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# The Lens Actin Filament Cytoskeleton: Diverse Structures for Complex Functions

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## Abstract

The eye lens is a transparent and avascular organ in the front of the eye that is responsible for focusing light onto the retina in order to transmit a clear image. A monolayer of epithelial cells covers the anterior hemisphere of the lens, and the bulk of the lens is made up of elongated and differentiated fiber cells. Lens fiber cells are very long and thin cells that are supported by sophisticated cytoskeletal networks, including actin filaments at cell junctions and the spectrin-actin network of the membrane skeleton. In this review, we highlight the proteins that regulate the diverse actin filament networks in the lens and discuss how these actin cytoskeletal structures assemble and function in epithelial and fiber cells. We then discuss methods that have been used to study actin in the lens and unanswered questions that can be addressed with novel techniques.

## Introduction

The eye lens is a remarkable spheroid organ composed of highly organized fiber cells covered by an anterior monolayer of epithelial cells (Figure 1). The lens presents a unique opportunity to study cell migration, elongation, packing, differentiation and aging all within the same tissue. Life-long lens growth is facilitated by the proliferation and differentiation of equatorial epithelial cells into fiber cells (Bassnett and Winzenburger, 2003; Kuszak, 1995; Kuszak et al., 2004a; Piatigorsky, 1981), followed by coordinated migration, elongation and stabilization of fiber cells (Kuszak et al., 2004b; Lovicu and Robinson, 2004; Piatigorsky, 1981; Taylor et al., 1996). Fiber cell morphogenesis is supported by three cytoskeletal systems: microtubules, intermediate filaments and actin filaments (F-actin). Single and bundled microtubules, which are arranged along the long axis of lens fibers, have been suggested to be important for cell elongation and vesicular transport (Kuwabara, 1968; Lo et al., 2003; Piatigorsky, 1975). Beaded intermediate filaments comprised of specialized intermediate filament proteins, CP49 (phakinin, *Bfsp2*) and filensin (CP115, *Bfsp1*) (Alizadeh et al., 2003; FitzGerald, 2009), are needed for mechanical integrity (Fudge et al., 2011; Gokhin et al., 2012) and maintaining life-long lens transparency (Gokhin et al., 2012; Oka et al., 2008; Sandilands et al., 1995). Of the F-actin networks, the best understood is the

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spectrin-actin membrane skeleton, composed of actin filaments cross-linked by $\alpha_2\beta_2$-spectrin, which is integral for fiber cell packing (Gokhin et al., 2012; More et al., 2001; Nowak et al., 2009; Nowak and Fowler, 2012) and mechanical stiffness (Gokhin et al., 2012). In this review, we focus on the organization, regulation and functions of F-actin networks and their associated actin-binding proteins in the lens (summarized in Tables 1 and 2). We also cover approaches for studying actin in the lens and discuss unanswered questions about actin's role in the lens.

## 1. Roles of actin in embryonic lens development and formation

Early lens development is characterized by invagination of a specialized region of the surface ectoderm (lens placode) to form the lens pit, which then pinches off from the overlying ectoderm to form the lens vesicle (Lovicu and Robinson, 2004; Piatigorsky, 1981). During formation of the optic cup (the presumptive retina and retinal pigmented epithelium) and invagination of the lens vesicle, actin-rich filopodia extend from the basal posterior surface of lens pit to the anterior surface of the retina, and contract to shape the embryonic eye (Chauhan et al., 2009; Mann, 1964; McAvoy, 1980). The Rho family of GTPases (RhoA, B and C; Rac 1, 2 and 3; Cdc42) is central to actin cytoskeleton regulation in epithelial morphogenesis, including cell adhesion, migration, filopodia extension, and apical domain contractions (Bishop and Hall, 2000; Heasman and Ridley, 2008; Linseman and Loucks, 2008; Nobes and Hall, 1994; Nobes and Hall, 1999; Villalonga and Ridley, 2006). Changes in Cdc42 cause a loss of actin-rich filopodia that leads to an abnormally shaped optic cup and lens pit, suggesting that F-actin filopodia transmit force to fine-tune embryonic eye morphogenesis (Chauhan et al., 2009). Three-dimensional culture of *Rx*+ (retinal homeobox gene) embryonic stem cells stimulates optic cup formation *in vitro*, and treatment of cultures with the myosin II ATPase inhibitor, blebbistatin, or the Rho-kinase (ROCK) inhibitor, Y-27632, blocks invagination of the optic cup (Eiraku et al., 2011). Lens pit filopodia also contain activated myosin II with phosphorylated myosin light chain (pMLC), and blebbistatin treatment to inhibit myosin II ATPase activity results in lengthening of these filopodia. In contrast, calyculin A treatment (inhibitor of myosin phosphatase, which leads to increased pMLC, thereby activating myosin II ATPase) results in shorter filopodia (Chauhan et al., 2009).

As the lens placode invaginates to form the lens vesicle, epithelial cells undergo apical constriction via contraction of an actomyosin network (Lang et al., 2014; Quintin et al., 2008), regulated by MLC phosphorylation and activation of myosin IIB at the apical surfaces of lens epithelial cells (Lang et al., 2014). Inhibition of myosin, actin dynamics or RhoA through specific inhibitors disrupts lens invagination (Borges et al., 2011; Plageman et al., 2011). Shroom3, a Rock1/2 and actin-binding cytoskeletal protein, is required for apical constriction of the lens placode, and loss of Shroom3 leads to reduced F-actin and myosin IIB at the apical surface of the lens placode (Plageman et al., 2010). Shroom3 likely recruits F-actin via the Mena-family of actin modulators that are known to play a role in apical constriction (Plageman et al., 2010; Roffers-Agarwal et al., 2008). Similar to the effects of Shroom3, loss of p120-catenin, a cadherin-binding protein that activates Rho GTPases to modulate actin dynamics (Pieters et al., 2012), also causes defects in lens invagination and leads to mislocalization of myosin IIB away from the apical surface (Lang et al., 2014). A

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new study shows that Cdc42 inhibits Shroom3-induced cell contraction in the lens placode to allow cell elongation, suggesting that a balance between these opposing forces on the actin cytoskeleton is needed for normal orientation and planar cell polarity in the lens placode (Muccioli et al., 2016). These studies indicate that contractility of the actomyosin network is important for early eye and lens formation.

In the lens vesicle, apical surfaces of epithelial cells are directed toward the lumen, and consequently, components of the basal lamina secreted by these cells surround the external surface of the vesicle, eventually creating a thin collageneous basement membrane (lens capsule) to encase the lens (Lovicu and Robinson, 2004). Posterior epithelial cells elongate at their apical ends into the vesicle lumen and differentiate into primary lens fiber cells to fill the bulk of the embryonic lens (Lovicu and Robinson, 2004). Rap1, a Ras-like small GTPase required for Cdc42 activation during cell-cell junction formation (Hogan et al., 2004), is needed for elongation of primary lens fibers and adhesion of fiber cell tips to the apical surface of the anterior epithelium (Maddala et al., 2015a). Rap1 also regulates E-cadherin at cell-cell junctions (Hogan et al., 2004), and E-cadherin junctions recruit cortactin and Arp2/3 to initiate F-actin assembly (Ehrlich et al., 2002; Helwani et al., 2004; Kovacs et al., 2002; Vasioukhin et al., 2000). Loss of Rap1 in the lens results in decreased F-actin and disrupts E-cadherin and β-catenin staining at the epithelial-fiber apical junction, leading to gaps between the apical surfaces of anterior epithelial cells and tips of elongating primary fiber cells (Maddala et al., 2015a). Transgenic lens expression of a Rho GTPase specific inhibitor C3-exoenzyme leads to small and very disrupted embryonic lenses and swollen fiber cells with decreased F-actin, adherens junctions and gap junctions (Maddala et al., 2004; Rao et al., 2002). Similarly, inhibition of Cdc42 activation due to transgenic lens expression of RLIP76/RALBP1, an effector of two Ras family GTPases, Ral and Rho/Rac (Jullien-Flores et al., 1995; Park and Weinberg, 1995), causes abnormal F-actin networks, small lenses and microphthalmia (Sahu et al., 2014).

## 2. Functions of actin in lens epithelial and fiber cell morphogenesis

### 2.1 Epithelial cells

The anterior hemisphere of the late embryonic and adult lens is covered by a monolayer of epithelial cells. Epithelial cells near the anterior pole are cuboidal in shape and quiescent. Basal surfaces of these epithelial cells contain actin stress fibers (Liou and Rafferty, 1988; Weber and Menko, 2006) (Figure 1G) and F-actin-rich lamellipodia (Weber and Menko, 2006) that are likely linked to the lens capsule through focal adhesions and integrin receptors (Schoenwaelder and Burridge, 1999). On apical and lateral surfaces, anterior epithelial cells (Bassnett, 2005) with adherens junctions (Zampighi et al., 2000) with adherens junctions (Weber and Menko, 2006) (Figure 1F). On their basal surfaces, actin stress fibers and lamellipodia likely aid the spreading of anterior epithelial cells on the lens capsule and may help to maintain the undifferentiated state as in other cell types (McBeath et al., 2004; Woods et al., 2005), while apical cortical actin stress fibers help stabilize the cuboidal cell shape (Weber and Menko, 2006). In addition to these typical actin structures, the apical domains of anterior epithelial cells also contain unusual polygonal arrays of F-actin associated with myosin II and sequestered actin bundles (SABs) (Rafferty, 1985; Rafferty

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and Scholz, 1984, 1985, 1989; Rafferty et al., 1990; Scholz and Rafferty, 1988). Polygonal arrays of F-actin have been found in mouse, rabbit, squirrel, monkey and human lens epithelial cells (Rafferty and Scholz, 1985, 1989; Rafferty et al., 1990; Scholz and Rafferty, 1988; Yeh et al., 1986). These geodesic domes of actin and myosin filaments are proposed to flatten in order to maintain epithelial cell integrity during lens accommodation, but this has not been experimentally tested (Rafferty and Scholz, 1985; Yeh et al., 1986). SABs (Figure 1E and 1F) are composed of a single, elongated bundle of F-actin that is attached to the apical plasma membrane at one end and interacts with a perinuclear network of intermediate filaments at the other end (Rafferty and Scholz, 1985). In mice, the presence and size of SABs are strain- and age-dependent (Liou and Rafferty, 1988; Rafferty and Scholz, 1989). The function of SABs in maintaining lens epithelial cell shapes or behaviors is unclear.

Life-long lens growth depends on the proliferation and differentiation of equatorial epithelial cells into secondary fiber cells (Lovicu and Robinson, 2004). Epithelial cells in the anterior-most region of the lens equator disassemble their F-actin stress fibers and only have dispersed F-actin on their basal surfaces, retaining cortical actin fibers at their apical surfaces (Weber and Menko, 2006). As equatorial epithelial cells proliferate and start to differentiate, they increase in height in the apical-basal dimension but decrease in diameter, thus maintaining their volume (Bassnett, 2005). Concomitantly, dispersed F-actin on their basal surfaces collects into bundles that radiate from the center of the cell toward the cell periphery, along with some cortical actin fibers (Weber and Menko, 2006). In the posterior-most region of the lens equator, epithelial cells undergo a remarkable morphogenesis to change from randomly packed cells into hexagonally packed cells arranged into organized meridional rows (Figure 1L) (Bassnett et al., 1999; Cheng et al., 2013; Wu et al., 2015). Cortical F-actin fibers appear on lateral membranes of these cells (Weber and Menko, 2006) and are likely important for stabilizing N-cadherin junctions between these differentiating cells (Ferreira-Cornwell et al., 2000; Leong et al., 2000). F-actin is also enriched at the vertices (tricellular junctions) of these hexagonal cells near their basal-lateral interface and at their apical tips (Cheng et al., 2013) to form the lens fulcrum (Figure 1H–J). The lens fulcrum (Sugiyama et al., 2009) or modiolus forms an anchor point where tips of many differentiating fiber cells are located as fiber cells rotate in orientation (Zampighi et al., 2000). Bidirectional signaling through EphA2 activates Src and cortactin, leading to the proper localization and accumulation of actin that is essential for hexagonal cell shape, organized packing of meridional rows and the formation of the lens fulcrum (Cheng et al., 2013) (Figure 1L).

### 2.2 Newly differentiating and elongating secondary fiber cells

Coordinated elongation and migration of differentiating fiber cells is a beautiful example of collective cell migration in tissues (Montell, 2008; Pocha and Montell, 2014). At the lens equator, newly formed fiber cells are hexagonally shaped in cross-section (Figure 2). Each fiber cell has 4 short sides and 2 broad sides and is contacted by 6 neighboring cells one at each of the 4 short sides, and at each of the 2 broad sides (1 cell from a more superficial layer and 1 cell from a deeper layer), so that each fiber cell is surrounded by 6 nearest neighbors, connected along their lateral membranes and by 6 tricellular junctions. This arrangement leads to the addition of new layers, or shells, of fiber cells (Kuszak, 1995;

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Kuszak et al., 1991; Kuszak et al., 2004a) on the lens periphery that surrounds previous generations of fiber cells (Figure 2D). Secondary fiber cells maintain hexagonal packing during elongation, as anterior tips of these cells migrate along the apical surfaces of epithelial cells, while posterior tips crawl along the lens capsule towards the anterior and posterior lens poles, respectively (Lovicu and Robinson, 2004). The tips of elongating fiber cells detach from the anterior epithelium or the posterior capsule at the poles and contact the tips of opposing fiber cells from the other side of the lens to form Y-sutures (Kuszak et al., 2004b) (Figure 1K). Differentiation of newly formed secondary lens fiber cells is accompanied by an increase in ratio of polymerized F-actin vs. monomeric globular G-actin (Ramaekers et al., 1981). Thus, newly formed fiber cells have an extensive cortical F-actin network along the entire lengths of their lateral membranes, and F-actin is enriched at the tricellular junctions (vertices) of hexagonally packed young fiber cells on their lateral surfaces (Leonard et al., 2011; Nowak et al., 2009). Treatment of primary cultures of chick lens epithelial cells with cytochalasin-D causes epithelial cells to disassemble F-actin stress fibers, initiate fiber cell differentiation and assemble cortical F-actin bundles (Weber and Menko, 2006), indicating that F-actin network rearrangement is necessary to drive fiber cell elongation and differentiation (Beebe and Cerrelli, 1989; Mousa and Trevithick, 1977; Weber and Menko, 2006).

F-actin bundles are important for stabilizing fiber cells during elongation and migration (Fischer et al., 2000; Lee et al., 2000). At the posterior tips of differentiating secondary fibers, F-actin forms a dense mesh at the basal surface (Al-Ghoul et al., 2003; Weber and Menko, 2006) (Figure 1M), and N-cadherin/F-actin complexes are enriched at the vertices of hexagonal fiber cells, with myosin II and caldesmon, a contractile regulatory protein, located at the center connected to the N-cadherin/F-actin complexes on the lateral membranes (Bassnett et al., 1999). These F-actin bundles are aligned from one cell to the next, connected via N-cadherin cell-cell junctions, with paxillin, myosin light chain kinase (MLCK) and focal adhesion kinase also detected in these membrane complexes (Bassnett et al., 1999). Basal F-actin bundles at posterior fiber cell tips are thought to aid in the collective and coordinated migration of fiber cells along the lens capsule (Al-Ghoul et al., 2003; Bassnett et al., 1999; Weber and Menko, 2006). A recent study in zebrafish indicates fibronectin1 is localized in small puncta at the apical-apical junctions between epithelial cells and elongating tips of secondary lens fiber cells, as well as in posterior portions of secondary fiber cells (Hayes et al., 2012). Loss of fibronectin1 in zebrafish lenses leads to abnormal F-actin distribution, disorganization of secondary lens fibers and disrupted Y-sutures (Hayes et al., 2012), suggesting that integrin signaling via focal contacts leads to altered F-actin organization and impaired fiber cell migration.

As fiber cells differentiate, the discontinuous and irregular spectrin-actin network becomes smooth and continuous along fiber cell membranes (Figure 2E–2I), likely due to tropomodulin 1 (Tmod1) stabilization of F-actin after its assembly (Lee et al., 2000; Nowak et al., 2009; Nowak and Fowler, 2012). Tmod1, an actin pointed end capping protein first identified in human erythrocytes (Fowler, 1987; Moyer et al., 2010), is also required for maintaining the hexagonal geometry and packing of differentiating lens fiber cells in the mouse lens (Gokhin et al., 2012; Nowak and Fowler, 2012). The spectrin-actin network, also known as the membrane skeleton, and Tmod1 are associated in a macromolecular complex

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with CP49 and filensin, which form beaded intermediate filaments (Fischer et al., 2003b; Gokhin et al., 2012). Loss of either Tmod1 or CP49 causes decreased lens mechanical stiffness, presumably due to disruption of the spectrin-actin or beaded intermediate filament networks, respectively (Gokhin et al., 2012; Nowak et al., 2009). Unexpectedly, the membrane skeleton and beaded intermediate filaments synergize to promote the formation of large micron-size gap junction plaques in differentiating fiber cells that are crucial for normal lens ion and fluid homeostasis (Cheng et al., 2015). Gap junction plaques rest in lacunae within the spectrin-actin network along fiber cell membranes, suggesting that the membrane skeleton plays a role in accretion or stability of large gap junction plaques in the lens (Cheng et al., 2015). It remains unclear whether the membrane skeleton interacts directly with gap junction plaques, or if gap junction plaque stability/assembly is affected indirectly by membrane skeleton/beaded filament network interactions through a linker protein (Fuchs and Yang, 1999; Wiche et al., 2015; Wiche and Winter, 2011).

The entire spectrin-actin network is proposed to be tethered to the plasma membrane through spectrin interactions with ankyrin-B (Bennett and Baines, 2001; Bennett and Stenbuck, 1979, 1980; Luna and Hitt, 1992; More et al., 2001) as well as by ezrin-periplakin-periaxin-desmoyokin (EPPD)-actin anchorage complexes (Maddala et al., 2011b; Maddala et al., 2015b; Straub et al., 2003). Ankyrin-B links the membrane skeleton to NrCAM (More et al., 2001) and possibly N-cadherin, as shown for E-cadherin in other epithelial cell types (Kizhatil et al., 2007). The loss of NrCAM or ankyrin-B in the lens causes cataracts and disrupts the actin cytoskeleton (More et al., 2001), and a recent study shows that haploinsufficiency of ankyrin-B leads to a disrupted spectrin-actin network, abnormal lens fiber cell shape and decreased lens stiffness (Maddala et al., 2015b). Interestingly, ankyrin-B disruption in the lens also affects the levels of perixain. Similarly, in periaxin knockout lenses, there is a dramatic decrease in actin, spectrin and ankyrin-B protein levels and staining signals at the fiber cell membrane (Maddala et al., 2011b; Maddala et al., 2015b). In periaxin and ankyrin-B mutant lenses, packing and shape of fiber cells appears normal at 3 weeks of age, and dramatic defects in cell shape occur with age, indicating that EPPD complexes and ankyrin-B are not needed for the initial packing of fiber cells, but are required for maintaining fiber cell shape, perhaps by helping to stabilize the membrane skeleton (Maddala et al., 2015b).

Cadherin-based adherens junctions interact with the lens actin cytoskeleton to maintain epithelial cell morphology and polarity and to influence fiber cell differentiation (Leonard et al., 2011; Pontoriero et al., 2009). F-actin and adherens junctions exist in complexes between epithelial-epithelial, epithelial-fiber and fiber-fiber cell membrane contacts (Lo, 1988). Lens epithelial cells mainly express E-cadherin, while fiber cells utilize N-cadherin (Xu et al., 2002), and these cadherin junctions are associated with the actin cytoskeleton via the β-catenin linker (Cain et al., 2008; Leonard et al., 2011; Straub et al., 2003). N-cadherin becomes increasingly associated with cytoskeletal proteins during lens development (Leong et al., 2000), and the formation of N-cadherin junctions in epithelial cells undergoing differentiation into lens fiber cells triggers changes in the actin cytoskeleton (Ferreira-Cornwell et al., 2000). Dlg-1, a homolog of *Drosophila* tumor suppressor *discs-large* (*dlg*), colocalizes with N-cadherin and E-cadherin in mouse lens epithelial and fiber cells (Nguyen et al., 2005). Similar to lens N-cadherin conditional knockouts (Pontoriero et al., 2009), loss

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of Dlg-1, a PDZ (PSD-95-Dlg-ZO-1) domain protein, in lenses leads to vacuole formation and abnormal orientation of secondary fiber cells (Rivera et al., 2009). Dlg-1 knockout lens fibers display disrupted F-actin and N-cadherin staining along with decreased levels of α-catenin, a linker between cadherins and the actin cytoskeleton (Rivera et al., 2009). This study suggests that Dlg-1 is needed for normal adherens junctions and actin cytoskeleton organization in the lens.

Cortactin binds to and regulates assembly of F-actin by activating the actin nucleator Arp2/3 (Higgs and Pollard, 2001; Pollard et al., 2000; Ren et al., 2009; Uruno et al., 2001; Weaver et al., 2001). Both Arp3 and cortactin are recruited to N-cadherin junctions as fiber cells begin to undergo differentiation and are enriched at the vertices of these hexagonal cells. Arp3 only co-immunoprecipitates with N-cadherin and cortactin in lens fibers but not epithelial cells, suggesting that Arp3 plays a role in fiber cell differentiation to remodel cell-cell adhesions (Leonard et al., 2011). Experiments with an N-cadherin function-blocking antibody in embryonic chick lens explants shows that N-cadherin-directed F-actin assembly is required for fiber cell elongation (Leonard et al., 2011).

Rho GTPase family members regulate both cadherin junctions and F-actin assembly (Fukata et al., 1999; Nagafuchi et al., 1994). Activated Rac GTPase stimulates the c-abl-interactor (Abi) family of adaptor proteins that interact with c-abl kinase to regulate F-actin assembly and dynamics via WAVE-mediated Arp2/3 activation (Leng et al., 2005; Miki and Takenawa, 2003; Stradal et al., 2004; Stuart et al., 2006). Loss of either Rac1 or Abi-2 causes secondary fiber cell migration and orientation defects in neonatal lenses (Grove et al., 2004; Maddala et al., 2011a), demonstrating the importance of F-actin polymerization and organization during lens fiber cell migration and elongation. Abi may function as a link between adherens junctions and actin polymerization by interacting with both diaphanous (Dia)-related formins, which act as F-actin nucleators, and β-catenin, which regulate cadherin-based actin networks (Ryu et al., 2009). Interestingly, the overexpression of secreted frizzled-related protein 2 (Sfrp2), a Wnt signaling antagonist, also leads to secondary fiber cell orientation defects very similar to Rac1 or Abi-2 knockout lenses (Chen et al., 2008). There is a decrease in Rac1 staining in the cortex of transgenic Sfrp2 lenses along with a loss of RhoA and Cdc42 enrichment at fiber cell tips (Chen et al., 2008). Decreased protein levels of Rho GTPases in these Sfrp2 transgenic lenses provides evidence that Wnt/planar cell polarity pathways are important for F-actin cytoskeletal organization during fiber cell differentiation (Chen et al., 2008).

### 2.3 Mature secondary fiber cells

As secondary fiber cells undergo differentiation and maturation, domains of interlocking membrane protrusions with various conformations (balls-and-sockets, protrusions, paddles and square arrays) form between neighboring fiber cells along the broad and short sides (Dickson and Crock, 1972; Kistler et al., 1986; Kuszak et al., 1980; Kuwabara, 1975; Lo and Harding, 1984; Willekens and Vrensen, 1981, 1982, 1985). Along broad sides of cortical differentiating fiber cells, ball-and-socket membrane interdigitations consist of a small protrusion on one cell precisely fitted into the socket indentation of the neighboring cell, and these processes increase in size as fiber cells mature (Harding et al., 1976; Kuszak et al.,

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1980; Lo and Reese, 1993; Willekens and Vrensen, 1981, 1982, 1985). In addition to balls-and-sockets along broad sides, cortical fiber cells also develop small interlocking protrusions at their vertices (tricellular junctions) adjacent to the short sides (Kistler et al., 1986; Kuszak et al., 1980; Kuwabara, 1975; Leeson, 1971; Lo et al., 2014; Zhou and Lo, 2003). Actin is highly enriched in small interlocking protrusions at these vertices (Lo et al., 1997; Zhou and Lo, 2003), but its role has not been well studied in balls-and-socket protrusions on the broad sides.

Early stages in small protrusion formation at the vertices are believed to involve plasma membrane invagination similar to clathrin-AP2-mediated endocytic pit formation (Kirchhausen, 1999, 2000; Kirchhausen et al., 1986; Pearse et al., 2000; Smith and Pearse, 1999). Indeed, clathrin and AP2 coat the cytoplasmic surface of fiber cell membrane indentations, and F-actin is observed on the cytoplasmic surface of the neighboring fiber cell membrane inside each protrusion (Zhou and Lo, 2003). It has been hypothesized that Arp2/3 may drive branching of F-actin in protrusions to generate a pushing force that would be coordinated with the pulling force generated by clathrin complexes along the concave membrane surface enveloping protrusions from the adjacent cell. The branched F-actin network would then presumably stabilize the interlocking protrusion/invagination structure (Zhou and Lo, 2003). Loss of Tmod1 and CP49 leads to a reduction in small protrusions at the vertices of mature fiber cells (Nowak et al., 2009), suggesting that both actin and intermediate filaments are needed for normal formation or stabilization of these interdigitations. Recent studies have localized aquaporin-0 and N-cadherin to small protrusions in mature fiber cells (Biswas et al., 2015; Lo et al., 2014), suggesting that these membrane proteins may be required for normal formation of protrusions at fiber cell vertices. However, the pathways and mechanisms that regulate the formation of these complex fiber cell interdigitations remain unclear.

As fiber cells continue to mature, they undergo complex degradative processes to eliminate all intracellular organelles, including nuclei, to remove light-scattering structures from the light path (Bassnett, 1992, 1995; Bassnett and Beebe, 1992; Bassnett and Mataic, 1997). Along with the removal of cellular organelles, there is degradation and/or proteolytic trimming of many cytoskeletal proteins, including microtubules, vimentin intermediate filaments and beaded intermediate filaments (Blankenship et al., 2001; Bradley et al., 1979; FitzGerald, 2009; Kuwabara, 1968; Oka et al., 2008; Rafferty, 1985; Sandilands et al., 1995). While α2- and β2-spectrin are cleaved by calpains and caspases into two large fragments (De Maria et al., 2009; Lee et al., 2000; Lee et al., 2001; Nowak et al., 2009), F-actin and Tmods (Tmod1 in mouse lenses and Tmod4 in chicken lenses) are not proteolysed and remain associated with the plasma membranes of mature fiber cells after denucleation and organelle loss (Lee et al., 2000; Lee et al., 2001; Nowak et al., 2009). These data suggest that the F-actin network likely plays a role in stabilizing and maintaining membrane integrity of mature fiber cells.

Maintenance of the actin cytoskeleton in mature fiber cells may rely on appropriate interactions with lens crystallin proteins. Crystallins comprise 90% of total lens proteins and are classified as α-, β- and γ-crystallins (Bloemendal et al., 2004). Alpha-crystallins, consisting of heteromers with αA- and αB-crystallin subunits, belong to the small heat

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shock protein family and are hypothesized to help maintain life-long lens transparency by sequestering denatured proteins and thereby preventing abnormal protein aggregation (Horwitz, 1992). Alpha-crystallins are reported to be associated with actin in lens lysates (Bloemendal et al., 1984; Del Vecchio et al., 1984; Gopalakrishnan and Takemoto, 1992; Kibbelaar et al., 1979), and mutations in αA-crystallin alter its association with actin (Andley et al., 2014; Brown et al., 2007), suggesting that actin may be a native substrate of α-crystallins. Beta- and γ-crystallins are members of the β/γ superfamily of proteins and function as structural proteins in the lens (Bloemendal et al., 2004). Immunoprecipitation experiments reveal a possible link between β- and γ-crystallins and major cytoskeletal proteins in the lens, including actin (Rao et al., 2008). Gamma-crystallins may play a role in stabilizing F-actin in mature lens fiber cells. In mature fiber cells, the γB-S11R mutation leads to reduced F-actin staining (Li et al., 2008) while loss of γS-crystallin in mouse lenses leads to F-actin depletion and aggregation (Fan et al., 2012). *In vitro* experiments suggest that γS-crystallins stabilize F-actin and protect filaments against depolymerization (Fan et al., 2012).

## 3. Approaches and future directions for studying actin in lens cells

### 3.1 Actin cytoskeleton networks and actin-associated proteins in the lens

While it is clear that actin plays important and diverse roles during lens development and in maintaining lens integrity, there remain a host of unknowns about actin organization in the lens. In other systems, such as striated muscle, precise locations of actin filament ends and associated cross-linking and binding proteins have been documented in detail (Clark et al., 2002; Ono, 2010). We can use similar techniques to reveal the structural organization of the actin cytoskeleton in the lens. Comparing the localization of barbed-end capping proteins, such as adducin (Kaiser et al., 1989; Matsuoka et al., 2000), CapZ (dos Remedios et al., 2003) or gelsolin (Andley et al., 2014; Nag et al., 2013), vs. the pointed-end capping protein Tmod1 (Nowak et al., 2009; Woo and Fowler, 1994) could help establish locations of barbed and pointed ends of actin filaments, respectively, although this may require super-resolution microscopy approaches if filaments are short and/or arranged in irregular orientations (see below).

The locations of actin filament side-binding proteins can also shed light on the organization of actin cytoskeletal networks. Alpha-actinin is a crosslinking protein for anti-parallel actin filaments that interact with myosin II bipolar filaments in loose bundles to generate contractile force (FitzGerald and Casselman, 1990; Lo et al., 1997; Sjoblom et al., 2008). F-actin bundles containing α-actinin are found in cortical lens fiber cells and are commonly localized near the vertices on the short sides of hexagonal fiber cells, perhaps serving to stabilize the hexagonal cell shape (Lo et al., 1997). The functions of these bundles, or whether other actin-associated proteins are associated with bundled or non-bundled F-actin in fiber cells, remain to be studied. For example, the presence of other actin-bundling proteins, such fascin (Jayo and Parsons, 2010) and fimbrin (aka plastin) (Delanote et al., 2005), would indicate bundles of filaments aligned in parallel orientations, while the presence of filamin (Hirata et al., 2014), would indicate looser networks of isotropically

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oriented filaments, each with distinct structural, mechanical and cellular functions (Matsudaira, 1994; Ridley, 2011; Tseng et al., 2005).

Locations of actin-nucleating proteins, such as Arp2/3 or formins, or depolymerizing proteins, such as ADF/cofilin, could indicate F-actin networks undergoing active assembly/disassembly and remodeling in specific cells or subcellular regions (Carlier et al., 2015; Ono, 2007; Pollard, 2007; Pollard and Cooper, 2009). Current data suggest that dynamic branched F-actin networks nucleated by Arp2/3, and α-actinin-stabilized anti-parallel actin filament bundles are both present at or near the vertices of fiber cells (Leonard et al., 2011; Lo et al., 1997), implying the complexity of F-actin organization and function at these fiber cell domains. While formins have been detected in developing lens tissue, and loss of formins may lead to fiber cell degeneration (de la Pompa et al., 1995; Woychik et al., 1990), it remains unclear which isoforms of this large and diverse family are required for establishing and maintaining the lens.

Another important and relatively poorly understood area is how F-actin networks are linked to plasma membranes of lens cells to control their complex topologies and functional domains. Ezrin-radixin-moesin (ERM) proteins, which link actin filament networks to the plasma membrane (Bretscher et al., 2000) and interact with N-WASP, an activator of Arp2/3-nucleated actin assembly (Manchanda et al., 2005), are present in the lens (Bagchi et al., 2004; Ingraffea et al., 2002; Rao et al., 2008; Straub et al., 2003). Immunoprecipitation experiments (Straub et al., 2003) indicate that plectin may serve as an important linker between ERM proteins, intermediate filament (Wiche et al., 2015; Wiche and Winter, 2011) and actin filament networks (Andra et al., 1998; Fontao et al., 2001; Jiu et al., 2015) in the lens. A recent study indicates that polymorphisms and genetic variations in ezrin are linked to human age-related cataracts (Lin et al., 2013), but the mechanism for these opacities and any links to the actin cytoskeleton will require further study. In addition, more work is needed to understand the subcellular localization and functions of other components of the spectrin-based membrane skeleton in the lens, such as tropomyosin (Nowak et al., 2009; Woo et al., 2000), band 3 (Allen et al., 1987), band 4.1 (Aster et al., 1986; Aster et al., 1984; Bagchi et al., 2004; Beebe et al., 2001), band 4.2 (Sung and Lo, 1997) and band 4.9 (Faquin et al., 1988).

Super-resolution microscopy techniques, such as structured illumination microscopy (SIM) (Brown et al., 2011; Gao et al., 2012; Gustafsson, 2000) and stimulated emission depletion (STED) microscopy (Chereau et al., 2015; Hell and Wichmann, 1994), could reveal more precise localization of F-actin and actin-binding proteins to determine whether multiple types of actin networks overlap at fiber cell vertices and/or along cell broad and narrow sides, or form distinct subdomains. In addition, recent advances in correlative light and electron microscopy (CLEM) can couple information about protein localization obtained using fluorescence microscopy with highly detailed ultrastructural electron microscopy information (de Boer et al., 2015) to revolutionize the study of F-actin and other proteins required to form the complex membrane geometry of fiber cell interdigitations, and to coordinate diverse F-actin structures at the interfaces between migrating fiber cell tips and the apical domains of lens epithelial cells.

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### 3.2 F-actin dynamics and stability

In most cells (Pollard et al., 2000; Pollard and Borisy, 2003), including lens cells, a large fraction of actin remains unassembled and associated with monomer-sequestering proteins, but is available for filament assembly upon appropriate signals, to coordinate cell migration, adhesion and other morphogenesis events (Ireland et al., 1983; Ramaekers et al., 1981). While previous studies have demonstrated that G- to F-actin assembly driven by signaling through the Rho family of proteins (Sections 1 and 2.2) is needed for lens development and fiber cell elongation, it remains unclear whether the ratio of G-actin to F-actin is important to maintain adult lens homeostasis and integrity. By comparing relative proportions of cytosolic G-actin vs. membrane-bound F-actin in subcellular fractions of lens lysates (Nowak et al., 2009; Woo et al., 2000), or evaluating the G/F-actin ratio in lysates using DNaseI (Blikstad et al., 1978; Kibbelaar et al., 1979; Ramaekers et al., 1981), we can investigate whether genetic mutations or lens aging affects global actin polymerization or filament stability. In addition, comparison of relative fluorescence intensity levels of phalloidin vs. DNase I staining can be used to reveal the F- to G-actin ratio (i.e., relative extent of F-actin polymerization) (Fischer et al., 2003a) in specific layers of lens fiber cells (Fan et al., 2012). Here we note that actin has often been used as a loading control for Western blots and as a membrane marker for lens sections. However, as we have illustrated in this review, many proteins directly interact with or indirectly affect the actin cytoskeleton. Therefore, using actin as a control is likely not appropriate for all studies. We suggest the use of Ponceau S staining for total proteins as a loading control for Western blots (Gilda and Gomes, 2013), while fluorescently labeled wheat germ agglutinin is a bright and easy-to-use membrane marker that outlines lens epithelial and fiber cells in fluorescent microscope images (Bond et al., 1996).

Another powerful approach to elucidate F-actin network assembly, stability and functions in the lens is the use of actin- or myosin-disrupting drugs. For example, disruption of myosin II binding to F-actin via blebbistatin inhibition of myosin ATPase (Kovacs et al., 2004), or via ML-7 inhibition of MLCK activity, leads to a decrease in whole lens stiffness (Won et al., 2015) and changes in focal length in chick lenses (Luck and Choh, 2011), and causes nuclear cataracts in mouse lenses due to changes in fiber cell morphology (Maddala et al., 2007). Treatment of chick lenses with latrunculin A, which binds and sequesters G-actin, preventing actin polymerization (Coue et al., 1987; Morton et al., 2000; Spector et al., 1989), also leads to a decrease in lens stiffness (Won et al., 2015), suggesting that a dynamic F-actin network is important for maintaining lens mechanical integrity. In addition to actin- and myosin-disrupting agents, there are drugs that stabilize F-actin, such as jaspaklinolide (Allingham et al., 2006; Bubb et al., 1994; Bubb et al., 2000; Holzinger, 2009), or specifically inhibit Arp2/3, formins or Rho GTPases, that will provide useful information about the functions of lens actin networks (Blanchoin and Boujemaa-Paterski, 2009; Hetrick et al., 2013; Nolen et al., 2009; Rizvi et al., 2009; Shang et al., 2013; Shang et al., 2012). While treatment of whole lenses can indicate global functions for F-actin assembly pathways and structural networks, information about F-actin in the lens with respect to regional cellular specializations and functions will require careful microscopic analyses of epithelial and fiber cells after drug treatments.

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The availability of transgenic mice with GFP-tagged cytoskeletal proteins offers yet another approach to studying actin dynamics regulation in the lens. By coupling high-resolution confocal or multi-photon fluorescence microscopy and transgenic mice expressing GFP-tagged actin (Gurniak and Witke, 2007; Narayanan et al., 2015), or GFP- or mRFPruby-tagged Lifeact, a F-actin binding peptide (Riedl et al., 2010), or other fluorescently-tagged F-actin-binding proteins (e.g., moesin or paxillin) (Abe et al., 2011), it would be possible to study how the actin cytoskeleton is remodeled during the epithelial-to-fiber-cell transition and during fiber cell elongation, migration and maturation in live lenses. Recent advances in the CRISPR-Cas9 technology (Sternberg and Doudna, 2015) will also greatly increase the number of knockout, transgenic and mutant animal models that can be applied to the study of the lens actin cytoskeleton.

## Concluding remarks

While it is clear that F-actin plays important roles during lens development, many questions remain about the mechanisms that maintain actin cytoskeletal structures in quiescent anterior epithelial cells and those that drive F-actin remodeling as equatorial epithelial cells proliferate, differentiate and mature into fiber cells. Many components of F-actin networks have been detected in the lens, but the functions of most of these proteins in establishing or maintaining lens cellular architecture and functions remain unknown. Further studies are also needed to identify additional proteins that directly interact with the actin cytoskeleton to regulate dynamic G-to-F-actin transitions or network rearrangements during lens fiber cell differentiation, and during lens development and aging. Fiber cells at the center of the lens are formed during embryogenesis, and these long-lived cells must maintain homeostasis and transparency throughout the lifetime of an organism. Little is known about the role of the actin cytoskeleton in maintaining the life-long integrity of mature lens fiber cells, and whether changes in the actin cytoskeleton or the membrane skeleton affect age-related pathologies, including cataracts and presbyopia. New methods, including super-resolution and live-cell microscopy, transgenic and knockout mouse models and novel actin-disrupting agents, present opportunities to study and understand actin cytoskeletal regulation and dynamics in live lenses.

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## Highlights

- F-actin plays important roles during lens development and maintains fiber cells.
- Dynamic regulation of actin is needed for normal development and fiber elongation.
- Actin filament stability and reorganization are essential for fiber cell packing.
- Disruptions of actomyosin function affect lens biomechanical properties.

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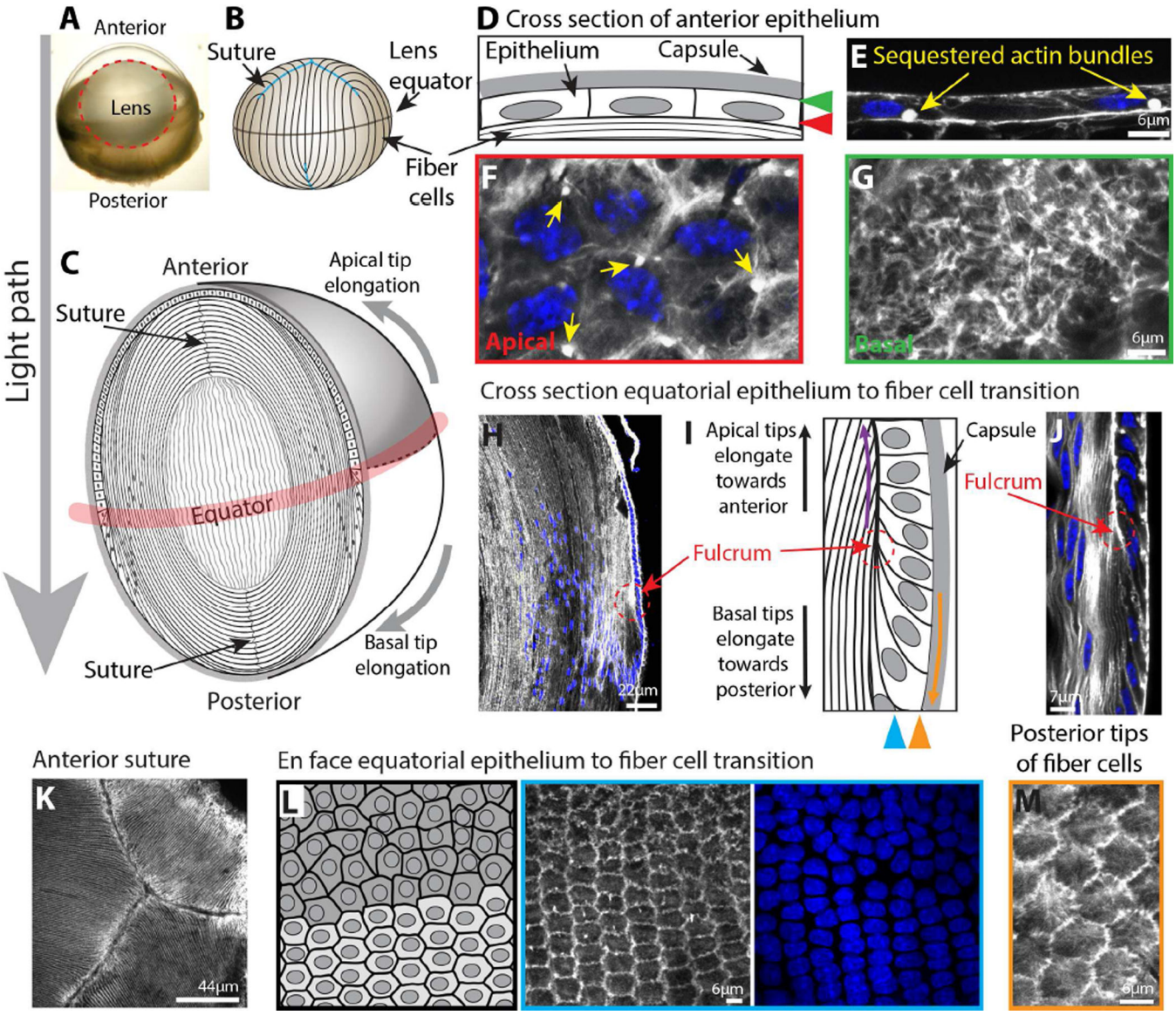


**Figure 1. Actin cytoskeletal organization in the lens**

A) Dissected mouse eye; red dotted circle outlines lens in the intact eye. B) Diagram of mouse lens (not drawn to scale). The lens equator is demarcated by black horizontal line, and the sutures are indicated by blue lines. C) Diagram of lens anatomy showing anterior epithelial cells and bulk elongated fibers. Secondary fiber cells elongate toward the anterior and posterior poles (gray arrows) and meet at the anterior and posterior lens sutures. Lens equator indicated by red shading. D) Diagram of cuboidal anterior epithelial cells. Red arrowhead indicates apical surface shown in F (*en face* view), and green arrowhead indicates basal surface shown in G (*en face* view). E) Phalloidin (F-actin) and Hoechst (nuclei, blue) staining of anterior epithelial cells in cross section. F-actin is abundant at apical and basal surfaces of these cells and forms sequestered actin bundles (yellow arrows) near the apical surface. F and G) Phalloidin and Hoechst staining of flat-mounted anterior epithelial cells *en face*. On their apical surfaces, anterior lens epithelial cells have cortical actin fibers and

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sequestered actin bundles (F, yellow arrows). In contrast, on the basal surfaces of these cells, abundant actin stress fibers are observed (G). H) Low-magnification sagittal section of a mouse lens stained with phalloidin and Hoechst showing lens epithelial and fiber cell morphology near the lens equator. The lens fulcrum (red dotted circle and arrow) forms an anchorage point for the tips of many differentiating fiber cells as fiber cells rotate in orientation. I) Diagram of equatorial epithelial-to-fiber cell transition. Red arrows and circle indicate the lens fulcrum. Purple arrow pointing up and orange arrow pointing down show the directions of apical and basal tip migration, respectively, of elongating secondary fiber cells. Blue and orange triangles indicate *en face* focal plane in L and M, respectively. J) Phalloidin and Hoechst staining of the lens fulcrum (red dotted circle and arrow) showing elongation of newly formed secondary fiber cells. F-actin is enriched at the lens fulcrum and the apical junction between epithelial and secondary fiber cells. K) Phalloidin staining of the anterior suture of a mouse lens where tips of elongating fiber cells meet at the pole (*en face* view). F-actin is enriched at fiber cell tips. L) Diagram, and phalloidin and Hoechst staining of an *en face* view of equatorial epithelial cells. Equatorial epithelial cells rearrange from randomly packed cells into organized meridional rows of hexagonal cells. F-actin is disorganized in randomly packed equatorial epithelial cells and becomes localized to the cell membrane in hexagonal meridional cells. M) *En face* view of newly formed, phalloidin-stained secondary fiber cell posterior tips (basal-lateral side) on the capsule showing the perpendicular organization of the actin stress fibers with respect to the cell boundary.

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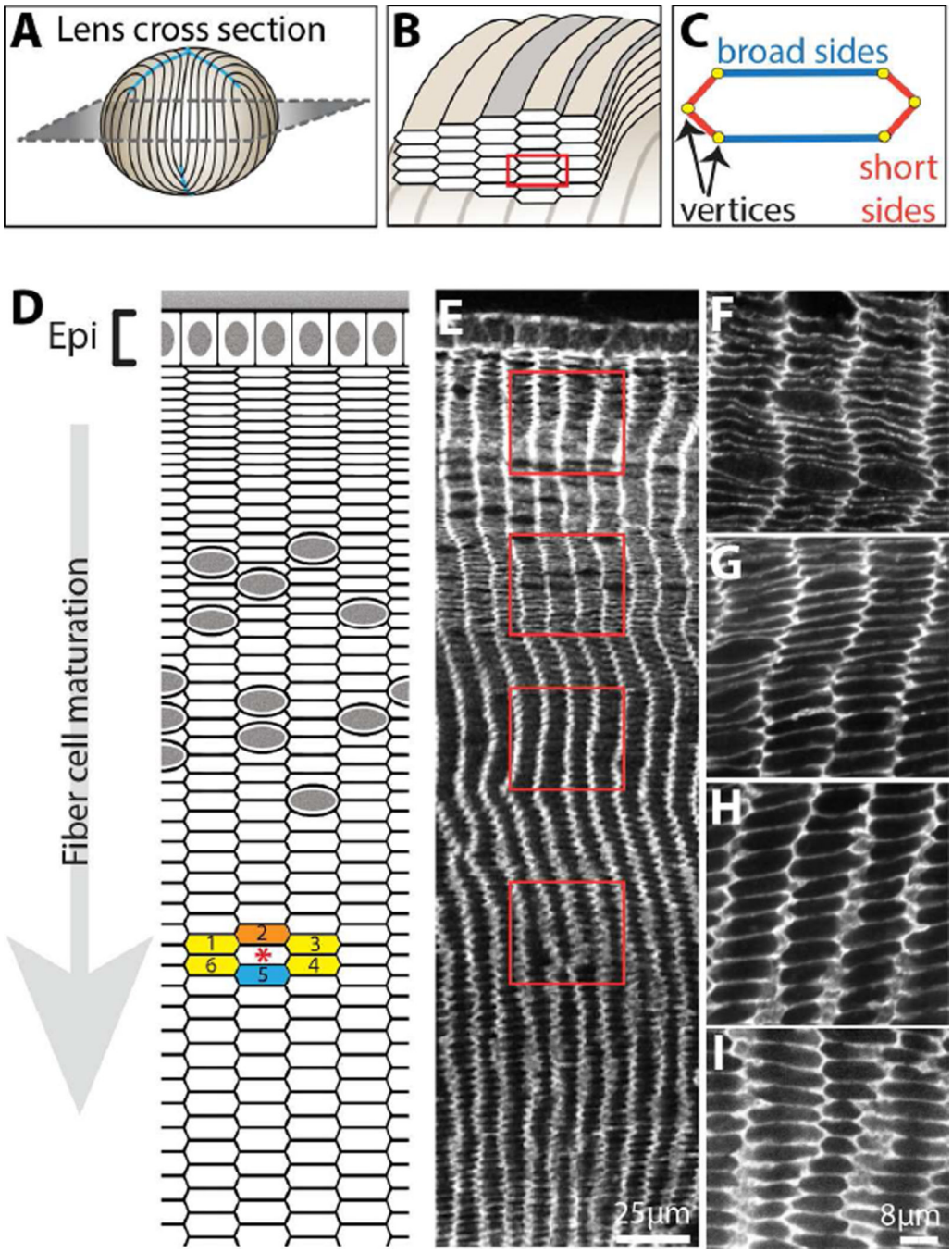


**Figure 2. Actin cytoskeletal organization in lens fiber cells**

A) Diagram of a lens showing orientation of a cross section through the lens equator. B) Cut-away diagram of cross-sections of organized hexagonally packed fiber cells, rotated 90° with respect to A. An individual fiber cell is outlined with a red box. C) Diagram showing the broad sides (blue), short sides (red) and vertices (yellow) of an individual hexagonal fiber cell in cross sectional orientation as in B, red box. D) Diagram of a lens equatorial cross-section. Epithelial cells (Epi) are on the periphery. Each hexagonal fiber cell (red asterisk) has 6 neighboring cells, colored orange, yellow or blue. There is a neighboring cell

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on each of the 4 short sides (1, 3, 4 and 6) and 2 broad sides (2 and 5). The orange cell (2) is a less mature cell (started elongating after the cell with the asterisk). The yellow cells (1, 3, 4 and 6) are about the same age, and the blue cell (5) is more mature (started elongating before the cell with asterisk). E–I) Phalloidin-stained cross-section showing hexagonal packing of cells as they mature. Red boxed regions in E indicate the approximate locations where (F–I) higher magnification images were obtained. F-actin surrounds the entire fiber cell membrane and is enriched at the vertices and short sides. In cortical fiber cells (F), F-actin staining appears discontinuous, but, as fiber cells mature (G–I), F-actin staining becomes smooth and continuous.

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**Table 1**

Actin Binding Proteins in the Lens

| Protein | Known functions | Epithelial Cell Localization (age, species) | Fiber Cell Localization (age, species) | Interactions With Lens Proteins | Lens Phenotype of Knockouts, Mutants or Inhibitor Treatment | References |
|---|---|---|---|---|---|---|
| α-actinin | Cross-links actin filaments in an anti-parallel orientation | - | Vertices of cortical fiber cells (neonatal and adult rat) | - | - | (Lo et al., 1997; Sjoblom et al., 2008) |
| Adducin | Caps actin filament barbed ends in actin-spectrin network | - | Broad and short sides of fiber cells (adult, rat) | - | - | (Fowler, 2013; Kaiser et al., 1989) |
| Arp2/3 complex | Actin nucleation. Initiates branched actin filament assembly from preexisting filaments | Cytoplasm (adult, mouse) | Enriched at vertices of fiber cells (adult, mouse) | N-cadherin and cortactin in fiber cells (not epithelial cells) | - | (Amann and Pollard, 2001; Blanchoin et al., 2000; Leonard et al., 2011; Mullins et al., 1998) |
| Band 4.1 | Binds to and stabilizes actin-spectrin network | Not present (embryo, chick) | Short sides of fiber cells (embryo, chick) | - | - | (Aster et al., 1986; Aster et al., 1984; Bagchi et al., 2004; Beebe et al., 2001; Fowler, 2013) |
| Band 4.9 (dematin) | Bundles actin filaments | - | Fiber cell membranes (adult, chick) | - | - | (Faquin et al., 1988; Fowler, 2013) |
| Cofilin | Binds to actin filaments causing depolymerization (cofilin is inactivated by phosphorylation) | - | - | - | Decreased phospho-cofilin and abnormal actin filament networks in RIP76/RALPB1 transgenic lenses; increased phospho-cofilin and abnormal secondary fiber cell migration and orientation in Rac1 conditional lens knockouts | (Bamburg et al., 1980; Maddala et al., 2011a; Nishida et al., 1984; Sahu et al., 2014) |
| Cortactin | Bind and stabilizes branched actin filaments and activated (phosphorylated) | Total and phospho-cortactin is enriched at vertices of hexagonal | - | - | Loss of EphA2 leads to changes in actin and cortactin localization in hexagonal | (Cheng et al., 2013; Uruno et al., 2001; Weaver et al., 2001) |

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| Protein | Known functions | Epithelial Cell Localization (age, species) | Fiber Cell Localization (age, species) | Interactions With Lens Proteins | Lens Phenotype of Knockouts, Mutants or Inhibitor Treatment | References |
|---|---|---|---|---|---|---|
| | cortactin recruits Arp2/3 to polymerize actin filaments | equatorial epithelial cells (adult, mouse) | | | equatorial epithelial cells of meridional rows | |
| Crystallin, α | Small heat shock proteins with chaperone-like functions | Cytoplasm (adult, mouse) | Cytoplasm (adult, mouse) | - | Mutations in α-crystallins alter association with actin, a possible native substrate of α-crystallins | (Andley et al., 2014; Bloemendal et al., 1984; Brown et al., 2007; Del Vecchio et al., 1984; Gopalakrishnan and Takemoto, 1992; Kibbelaar et al., 1979) |
| Crystallin, β/γ | Structural proteins | - | Cytoplasm (adult, mouse) | - | Interacts with actin, and γ-crystallins may help stabilize actin filaments in mature fiber cells | (Fan et al., 2012; Li et al., 2008; Rao et al., 2008) |
| Ezrin | In ERM/EPPD complexes that link actin filament networks with the plasma membrane | - | Broad and short sides of fiber cells (adult, bovine/mouse); enriched on sides of fiber cells (embryo, chick) | Aqp0, moesin, spectrin, plectin, periaxin, periplaxin, desmoyokin | Genetic variation linked to human age-related cataracts | (Bagchi et al., 2004; Bretscher, 1983; Lin et al., 2013; Maddala et al., 2011b; Straub et al., 2003; Wang and Schey, 2011) |
| Formin [isoform(s) unknown] | Nucleates unbranched actin networks by promoting barbed end filament assembly | Cytoplasm and nucleus (embryo, mouse) | Cytoplasm and nucleus (embryo, mouse) | - | Fiber cell degeneration | (de la Pompa et al., 1995; Goode and Eck, 2007; Pruyne et al., 2002; Sagot et al., 2002) |
| Gelsolin | Severs actin filaments and caps actin filament barbed ends | - | - | α-crystallins | - | (Andley et al., 2014; Yin and Stossel, 1980) |
| Myosin II | Bipolar filaments bind to anti-parallel actin filaments to generate filament sliding and contractility | Apical surface of lens vesicle (embryo, mouse); cytoplasm and concentrated at apical junction between epithelial and fiber cells (embryo and neonatal, mouse) | Cytoplasm (embryo and neonatal, mouse); basal membrane complexes (embryo, chick) | - | Mutations in humans and mice cause cataracts; inhibitor treatment leads abnormal eye cup and lens vesicle formation and nuclear cataracts (mouse) as well as decreased lens stiffness and | (Bassnett et al., 1999; Borges et al., 2011; Chauhan et al., 2009; De Rocco et al., 2013; Economou et al., 2012; Eiraku et al., 2011; Hao et al., 2012; Lang et al., 2014; Luck and Choh, 2011; Maddala et al., 2007; Plageman et al., 2011; Saposnik et al., 2014; Won et al., 2015; Zhang et al., 2012) |

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| Protein | Known functions | Epithelial Cell Localization (age, species) | Fiber Cell Localization (age, species) | Interactions With Lens Proteins | Lens Phenotype of Knockouts, Mutants or Inhibitor Treatment | References |
|---|---|---|---|---|---|---|
| | | | | | changes in focal length (chick) | |
| Plectin | Links actin and intermediate filaments to ERM/EPPD complexes | - | Broad and short sides of fiber cell membranes (adult, bovine) | Ezrin, moesin, periaxin, periplakin, desmoyokin, spectrin | - | (Andra et al., 1998; Fontao et al., 2001; Jiu et al., 2015; Sandilands et al., 1995; Straub et al., 2003; Weitzer and Wiche, 1987) |
| $\alpha_2\beta_2$-spectrin | Spectrin tetramers cross-link actin filaments to form an isotropic network at the membrane, the membrane skeleton | - | Broad and short sides of fiber cells (adult, mouse) | Ezrin, moesin, periaxin, periplaxin, desmoyokin, ankyrin-B | - | (Cheng et al., 2015; Fowler, 2013; Gokhin et al., 2012; Nowak et al., 2009; Nowak and Fowler, 2012; Straub et al., 2003) |
| Tmod1 (mouse) Tmod4 (chicken) | Caps actin filament pointed ends, inhibiting actin association and dissociation, promoting length regulation and stability | Cytoplasm in differentiating equatorial epithelial cells (adult, mouse) | Broad and short sides of fiber cells; not at vertices (adult, mouse) | γTM (mouse), filensin (chick), spectrin, CP49 (indirect, mouse) | Abnormal actin-spectrin network, disrupted fiber cell packing, decreased lens stiffness and gap junction coupling | (Almenar-Queralt et al., 1999; Cheng et al., 2015; Fischer et al., 2003; Fischer et al., 2000; Fowler, 1996, 2013; Gokhin et al., 2012; Lee et al., 2000; Nowak et al., 2009; Nowak and Fowler, 2012; Sussman et al., 1996; Weber et al., 1994; Woo et al., 2000; Yamashiro et al., 2012) |
| Tropomyosin (γTM) | Binds along sides of actin filaments to prevent severing and depolymerization | Cytoplasm (adult, mouse) | Broad and short sides of fiber cells (adult, mouse) | Tmod1, Tmod4 | - | (Fischer et al., 2000; Fowler, 2013; Lee et al., 2000; Nowak et al., 2009; Woo and Fowler, 1994) |

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**Table 2**

Actin Regulating Proteins in the Lens

| Protein | Known functions | Epithelial Cell Localization (age, species) | Fiber Cell Localization (age, species) | Interactions With Lens Proteins | Lens Phenotype of Knockouts, Mutants or Inhibitor Treatment | References |
|---|---|---|---|---|---|---|
| Abi-2 (c-abl-interactor) | Adaptor that interacts with c-abl kinase to regulate WAVE-mediated Arp2/3 activation-driven actin assembly and dynamics | Cytoplasm and enriched at the apical epithelial-fiber cell junction, especially near lens equator (embryo, mouse) | Cytoplasm (embryo, mouse) | Co-localizes with WAVE-2, cadherins and β-catenin at apical interface between epithelial and fiber cells | Secondary fiber cell migration and orientation defects | (Grove et al., 2004; Maddala et al., 2011) |
| Cdc42 | Filopodia formation | Cytoplasm (neonatal, mouse) | Cytoplasm, especially in the cortex at the basal and apical tips of fibers (neonatal, mouse) | - | Loss of actin filopodia between the lens placode and developing retina leads to abnormal optic cup and lens pit shape | (Chauhan et al., 2009; Chen et al., 2006; Chen et al., 2008) |
| p120-catenin | Binds cadherins to activate Rho GTPases to modulate actin dynamics | - | - | - | Mislocalization of myosin IIB away from apical surface of lens placode leading to placode invagination defect | (Lang et al., 2014) |
| Rac1 | Actin stress fiber formation | Cytoplasm (neonatal, mouse) | Cytoplasm (neonatal, mouse) | - | Secondary fiber cell migration and orientation defects | (Chen et al., 2006; Chen et al., 2008; Maddala et al., 2011) |
| Rap1 | Ras-like small GTPase needed for Cdc42 activation during cell-cell junction formation | - | - | - | Incomplete elongation of primary fibers with gap between anterior tips of primary fibers and apical surface of anterior epithelium, decreased actin filaments and | (Maddala et al., 2015) |

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| Protein | Known functions | Epithelial Cell Localization (age, species) | Fiber Cell Localization (age, species) | Interactions With Lens Proteins | Lens Phenotype of Knockouts, Mutants or Inhibitor Treatment | References |
|---|---|---|---|---|---|---|
| | | | | | disrupted E-cadherin and β-catenin staining | |
| RhoA/B/C | Promotes actin stress fiber formation | Cytoplasm (RhoA, neonatal, mouse) | Cytoplasm, especially in the cortex at the basal and apical tips of fibers (RhoA, neonatal, mouse) | - | Transgenic lens expression of C3 Rho GTPase inhibitor leads to small lenses with swollen fibers and decreased actin filaments, adherens junctions, gap junction and water channels | (Chen et al., 2006; Chen et al., 2008; Maddala et al., 2004; Rao et al., 2002) |
| RLIP76/RALBP 1 | Effector of Ral1 and Rho/Rac | - | - | - | Transgenic lens expression leads to small lenses and microphthalmia with abnormal actin filament networks, Cdc42 inactivation and lower levels of phospho-cofilin | (Sahu et al., 2014) |
| Shroom3 | Recruits actin filaments via Mena-family of actin modulators that are needed for apical constriction | - | - | Rock1/2 | Reduced actin filaments and myosin IIB at apical surface of lens placode leading to abnormal placode invagination | (Plageman et al., 2010) |
| WAVE-2 | Activates Arp2/3 leading to actin polymerization | Cytoplamic and enriched at the apical epithelial-fiber cell junction, especially near the lens equator (embryo, mouse) | Cytoplasmic (embryo, mouse) | - | - | (Maddala et al., 2011) |