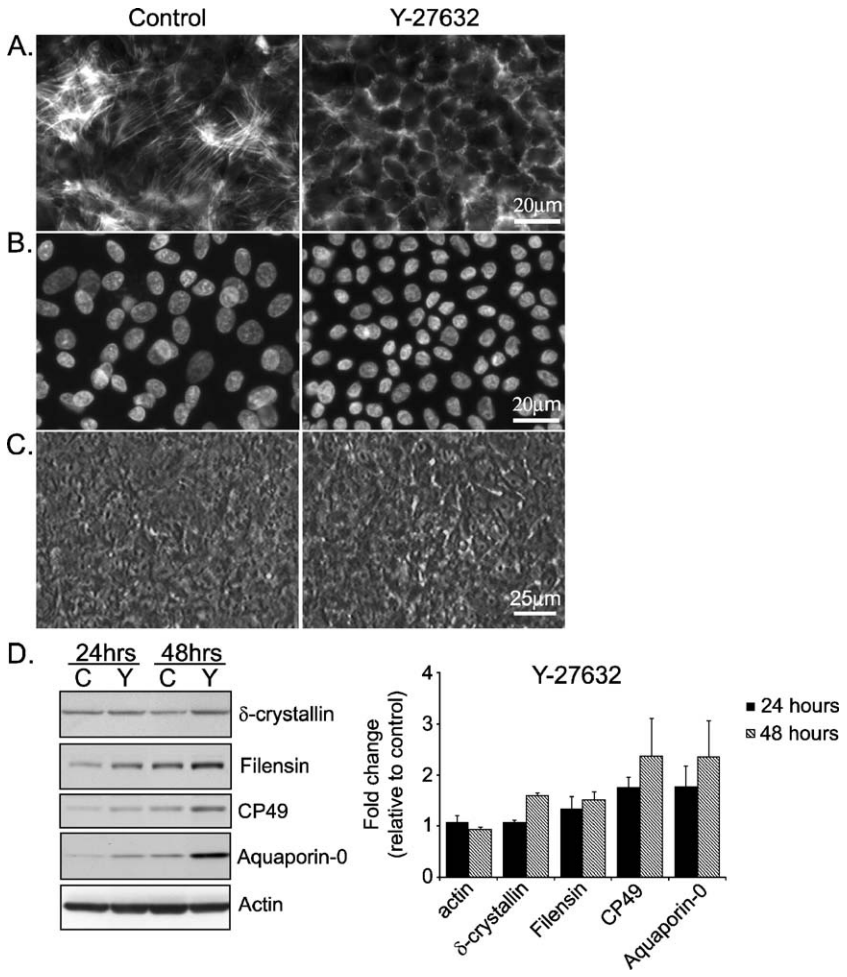


Fig. 6. Suppression of the ROCK pathway induces lens cell differentiation. Primary lens epithelial cell cultures were treated with the ROCK inhibitor Y-27632 (10 μM) for 24 h to disassemble actin stress fibers without interfering with the assembly of actin filaments or the formation of other actin structures. (A) Cultures were labeled with fluorescent-conjugated phalloidin to visualize actin filaments. Control cultures exhibited characteristic actin stress fibers. Exposure to Y-27632 induced the disassembly of these actin stress fibers and the formation of cortical actin fibers. (B) Nuclear labeling with DAPI in Y-27632 treated cells showed no evidence of pyknotic nuclei as occurs in apoptosis. (C) In addition, in the presence of Y-27632 cell morphology appeared normal with no evidence of plasma membrane blebbing as is typical of apoptotic cells. These results demonstrated that Y-27632 did not induce apoptosis in lens epithelial cells. (D) To examine the induction of lens cell differentiation following disassembly of stress fibers, protein extracts from lens epithelial cell cultures treated with Y-27632 were immunoblotted for δ-crystallin, filensin, CP49, and aquaporin-0. Samples were immunoblotted for actin as a control. Disassembly of actin stress fibers by exposure to Y-27632 induced expression of lens differentiation markers. All studies presented in this figure were repeated at least 3 times with similar results.

including δ-crystallin, filensin, CP49, and aquaporin-0 (Fig. 6D). These lens cells did not undergo apoptosis; no condensed nuclei or membrane blebbing were observed in the presence of Y-27632 (Figs. 6B, C). It is possible that the reassembly of cortical actin filaments protected these cells against the apoptotic pathways activated by disassembly of actin stress fibers.

Actin filaments may signal survival by promoting expression of Bcl-2

In order to investigate the mechanism of actin filament-dependent survival during lens differentiation, we examined whether a loss of expression of survival proteins correlated with CD induction of apoptotic pathways. Many pro-survival proteins are highly expressed as lens cells initiate their differentiation in vivo including Bcl-2, Bcl-X_L and the inhibitor of apoptosis proteins (IAPs) ch-IAP1, IAP3 and survivin (Weber and Menko, 2005). We found a similar temporal induction of pro-survival pathways as differentiation was initiated in lens epithelial cell cultures; expression of both Bcl-2 and survivin increased dramatically with lens differentiation in culture (Fig. 7A, day 4–5). Interestingly, the loss of actin stress fibers had no significant effect on expression of the IAPs ch-IAP1 or IAP3 and even caused an increase in expression of survivin (Fig. 7B). Phosphorylation of GSK3, another pro-survival signal, also was increased in CD treated lens epithelial cells (Fig. 7B). In contrast, CD exposure had marked effects on Bcl-2 family members known to regulate lens differentiation initiation and apoptosis. While the overall level of expression of the pro-survival protein Bcl-X_L did not change, CD exposure increased the generation of Bcl-X_L cleavage products and the expression of Bcl-X_S (Fig. 7C), both pro-apoptotic signals. Of the many pro-survival molecules we

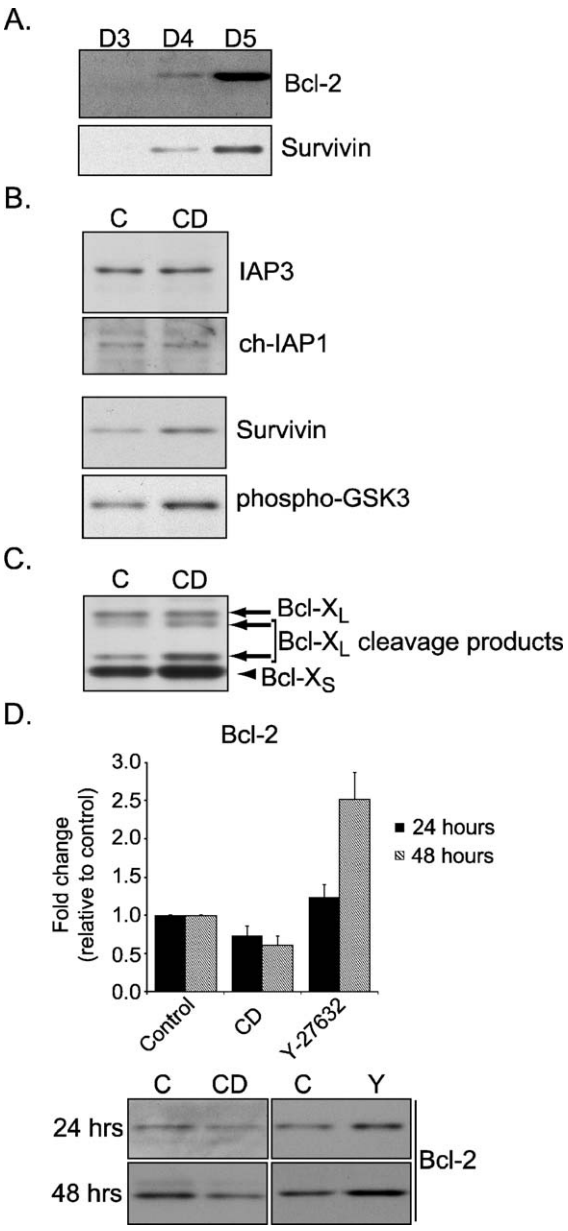


Fig. 7. Actin filaments promote expression of the pro-survival molecule Bcl-2. (A) Protein extracts from primary lens epithelial cells cultured for 3, 4, and 5 days were immunoblotted for the survival molecules Bcl-2 and survivin. Both Bcl-2 and survivin expression increased as differentiation was induced. To examine potential mechanisms for actin filament promotion of survival, lens epithelial cell cultures were treated on day 3 with 1 μM cytochalasin D (CD) for 24 h, and protein extracts were immunoblotted for (B) IAP3, ch-IAP1, survivin, GSK3, and (C) Bcl-2 family members Bcl-$X_{L/S}$. Bands corresponding to Bcl-X_L and its cleavage products are denoted with an arrow, and the band corresponding to Bcl-X_S is marked with an arrowhead. Treatment of lens epithelial cell cultures with CD increased the expression of Bcl-X_S and the formation of Bcl-X_L cleavage fragments. (D) Protein extracts from Y-27632 and CD treated lens epithelial cell cultures were immunoblotted for the pro-survival molecule Bcl-2, and densitometric quantification of the Bcl-2 immunoblots was performed. These data indicate that actin filaments specifically promote the expression of Bcl-2. All studies presented in this figure were repeated at least 3 times with similar results.

examined, Bcl-2 was the only one suppressed when actin filaments were disassembled by exposure to CD (Fig. 7D). In support of an actin-linked Bcl-2 survival signal in lens cells was our finding that Bcl-2 expression was increased in Y-27632 treated lens cells which lose their actin stress fibers but organize cortical filaments and survive (Fig. 7D). Together, these results suggest that the actin filament survival signal in differentiating lens cells may be conveyed through Bcl-2.

Rac signaling of cortical actin filament organization protects lens cells during differentiation

Our 3D in vivo mapping of actin in the region of lens differentiation demonstrated that the loss of actin stress fibers was followed by the formation of cortical actin filaments. Cortical actin fibers became more extensive as these cells differentiated into lens fiber cells. The formation of cortical actin filaments is signaled by the small RhoGTPase Rac (Nobes and Hall, 1995). We hypothesized that Rac signaling had a role in the formation of cortical actin filaments formed during lens differentiation and the actin-dependent survival of differentiating lens fiber cells. The inhibitor NSC23766, a specific inhibitor of Rac interaction with Rac GEFs such as Trio and Tiam1 (Gao et al., 2004), was used to block Rac activity in differentiating lens cell cultures. Note that this inhibitor does not inhibit Rac activation by the GEF Vav and other GEFs that bind to alternative sites on Rac (Gao et al., 2004).

Treatment of undifferentiated lens epithelial cells for 48 h with 100 μM NSC23766 did not affect the formation of cortical actin fibers during the initiation of lens cell differentiation, and there was no induction of apoptosis as determined by phase microscopy or TUNEL staining (Fig. 8A). This result suggests that Rac activated by a GEF with a different mechanism than Trio or Tiam1 may signal the switch from actin stress fibers to cortical actin fibers. To further explore whether Rac mediated organization of cortical actin fibers may provide a survival signal during lens differentiation, we exposed differentiated lens cultures, whose lens fiber cells exhibited prominent cortical actin fibers, to NSC23766 for 48 h. Treatment with NSC23766 caused extensive loss of cortical actin filaments (Fig. 8B, phalloidin). Inhibition of this Rac pathway resulted in blebbing of the plasma membrane (Fig. 8B, phase) and the appearance of TUNEL positive nuclei (Fig. 8B, TUNEL), both characteristics of apoptotic cells. These data demonstrate that Rac signaling of cortical actin filaments is essential for the survival of differentiating lens fiber cells.

Discussion

Disassembly of actin stress fibers signals initiation of lens cell differentiation

3D imaging of the embryonic lens revealed that the predominant actin filament structure of undifferentiated lens epithelial cells in vivo were actin stress fibers. In a recent study by the Bassnett laboratory, a GFP space-filling model was used to examine the cellular shape changes that occur during lens cell differentiation (Bassnett, 2005). They demonstrated that the basal surface of anterior lens epithelial cells has an irregular morphology with cellular projections that spread along the lens

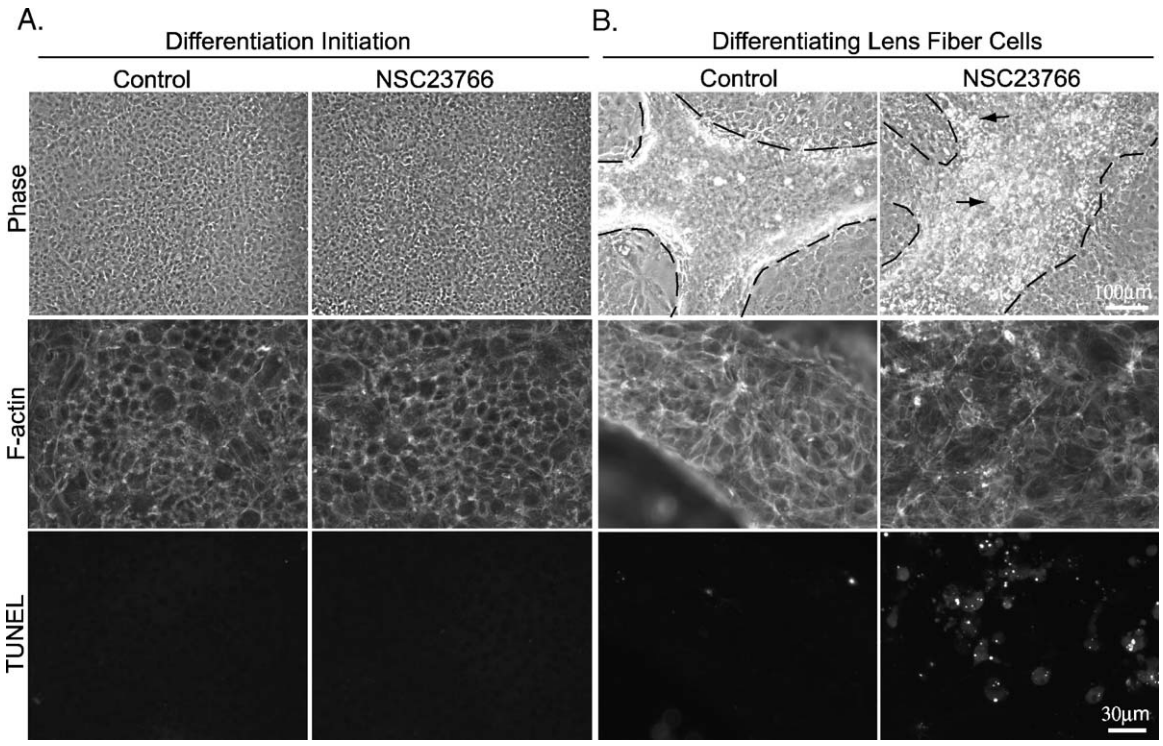


Fig. 8. Rac is required for cortical actin organization and lens fiber cell survival. Primary lens cell cultures containing either (A) undifferentiated lens epithelial cells or (B) differentiating lens fiber cells were treated for 48 h with the Rac inhibitor NSC23766. Pictures of live cultures were acquired by phase microscopy. Cultures were fixed and labeled with fluorescent-conjugated phalloidin for actin filaments and using the TUNEL assay which binds DNA of apoptotic cells. Exposure of lens epithelial cells to NSC23766 had no effect on the formation of cortical actin fibers during differentiation initiation or survival of these cells. In contrast, exposure of lentoid-containing cultures to NSC23766 for 48 h disrupted cortical actin fibers and induced membrane blebbing (arrows) and positive TUNEL labeling, both markers of apoptosis.

capsule. In support of this model, we found both actin-containing lamellipodial extensions and actin stress fibers in undifferentiated lens epithelial cells in vivo, providing evidence that actin filaments may form cellular processes which provide cell attachment to the extracellular matrix components of the lens capsule.

The maintenance of actin stress fibers was dependent on signaling through the RhoA/ROCK pathway. This signaling pathway has been shown to be important not only for the maintenance of actin stress fibers but also for keeping cells in their undifferentiated state. For example, the expression of constitutively active RhoA or ROCK in chondrogenic and adipogenic precursors maintains their actin stress fibers and prevents their differentiation (McBeath et al., 2004; Woods et al., 2005). We propose that ROCK activity in lens epithelial cells maintains actin stress fibers, promoting an undifferentiated phenotype.

Actin stress fibers were exclusive to the undifferentiated lens epithelium, and as lens cells entered the equatorial region where they began the differentiation program, both the cellular projections (Bassnett, 2005) and actin stress fibers were lost. Both in vivo and in culture, the initiation of lens differentiation was coincident with the loss of actin stress fibers and the reorganization of actin filaments as cortical fibers. Inhibitor studies showed a direct connection between stress fiber disassembly and initiation of lens differentiation. Exposure of lens cells to CD, which disassembles actin filaments, or the ROCK inhibitor Y-27632, which disassembles actin stress fibers, was sufficient to induce lens cell differentiation. These studies demonstrated that the loss of actin stress fibers during lens development is an inductive signal for lens cell differentiation. Similarly, disassembly of actin stress fibers by Y-27632 or CD promotes chondrogenesis and adipogenesis (McBeath et al., 2004; Woods et al., 2005). The affect of actin depolymerization on lens differentiation is similar to that of the broad spectrum kinase inhibitor staurosporine, an apoptogen that we previously showed activates both biochemical and morphological differentiation of lens cells (Weber and Menko, 2005). We now know that staurosporine disassembles actin stress fibers in lens epithelial cells. Staurosporine induction of biochemical lens differentiation may involve its ability to disassemble actin stress fibers. Interestingly, one mechanism by which staurosporine is known to disassemble actin stress fibers involves the dephosphorylation of myosin II (Mobley et al., 1994), a primary substrate for ROCK. It is likely that in vivo the downregulation of ROCK activity and consequential decrease of myosin phosphorylation causes the loss of actin stress fibers that initiates lens cell differentiation.

Reorganization of the actin cytoskeleton may initiate ABC differentiation

Our laboratory previously demonstrated that low-level activation of the canonical mitochondrial death pathway was

required for the initiation of lens cell differentiation through a process we called ABC differentiation (Weber and Menko, 2005). We now report that actin stress fibers provide a cell survival signal in the differentiating lens and that the disassembly of actin stress fibers is closely connected to the activation of the mitochondrial death pathway, the same pathway involved in ABC differentiation. Temporally, the disassembly of actin stress fibers during lens cell differentiation in vivo was coincident with the activation of caspase 3-like proteases. In addition to activation of apoptotic-related pathways, we have shown that the disassembly of actin stress fibers plays an essential role in the induction of lens cell differentiation. We propose that the roles for actin stress fibers in lens differentiation and survival are related and suggest that the disassembly of actin stress fibers may signal the initiation of differentiation, in part, through its activation of the ABC differentiation pathway.

Relationship of cell adhesion receptors to actin stress fiber organization and lens cell differentiation

In adherent cells, the formation of stable actin cytoskeletal structures is closely associated with cell adhesion to the extracellular matrix (Wiesner et al., 2005) and to neighboring cells (Angres et al., 1996). Actin filaments organized as stress fibers interact with integrin receptors at focal adhesion complexes where they mediate both integrin/matrix adhesion and integrin signaling function (Miyamoto et al., 1995; Nojima et al., 1995; Shattil et al., 1994). The lens epithelium is the only region of the embryonic lens that expresses high levels of α5β1 integrin and its extracellular matrix ligand fibronectin (Menko and Philp, 1995; Menko et al., 1998; Takada et al., 1987), whose interaction activates Rho, which promotes actin stress fiber organization (Defilippi et al., 1997; Gu et al., 2001). Our finding of actin stress fibers along the basal surfaces of undifferentiated lens epithelial cells suggests that they are likely signaling intermediates of fibronectin/α5β1 integrin. The loss of fibronectin and its receptor α5β1 integrin as lens cells initiate their differentiation (Menko and Philp, 1995; Menko et al., 1998) could provide the instructive signal for the disassembly of actin stress fibers that activates lens cell differentiation.

The disassembly of actin stress fibers and formation of cortical actin fibers is also dependent, in part, on the assembly of cadherin-based cell–cell adhesions. In lens cell cultures, if the establishment of stable N-cadherin cell–cell junctions is blocked, cortical actin fibers do not form, actin stress fibers are retained, and the cells do not differentiate (Ferreira-Cornwell et al., 2000). While in undifferentiated lens epithelial cells in vivo cell adhesion is defined primarily by integrin–matrix interactions (Menko and Philp, 1995), these cells lose their tight association with the capsule as they differentiate and N-cadherin cell–cell junctions become their principal adhesion system (Ferreira-Cornwell et al., 2000; Leong et al., 2000). We suggest that this switch from integrin-based adhesion, which is linked to actin stress fibers, to cadherin-based adhesion, which is linked to cortical actin fibers, promotes the disassembly of actin stress fibers and induces lens cell differentiation.

Actin filaments promote the Bcl-2 pro-survival signal

While many survival molecules in the IAP and Bcl-2 families are expressed during lens cell differentiation (Weber and Menko, 2005), we found that the only one directly regulated by the state of actin filament organization was Bcl-2. Specifically, the depolymerization of actin filaments inhibited Bcl-2 expression and Bcl-2 expression was increased when cortical actin filaments were allowed to form. Therefore, we suggest that the mechanism by which actin filaments signal lens cell survival involves induction of Bcl-2.

In addition to their role as regulators of actin organization, adhesion molecules are likely upstream of the actin-linked Bcl-2 survival signal. Bcl-2 expression is promoted by both cadherin and integrin signaling pathways (Matter and Ruoslahti, 2001; Tiberio et al., 2002; Tran et al., 2002; Zhang et al., 1995). As actin filaments are important mediators of signals initiated by adhesion molecules (Miyamoto et al., 1995; Nojima et al., 1995; Shattil et al., 1994; Tran et al., 2002), it is likely that actin filaments promote Bcl-2 expression and regulate lens cell differentiation and survival through their role as downstream effectors of adhesion receptors.

Role of cortical actin organization in lens fiber cell survival

Although the Rac inhibitor NSC23766 had no effect on the cortical actin fibers formed during differentiation initiation, treatment of lens fiber cells with NSC23766 disassembled cortical actin fibers and induced apoptosis. These data indicated that Rac activation in differentiating lens fiber cells, likely by Trio or Tiam1, was required for signaling cortical actin filament organization and lens fiber cell survival.

Rac activation by Tiam1 and the physiological role of Rac activation is highly dependent on cell adhesion to extracellular matrix (Sander et al., 1998). Such cell–matrix interactions are principally dependent upon the binding of integrin receptors to their extracellular matrix ligands. As lens cells differentiate, one of the few integrins highly expressed is α6 integrin (Walker and Menko, 1999), whose function is required for lens cell differentiation (Walker et al., 2002b). Cell adhesion to laminin-10/11, a ligand for α6β1 integrin, induces potent activation of Rac and the formation of cortical actin fibers (Gu et al., 2001). Activation of Rac by overexpression of the Rac GEF Tiam1 causes formation of cortical actin fibers and localization of α6β1 to the cell periphery (Leeuwen et al., 1997), a pattern evocative of what occurs during differentiation in the embryonic lens (Walker and Menko, 1999). Since α6 integrin activates Rac and cortical fiber formation (Mainiero et al., 1997; Shaw et al., 1997) and Rac/cortical actin protects cells from apoptosis (Fig. 8; Cheng et al., 2004; Coniglio et al., 2001), α6 integrin is a likely upstream activator of Rac signaling pathways that promote cortical actin filament organization and survival in lens fiber cells.

Our studies have identified actin filaments as the upstream regulators of both lens cell differentiation and survival. Actin stress fibers are disassembled to initiate differentiation pathways, and actin is then organized as cortical fibers. As differentiation progresses, cortical actin fibers play a central role in protecting differentiating lens fiber cells from apoptosis. Our findings in lens cells are expected to provide insights into the instructive role of actin dynamics in signaling differentiation and survival of other cell types.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2006.03.056.

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