Tropomodulin and Tropomyosin Mediate Lens Cell Actin Cytoskeleton Reorganization In Vitro

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PURPOSE. To determine the role of the actin cytoskeleton regulatory proteins tropomyosin and tropomodulin (Tmod) in the reorganization of the actin cytoskeleton during lens epithelial cell differentiation.

METHODS. Primary cultures of chick lens epithelial cells were allowed to differentiate in vitro to form lentoid bodies. Localization of F-actin, Tmod, and tropomyosin were determined by immunofluorescent staining followed by confocal microscopy. Tropomyosin and Tmod isoform expression was determined by immunoprecipitation and western blot analysis.

RESULTS. In undifferentiated epithelial cells F-actin was organized in polygonal arrays of stress fibers and was also associated with the adherens belt. In contrast, F-actin in differentiated cells was predominantly associated with membranes in a reticular or fibrillar pattern and was organized in curvilinear fibrils in the cytoplasm. Tmod was not detected in the undifferentiated epithelial cells but was expressed upon cell differentiation and assembled into F-actin and non-F-actin structures. Tmod isoforms expressed in the lens cell cultures were identical with those expressed in the embryonic chick lens fiber cells. Tropomyosin was associated with the polygonal arrays of stress fibers in the undifferentiated epithelial cells and was recruited to cortical F-actin at the cell periphery during differentiation. This occurred coincident with a shift in tropomyosin isoform expression.

CONCLUSIONS. Expression and sequential assembly of low-molecular-weight tropomyosin and Tmod into the cortical actin cytoskeleton of differentiated lens cells may help to reorganize the actin cytoskeleton during morphogenetic differentiation. Moreover, lens epithelial cell differentiation may include the generation of novel Tmod-containing, non-F-actin cytoskeletal structures. (Invest Ophthalmol Vis Sci. 2000;41:166–174)

Cell morphogenesis is an integral component of many cellular differentiation programs, underscoring the dependence of cell function on cell shape. Cell morphology is, in turn, critically dependent on the regulation of the actin cytoskeleton.1–3 These concepts are well illustrated in the vertebrate eye, where the process of lens fiber cell elongation parallels actin filament assembly on the membrane4,5 and may require functions of the family of guanosine triphosphatases (GTPases).6 Furthermore, cytochalasins can specifically block elongation of lens epithelial cells in vitro.7,8

The F-actin organization of lens epithelial cells is characterized by adherens belts typical of epithelial cells as well as unique, polygonal arrays of stress fibers underlying the apical membrane.9–12 The actin cytoskeleton of lens fiber cells is markedly different. Prominent bundles of F-actin are aligned along the vertices of the hexagonal fiber cells,13 but there is also a continuous F-actin network underlying the entire plasma membrane of these cells.14,15 The mechanisms responsible for generating and maintaining this change in F-actin organization during lens cell morphogenesis have not been elucidated, but presumably depend on actin-binding proteins, which regulate actin filament dynamics and associations.16,17

Changes in the composition and subcellular distribution of specific actin-binding proteins are often crucial events in normal cell morphogenesis and differentiation.18–20 In lens morphogenesis, the actin filament-capping protein Tmod is not present in the anterior epithelium but is expressed and assembled on the membrane as fiber cells elongate.15,21 This is significant because Tmod stabilizes actin filaments both in vitro and in vivo and is expressed only by postmitotic differentiated cell types in vertebrates.16,22 In vivo, altered expression of Tmod leads to dilated cardiomyopathy23 in mice and defects in neuronal differentiation in invertebrates.24,25

In vitro, the affinity of Tmod for actin filaments is enhanced 1000-fold by tropomyosin.26 Tropomyosins are a family of actin-binding proteins that bind along the lengths of actin filaments, thereby modulating the stability and localization properties of actin filaments with dramatic consequences to cellular behavior.20,27,28 For instance, altered expression of tropomyosin isoforms is correlated with metastatic cell behavior in transformed kidney cells,29–31 and is specifically induced by oncogenic signals.32,33 At least 16 distinct tropomyosin isoforms expressed in vertebrates can be categorized into high-molecular-weight (HMW) and low-molecular-weight (LMW) groups.27 These groups bind to distinct populations of actin filaments in epithelial cells. The LMW tropomyosins are found.

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in the cortical cytoskeleton at the adherens belt, whereas HMW tropomyosins are found exclusively along stress fibers.\(^{34}\)

Lens fiber cells express a tropomyosin isoform similar to that of erythrocytes, an LMW isoform.\(^{31}\) However, it is not understood how tropomyosin expression and localization may change during differentiation or how these changes correlate with Tmod expression and localization to the actin cytoskeleton. To investigate these questions, we used a primary chick lens cell culture system to study the expression of tropomyosin and Tmod and their subcellular distribution during differentiation and morphogenesis.

Primary cultures of isolated lens epithelial cells have been successfully used to understand many processes essential to lens biology.\(^{55-57}\) This is largely because these cultures replicate many of the molecular features of lens fiber cell differentiation observed in vivo.\(^{37,45\text{ to } 46}\) Moreover, culture models of lens cell differentiation have provided insights on aspects of ion channel regulation,\(^{47}\) gap junction regulation,\(^{48,49}\) growth factor responses,\(^{50-52}\) and contractile activity\(^{53}\) common to many cell and tissue types. However, although these processes are known to be critically dependent on the actin cytoskeleton,\(^{54}\) the assembly and organization of the actin cytoskeleton in differentiating lens cell cultures remains poorly understood. We show here that the actin filaments of these cells are organized into different types of unique structures that include Tmod and/or tropomyosin and that these structures exhibit some striking morphologic and biochemical similarities with those of the lens.

**Materials and Methods**

**Primary Cell Culture**

Cells were isolated as previously described,\(^{44-46}\) with minor modifications. Briefly, lenses were isolated from 11-day-old chicken embryos, and the vitreous and ciliary epithelia were dissected. Capsules were ripped open and the lenses trypsinized with 0.25% trypsin-EDTA for 5 minutes at 37°C. Digestion was stopped with 10% fetal calf serum (FCS) in Media 199 (Gibco, Grand Island, NY), after which cells were harvested by centrifugation. Cells were resuspended in 10% FCS-Media 199 supplemented with penicillin-streptomycin, and plated at approximately 2 × 10^5 cells/cm² on collagen\(^{55}\) or Matrigel (Collaborative Biomedical Products, Bedford, MA). Similar culture results were obtained with either coating. For data shown herein, cells were plated on Matrigel, which exhibited lower autofluorescence. Media was changed after epithelial cell attachment to remove unattached fiber cells,\(^{46}\) and every 2 days thereafter.

**Antibodies**

Rabbit polyclonal antibodies against Tmod were produced as described previously,\(^{56,57}\) as were polyclonal antibodies against tropomyosin.\(^{58}\) The C4 monoclonal anti-actin antibody was the kind gift of James Lessard, Children’s Hospital Medical Center, Cincinnati, Ohio. (C)x56-specific antibodies were the generous gift of Jean Jiang, University of Texas Health Science Center, San Antonio, Texas. Anti-chicken filensin rabbit polyclonal antibodies were kindly provided by Mark E. Ireland, Wayne State University, Lansing, Michigan.

**Immunofluorescence Staining and Confocal Microscopy**

Cultured cells were rinsed in phosphate-buffered saline (PBS) twice, and then fixed with 3.0% paraformaldehyde at room temperature for either 10 or 15 minutes, rinsed with PBS, and then quenched for 15 minutes in 20 mM NaBH₄ in PBS. Fixed cells were permeabilized for 15 minutes at room temperature with 0.2% or 0.4% Triton X-100 in PBS. Cells were blocked for 1 hour in 2% bovine serum albumin and 1% heat-inactivated FCS in PBS. Two to 10 μg/ml primary antibodies were incubated in blocking buffer for 12 to 18 hours at 4°C, followed by three 2-hour washes in PBS. Primary antibodies were detected by incubation with rhodamine-conjugated anti-rabbit or antimouse antibodies for 4 hours at 4°C, followed by washes as above (Boehringer–Mannheim, Indianapolis, IN). F-actin and nuclei were detected with BODIPY-phallacidin and DAPI respectively (Molecular Probes, Eugene, OR). For membrane staining, cells were fixed for 2 hours in 3% paraformaldehyde-0.5% glutaraldehyde in PBS, followed by quenching and incubation in 1 μg/ml BODIPY-SPDiOC18 (Molecular Probes) in PBS for 4 hours at room temperature. Confocal microscopy was performed with a confocal laser scanning microscope unit (model 1024; Bio-Rad, Cambridge, MA) mounted on an inverted microscope (Axiovert; Zeiss, Oberkochen, Germany), with a ×40 oil-immersion plan-apochromatic lens (numerical aperture 1.5). Single wavelength excitation was used, with both channels collected to ensure no bleed-through signal was obtained. Images in either channel were then collected sequentially and recombined to yield multiwavelength images for colocalization purposes. The approximate optical slice thickness (R₀) was 0.965 μm for all confocal images shown.

**Biochemical Procedures**

To detect Tmod in whole lenses or cultured lens cells, quantitative immunoprecipitations were used to enrich for Tmod, to maximize Tmod signal, and to remove actin and β-crystallin, abundant proteins that interfere with western blot analyses. For quantitative immunoprecipitation of Tmod from cell extracts, cell lysates were prepared at various times after plating, essentially as described.\(^{59}\) Cell culture lysate volumes were normalized to approximately equivalent cell numbers. Lysates of whole lenses or fiber cell masses were prepared as described.\(^{15}\) Lens homogenates were prepared for immunoprecipitation by addition of an equal volume of 0.8% sodium dodecyl sulfate (SDS) lysis buffer followed by boiling, sonication and addition of TX-100 to 2%.\(^{60}\) All procedures were performed on ice in the presence of the following protease inhibitors: polymethylsulfonyl fluoride (100 μg/ml; Sigma, St. Louis, MO), apro tinin (1 μg/ml; Sigma), leupeptin and pepstat in A (5 μg/ml each, Boehringer–Mannheim), and tosyl-L-lysyl chloromethyl ketone (100 μg/ml; Calbiochem, San Diego, CA). The appropriate amount of antibody for quantitative immunoprecipitation of Tmod was determined in separate experiments. Immunoprecipitated proteins were separated by standard SDS-polyacrylamide gel electrophoresis (PAGE) and assayed by western blot analyses.

Triton X-100 extractions were performed essentially as previously described.\(^{59}\) Aliquots of fractions were mixed with an equal volume of 2× Laemmli sample buffer\(^{61}\) to be analyzed by SDS-PAGE. The remainder of each fraction was used for Tmod immunoprecipitations, as described.\(^{59}\) For western blot
analysis of total cell homogenates (without prior immunoprecipitation), plates were rinsed with PBS, and boiling 1× Laemmli sample buffer was added to each dish. Lysates were scraped into microcentrifuge tubes and boiled for 7 minutes. Insoluble material was removed by centrifugation at 14,000 g for 10 minutes, and the supernatant was analyzed by SDS-PAGE. As a standard for erythrocyte tropomyosin, human erythrocyte membranes were prepared as previously described.58 Two-dimensional electrophoresis was performed as previously described.57 Additionally, purified skeletal muscle actin (0.5 μg) was added as a positional marker so that gels could be compared with each other. Skeletal muscle extracts were prepared from adult or embryonic chicken pectoralis muscle, as described.57 Western blot analyses were performed as previously described,60 with some modifications. Blots were blocked overnight at 4°C in 4% BSA in PBS. Blots were probed in 20 mM HEPES (pH 7.4), 150 mM NaCl, 0.1% Tween-20 (HBST), supplemented with 3% fish gelatin. Blots were probed for 1 hour at room temperature with polyclonal Tmod antibodies, anti-chicken filensin antibody or anti-tropomyosin antibody. For anti-actin analyses, blots were probed with the C4 anti-actin monoclonal antibody, followed by a polyclonal anti-mouse IgG antibody. After antibody or secondary probe incubation, blots were washed with HBST at room temperature. Antibodies were detected with protein A-horseradish peroxidase (Sigma), followed by standard chemiluminescence detection methods.

RESULTS

Lens epithelial cells proliferated to fill the culture dish within 3 days after plating, eventually forming a confluent monolayer in which the cells maintained a characteristic polygonal, epithelial appearance (Fig. 1A). These epithelial cells then differentiated to form lentoid bodies beginning at 3 to 4 days after plating. During the early stages of differentiation into lentoid bodies, cells appeared to elongate toward a central focus (Fig. 1B), as previously observed.37,44 At later stages of differentiation, lentoid bodies were round and quite refractile (Fig. 1C), presumably due to their thickness (approximately 15–50 μm, observed by confocal microscopy). Staining membranes with a lipid dye showed that the undifferentiated epithelial cells had a rough plasma membrane contour along their extensive intercellular contacts and punctate membrane staining in their cytoplasm (Fig. 1D). As cells elongated into lentoids, the plasma membrane contours became smoother, although some small membrane-rich protrusions appeared to persist along the length of the cells (Fig. 1E, arrowheads). Later in the differentiation process, cell membranes displayed complex topologies (Fig. 1F, arrows), delineating very irregular cell shapes reminiscent of that observed in nuclear fiber cells in vivo.62 The later-stage cells were nearly devoid of the punctate, intracellular membrane staining observed in the undifferentiated epithelial cells but displayed large membrane infoldings and protrusions along their peripheries (Fig. 1F). The authenticity of this morphogenetic differentiation process was supported by the
fact that the cells in lentoids expressed Cx56, a fiber cell-specific protein, which localized to bright puncta on their plasma membranes (Figs. 1H, 1J) and was not detected in the undifferentiated epithelial cells (Fig. 1G). Furthermore, the nuclei of cells in intermediate to late lentoids appeared pyknotic as detected by DAPI staining of cultures (data not shown). Therefore, based on these morphologic observations, we categorized the differentiation process into three stages: undifferentiated epithelial cells, early to intermediate lentoids, and late lentoids. The localization of actin cytoskeletal components was thus assessed at each of these stages.

The undifferentiated epithelial cells displayed characteristic F-actin stress-fiber polygonal arrays (Figs. 2A, 5A, 6A, arrows) remarkably similar to that which has previously been observed in the anterior epithelium of some species in vivo, as well as an adherens belt of F-actin typical of that is, in cultures of undifferentiated epithelial cells (Fig. 3A, day 2). Tmod expression was first detected within 3 to 4 days after plating (Fig. 3A), concomitant with early lentoid formation (Fig. 1B), and Tmod expression continued to increase with the accumulation of lentoids in the culture (Fig. 3A). This accumulation of lentoids was also accompanied by expression of filensin (Fig. 3A), a lens fiber cell-specific intermediate filament protein (reviewed in References 66,67). Moreover, the Tmod that was expressed was associated with the cytoskeleton, because Triton X-100 fractionation of cells revealed that most of the Tmod was found in a Triton X-100-insoluble pool (Fig. 3B), presumably in association with the membrane skeleton. This is consistent with observations in whole chick lenses, but differs from observations in rat lenses.15,21 In contrast, actin expression remained constant (Fig. 3A) and was approximately equally distributed between soluble and insoluble fractions (Fig. 3B), as expected.15,21

There are at least three vertebrate isoforms of tropomodulin, which are named by the tissue from which they were first identified: E (erythrocyte [human]), N (neural), and Sk (skeletal) muscle. We used two-dimensional gel western blot analyses to determine which isoforms of Tmod were expressed in the cultures and in vivo (Fig. 4). In 11-day-old embryonic chick lenses and differentiated lens epithelial cell cultures, Sk-Tmod is the predominant isoform, but a small amount of E-Tmod was detected as well (Fig. 4). Whereas adult chicken lenses expressed only the Sk-Tmod isoform, rat and mouse lenses expressed only E-Tmod (Fig. 4). N-Tmod was not found in either the cultures or the adult lenses from the species tested (data not shown).

Immunofluorescent staining for Tmod confirmed that the expression of Tmod was limited to differentiated cells, because the epithelial cells did not exhibit any detectable specific staining with anti-Tmod antibodies at any time in the culture (Fig. 5A). In contrast, as cells began to differentiate into lentoids, diffuse staining was observed throughout the cytoplasm (Fig. 5B, asterisks), along with intense staining in some coarse, fibrillar structures (Fig. 5B, white arrows). There was also prominent cortical Tmod at this stage, but Tmod was not yet detected in these regions (Fig. 2B, 5B, black arrows). As the differentiation process continued, cells exhibited less diffuse staining, and more condensed Tmod staining in fibrillar structures (Fig. 5C). Preincubation of anti-Tmod antibodies with purified human erythrocyte Tmod eliminated both the cytoplasmic and fibrillar staining patterns (data not shown), indicating that the staining was specific for Tmod. Interestingly, these fibrillar structures did not always colocalize with phalloidin staining for F-actin, particularly in early to intermediate lentoids (Fig. 5B, 5C, compare black arrows for cortical actin and white arrows for Tmod). In late lentoids, however, Tmod antibody staining was frequently localized to large, Factin

**Figure 2.** F-actin organization in differentiating lens epithelial cell cultures. Confocal microscopy images of phallacidin-stained undifferentiated epithelial cells (A), intermediate lentoid cells (B), and late lentoid (C) cells. Arrows indicate regions of polygonal arrays of filament bundles, arrowheads indicate adherens belt actin (A) or cortical actin (B, C), and asterisks indicate position of the nuclei determined by DAPI staining (not shown). Optical slice through apical region of epithelial cells (A), base of lentoid (B), and center of lentoid (C). Bar, (A) 8.5 μm; (B, C) 15 μm.
bundles that followed the irregular contours of the differentiated cells (Fig. 5D, arrowheads).

Because Tmod binds tightly to tropomyosin-coated actin filaments, we also investigated the localization of tropomyosin during the differentiation process. In undifferentiated epithelial cells, tropomyosin was associated with the prominent phalloidin stained polygonal arrays of stress fibers (Fig. 6A, arrows) but was not detected in the cortical cytoskeleton along the adherens belt (Fig. 5A, arrowheads). On differentiation, tropomyosin was redistributed to the cell periphery (Fig. 6B, arrowheads) along with F-actin (Figs. 2B, 6B, arrowheads). However, some stress-fiber–like structures persisted in cells located at the top of the lentoids, which also contained both F-actin and tropomyosin (data not shown). Western blot analysis of cell homogenates on various days of culture revealed that in early cultures before lentoid formation occurs, the predominant isoform of tropomyosin (>90% of total tropomyosin by densitometry analysis) was an HMW tropomyosin (~34 kDa, Fig. 7 left). At early to intermediate stages, 36- and 34-kDa HMW tropomyosins were also detected (days 4 and 6, Fig. 7, lane L, right). As cultures differentiated to form lentoids, these HMW tropomyosins were lost relative to an LMW isoform (~28 kDa, Fig. 7). This LMW tropomyosin comigrates with the LMW tropomyosin expressed by lens fiber cells in vivo (Fig. 7, lane F, right). At late stages of differentiation, the predominant isoform (>60% of total tropomyosin by densitometry analysis) was the LMW isoform (Fig. 7A). Some HMW tropomyosin was still expressed, consistent with the persistence of patches of undifferentiated epithelial cells in the cultures.44–46

**DISCUSSION**

The data reported here describe a change in the organization of the actin cytoskeleton during the morphogenesis and differentiation of chick lens epithelial cells in vitro, concomitant with changes in the expression of specific actin regulatory proteins. The organization of F-actin changes from an epithelial-type cytoskeleton where actin stress fibers are organized in polygonal arrays that intersect with an adherens belt of F-actin, to one that is dominated by a cortical F-actin network. This specialized actin organization is paralleled by dramatic changes in cell shape and topologic complexity of the cell membranes,
suggesting that regulation of the organization and associations of the actin cytoskeleton may facilitate the observed morphogenesis. Moreover, our data suggest that induction of Tmod expression and a shift in tropomyosin isoform expression are part of this regulatory process.

Although the morphogenetic differentiation observed in these cultures clearly does not reproduce all the lens structural features, the data presented here indicate that these cultures replicate some of the changes in molecular composition and architectural features of the differentiated lens fiber cell actin cytoskeleton. First, the shift from polygonal stress fiber arrays in epithelial cells to cortical actin filaments in fiber cells that is observed in the lens also takes place in these cultured cells as they differentiate.9,13,70 Second, persistence of the cortical actin cytoskeleton in the differentiated cultured cells is consistent with numerous immunolocalization studies that have shown F-actin primarily associated with the lateral membranes of the fiber cells (e.g., references 4, 15, 21, 71). Interestingly, generation of an irregular cell shape (reminiscent of older fiber cells15,62) in these cultures occurs after the loss of stress fibers and the accumulation of F-actin along cell surfaces. That lens epithelial cells both in vivo and in vitro preferentially assemble F-actin near cell membranes suggests a central requirement for this structural feature in the differentiation program.

Another aspect of lens cytoskeletal regulation that appears to be replicated by these cultures is that Tmod is not detected in the undifferentiated epithelium, but is appropriately expressed after the initial stages of differentiation, as in the lens.15,72 At early stages, Tmod appears to be localized diffusely in the cytoplasm, in agreement with the diffuse staining observed in annular pad cells.15 The progression from no expression, to diffuse staining, to Tmod in non–F-actin structures and finally to discrete F-actin localization with Tmod antibodies in the lens cell cultures mirrors what is observed in the embryonic and adult chicken lens regions of the anterior epithelium, annular pad, cortex, and nucleus respectively.15 A previous study has demonstrated that Tmod mRNA is upregulated in rat lens fiber cells, but only general, diffuse staining with Tmod antibodies was obtained in differentiating rat lens culture systems,72 as was observed here in early lentoids (Fig. 5B). This may be due to more complete differentiation of the chick lens cell cultures in connection with assembly of Tmod into the cytoskeleton, because immunolocalization of Tmod in the rat lens shows discrete localization to the cortical cytoskeleton.21

**FIGURE 5.** Colocalization of Tmod and F-actin in differentiating lens epithelial cells by confocal microscopy: (A) Undifferentiated epithelial cells, (B) early intermediate lentoid formation, (C) intermediate lentoid formation, and (D) late lentoid formation. *Left:* phalloidin-stained F-actin; *middle:* anti-Tmod staining; *right:* merged confocal image, with Tmod in red and actin in green. **Black arrows:** cortical Factin without Tmod; **asterisks:** diffuse cytoplasmic Tmod staining. **White arrows:** Tmod staining in the absence of Factin; **arrowbeads:** Tmod colocalization with Factin (yellow). Optical slice through apical region of epithelial cells (A), base of lentoid (B), and middle region of lentoid (C, D). Bar (A through C) 15 μm; (D) 30 μm.
Of particular interest is that Tmod assembles into discrete, non-F-actin structures, which has also been observed recently in chick lens cryosections. These structures may be a different filament system to which Tmod is able to bind, either exclusively or coordinately with F-actin. However, we cannot rule out the possibility that these structures may contain actin filaments that do not bind phallloidin. In either case, it is important to note that these cultures express more than one isoform of Tmod (Sk-Tmod and E-Tmod), raising the possibility that different isoforms may be associated with different structures in lens cells. This hypothesis is strengthened by our observation that rat lenses express the E-Tmod isoform, whereas chick lenses express predominantly the Sk-Tmod isoform. It has been shown that a significant soluble pool of Tmod exists in the rat lens. Conversely, only a relatively small percentage of the Tmod in the chick lens or in chick lens cell cultures is soluble under physiological salt conditions, suggesting that different isoforms may have unique properties in vivo, despite exhibiting remarkable similarity in vitro filament-capping assays. Indeed, in fast skeletal muscle fibers, Sk-Tmod is localized preferentially to the thin filament pointed ends in the sarcomere, whereas E-Tmod is localized to the costameres along the plasma membrane.

Finally, we provide evidence that tropomyosins may play a central role in regulating the actin cytoskeleton during lens epithelial cell differentiation in vitro. A shift in tropomyosin isoform content coincident with redistribution of tropomyosin and F-actin on differentiation suggests that the LMW tropomyosin may preferentially stabilize actin filaments in the cortical cytoskeleton. Interestingly, our data suggest a correlation between polygonal arrays of stress fibers found in the undifferentiated epithelial cells and localization of tropomyosin to stress fibers and expression of an HMW tropomyosin. Indeed, HMW tropomyosin isoforms have been shown to associate with stress fibers, whereas LMW isoforms are found on the adherens belt of LLC-PK1 kidney epithelial cells. These data lend support to the hypothesis that lens epithelial cell differentiation and morphogenesis may involve differential stabilization of specific actin filament populations through shifts in tropomyosin expression. Previous work from our laboratory has detected only an LMW tropomyosin in rat lens extracts and has localized this tropomyosin with the cortical F-actin along the lateral sides of fiber cells in the lens. We have been unable to detect HMW tropomyosins in chick epithelial cells in vivo. This may be due poor recovery or solubilization of the HMW tropomyosins or to differences in relative expression levels of LMW tropomyosins in vivo versus in vitro. It will be important to clone and sequence lens tropomyosins to precisely identify which isoforms are expressed where. Nevertheless, the sequential incorporation of LMW tropomyosin and Tmod into the cortical cytoskeleton suggests a progressive stabilization of this F-actin population. This stabilization of cortical actin structures presumably supports the adhesion and intercellular communication required by differentiated lens cells.

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**Figure 6.** Colocalization of tropomyosin (Tm) and F-actin in undifferentiated epithelial cells (A) and differentiated cells (B; day 8 lentoid). **Arrows** indicate cortical actin/adherens belt regions; **arrowheads** indicate stress fibers. (B) **Arrows** indicate stress fibers in an epithelial cell just outside the lentoid. Optical slice was taken through the level of the apical region of the epithelial cells in both (A) and (B), which corresponds to the base of the lentoid (B). Bar, 15 μm.

**Figure 7.** Expression of tropomyosin isoforms during lens epithelial cell differentiation. Left: comparison of tropomyosin expression at various days of culture; numbers indicate days in culture; Right: comparison of tropomyosin from human erythrocytes (Er), chick lens fiber cells (F), and 4-day-old lentoid cultures (L). Estimated molecular weights are shown at right, in kilodaltons. Er, erythrocyte membrane sample.
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