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Morphological Study of the Accommodative Apparatus in the Monkey Eye

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ABSTRACT

For more than a century there has been debate concerning the mechanism of accommodation—whether the lens capsule or lens material itself determines the functional relationship between ciliary muscle contractility and lens deformation during refractive adaptation. This morphological study in monkey eyes investigates the composition and distribution of several connective tissue components in the accommodative apparatus relaying muscle force to lens organization. Elastin distributes on the marginal surface of the ciliary process. A zonule is composed of fibrillin produced by epithelial cells of the process. In the progress of extension over the posterior chamber, fibrils unite into strands and possess longitudinal plasticity. By induction of the elastin network, strands extend in a concentric direction covering the equatorial region of the capsule. Upon tethering to the lens, the strand ramifies into fibrils, penetrating deeply close to the epithelial layer of the lens and binding with the collagen of the intercellular spaces. Tight linkage of the zonule with the capsule transmits precise contractility. Inside the lens, the cortical layer’s elastic connective tissue network forms widely spaced lamellae of crystalline fibers. In contrast, the central nuclear lamellae are tightly opposed. The accumulation of lamellae is greater in the anterior cortex than in the posterior, yielding a more variable anterior chamber depth in the visual axis. The plasticity of the zonule and connective tissue distribution inside the lens produces an adjustable configuration. Thus, tight linkage between the dynamism of the capsule with interaction of the lenticular flexibility provides a novel understanding of accommodation. Anat Rec, 298:630–636, 2015. © 2014 The Authors The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology Published by Wiley Periodicals, Inc.

Key words: accommodation; zonule; fibrillin; elastin; fibronectin; collagen

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Abbreviations used: CB-p = process of ciliary body; FBN = fibrillin; CM = ciliary muscle; ECM = extracellular matrix; EMG = electromyograms.

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Accommodation is accompanied by precise amplitude changes in the diameter of the anteroposterior axis of the lens in response to ciliary muscle (CM) contraction. The primary stimulus is the sensation of a retinal image, and the efferent pathway then transmits the active motor stimulus to the CM through autonomic nervous innervation. However, understanding the means by which the shape of the lens responds to contraction or relaxation of the CM, and the mechanisms for precisely transforming the lens configuration, is complicated by a lack of innervation within the lens itself.

In 1855 Helmholtz was the first to describe a mechanism for accommodation, although many theories have been proposed since then (Duke-Elder, 1970). The main disagreement in the present theories is whether the elasticity of the lens morphology is due to the lens substance or to the capsule. The Helmholtz theory (or lens theory) is that relaxation of the zonule permits the lens substance to return to a more convex, natural curvature. Another view, the capsular theory of Fincham (1955), is that the relaxation of the zonule makes the elastic capsule more convex. Both schools agree that the zonule acts to alter tension on the mechanical linkage between the CM and lens during accommodation. A variation of the capsular theory holds that the zonule compresses the equator of the lens (Wilson, 1993). Nevertheless, it remains to be determined whether the zonule is capable of changing tension in response to contractility of the CM, thereby causing lens deformation.

The lens contains fibrous networks of elastic extracellular matrix (ECM) around the crystalline fibers, which are composed of mainly collagen and fibronectin. These networks produce the dominant response that allows the lens to withstand tensile and repetitive stresses. The ECM (Hiraoka et al., 2002, 2006), intracapsular collagen-4. The three-step method was utilized for primary antibody staining with fibronectin rabbit polyclonal antibody, FBN-1 monoclonal antibody, and collagen-4. The three-step method was utilized for peroxiredoxin 4 conjugated streptavidin, followed by a secondary antibody and Rhodamine 555 (rabbit anti-goat IgG) or propidium iodide (DAPI). Specificity of staining was confirmed by omitting the primary antibody. To activate the reaction of the primary antibody, fixed-on-slide paraffin specimens were treated with microwaves for 15 min and blocked by 1% skim milk in PBS for 1 h. The primary and secondary antibodies and staining substances used in these experiments are listed in Table 1. Details of the method are published elsewhere (Hiraoka et al., 2002, 2013).

Images were examined and photographed using a brightfield and phase contrast microscope (Nikon Eclipse E600), Axioscope (Carl Zeiss) and fluorescent confocal laser microscope (Carl Zeiss, LSM410).

RESULTS

Macroscopic Composition of the Lens and Zonule

Representative examples of structures that were an object of this study and shown in all preparations are shown in the figures (Fig. 1). The lens was situated behind the iris and connected with the ciliary body by the zonules. The shape of the lens was not a simple disc. Instead, the superficial curvature was smoothly convex over its anterior surface, but the posterior surface was more convex in the central region than the periphery, resulting in a wider central horizontal width in the posterior half than in the anterior half (Fig. 1Aa). The visual axis in the anterior segment is the concentric area on the horizontal midline crossing the lens. The
Fluorescent staining: diluted 100 times with buffered saline; kept 45 min in room temperature

Secondary antibody: diluted 100 times with phosphate-burred saline and 1% cow serum albumin; kept 45 min in room temperature

Primary antibody: 1% cow serum albumin and 2% mouse, donkey, goat, rabbit serum diluted 100 times; incubated 12 hr in 4°C

TABLE 1. Materials used for immunofluorescence staining

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<th>Primary antibody</th>
<th>Mouse monoclonal</th>
<th>Goat polyclonal</th>
<th>Neomarkers (USA)</th>
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<tr>
<td>Anti-Fibrillin1</td>
<td>Mouse monoclonal</td>
<td>Rabbit polyclonal</td>
<td>Elastin Products (USA)</td>
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<tr>
<td>Anti-α-Elastin</td>
<td>Mouse monoclonal</td>
<td>Goat polyclonal</td>
<td>Progen Biotech (Germany)</td>
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<tr>
<td>Anti-α-Smooth muscle actin</td>
<td>Goat polyclonal</td>
<td>Rabbit polyclonal</td>
<td>Santa Cruz Biotech (USA)</td>
</tr>
<tr>
<td>Anti-collagen type IV</td>
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<tr>
<td>Anti-fibronectin</td>
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Secondary antibody: diluted 100 times with phosphate-burred saline and 1% cow serum albumin; kept 45 min in room temperature

Fluorescent staining: diluted 100 times with buffered saline; kept 45 min in room temperature

| Alexa Fluor 488 conjugated Streptavidin | Molecular Probe (Netherlands) |
| Propidium iodide                        | Wako Inc (Japan) |

variability of focusing depends upon the distance between the anterior and posterior midpoints of the superficial curvature. The zonules associated with the anterior capsule (a, Fig. 1Ab) were more abundant and thicker than those of the equatorial (e) and posterior (p) capsule (Fig. 1Ac). The lens was composed of a membrane with a single layer of epithelium and multilayered lens fiber lamellae of the lenticular matrices wrapped by the capsule. A single layer of epithelial cells was aligned between the capsule and the lens fiber lamellae. At a specific point in the mid-posterior equatorial region, single nuclei were aligned in an oblique row (Fig. 1B, two small arrows; see also Fig. 3Cb).

Connection of the Zonule Between the Circular Division of Ciliary Smooth Muscle and the Lens Capsule

The structure of these regions is visualized by the immunostaining patterns of α-smooth muscle actin for CM (Alexa 488) and of elastin (Rhodamine) for distribution of connective tissues (Fig. 2A). The CM was composed of longitudinally (CM-l) and circumferentially (CM-c) aligned muscle fibers. The contractile direction of CM-l is centrifugal, whereas the CM-c is centripetal. For lens accommodation, contraction of the CM-c positions the CB-p concentrically toward the anterior apex of the lens. The CB-p did not connect directly with the lens, but instead the ECM components were intercalated. Thus, the contraction of CM-c would bring CB-p indirectly onto the lens by the mediation of the connecting ECM, composed mainly of elastin. Zonules, the principle structures bridging the muscle and lens, were aggregates of fiber strands made of fibrillin (FBN) fibrils (Fig. 2Bb). To facilitate connection of zonules with muscles, elastin was distributed in the surrounding superficial layer of CM-c and internal core of each CB-p (Fig. 2Ba). On the superficial layer of CB-p, the FBN filaments sprouted up from the epithelium, and each fibril united with its neighbors, forming fibers (Fig. 2Bb). These fibers formed bundles throughout the long extension from the CB-p to the lens capsule, traversing the posterior chamber. Flaccid bundles had a transverse striped pattern on their bellies, whereas tensile bundles did not (Fig. 2Bc). On the capsule, zonules ramified into mainly longitudinal and circumferential branches in the process extending toward the lens (Fig. 2C). Thus, the contractile strength of CM-c was transmitted to the lens indirectly by the mediation of the zonule and associated ECM.

ECM in the Lens Capsule and Epithelial Layer of the Lens

The zonule formed a zonular stalk containing a variable number of bundles on the process extending to the lens capsule in the posterior chamber (Fig. 3A). The stalk divided into bundles near the surface of the capsule and attached superficially (Fig. 3Ba). The bundle ramified into fibers and extended on the lens surface in a concentric direction. In the lens epithelial layer the fibronectin encircled the cells (Fig. 3Ba, Bb) and a network of collagen connected the intercellular spaces between cells (Fig. 3Bc).

The composition of the superficial layer of the lens between the capsule and lens fiber differed with its location. The epithelium in the anterior zone had a layer with uniform cells (Fig. 3Ca), while that in the posterior zone lacked cells (Fig. 3Cc). In the equator, a row of large nuclei aligned in the fiber zone underneath the epithelial layer (Fig. 3Cb). The width of lighter purple staining with aldehyde-fuchsin demonstrated wider interspaces in the anterior fiber zone (Fig. 3Ca) than the posterior zone (Fig. 3Cc), suggesting differences in flexibility.

DISCUSSION

Characteristic Morphology of the Linkage Between CM and the Lens Capsule

We have used histological analysis of each structure related to lens dynamics to investigate the functional morphology underlying the mechanism of accommodation.

The components of the accommodative apparatus that work concomitantly with CM contraction are the coordination between the elasticity of the zonular bundle and the plasticity of the lens fiber laminae. The many zonular fibrils produced by the epithelial cells of the CB-p (Hiraoka et al., 2010) extended to the lens within the aggregated bundles in the posterior chamber. And the zonular bundles were tethered to the lens capsule in a
pattern of fibrils (Fig. 4). The fibrils were connected via components of the ECM, such as elastin (Fig. 2A), fibronectin (Fig. 3Ba, 3Bb), collagen (Fig. 3Bc, Hiraoka et al., 2002, 2010) and laminin (Hiraoka et al., 2013) to the surrounding epithelium of the lens capsule (Fig. 3B).

Elastin acts as a structural lattice over the FBN deposition, thereby determining the direction of zonular fiber growth by cross-linkage (Streeten 1982; Kielt et al., 2001; Sherratt et al., 2001; Kuchtey and Kuchtey, 2014). By this linkage, elastin cores in the stalk of ciliary

Fig. 1. Orientation of the accommodative components. A (a): Stereoscopic view of a sagittal section of adult monkey eye demonstrating the cornea (Cornea), process of the CM (CB-p, upper arrow), zonule (zonule, lower arrow) and lens (Lens) (Aldehyde-fuchsin stain). The shape of the disc lens is different between the anterior (a) and posterior (p) curvature. The anterior curvature is smooth concave, and the posterior curvature shows two steps of central concave with a rather flat peripheral configuration. The width of the middle axis between (a) and (e) is shorter than that between (e) and (p). A (b): The anterior zonule attaches to the anterior lens capsule (a) as a thick fan-shaped bundle and diverges in a concentric extension toward the lens apex. The posterior zonule (p) is thinner and shorter than the anterior zonule and sparse in the equator (e). B: Sagittal section of the equatorial region of the lens. Zones of the capsule (arrowhead), lens epithelium (large arrow) with nuclear inward alignment (right-to-left) demonstrating the possible process of lens fiber development (small arrows) and fiber lamellae (*) are identified. Interspaces of the newly formed fiber zone of the lens cortex (*) contain wider spaces between lamellae than in the internal zone (double asterisks), where lamellae are closely associated with each other (Trichrome stain).

Fig. 2. Linkage of the zonule between lens and CM. A: Double immunostaining of α-smooth muscle actin (green) with α-elastin (red) reveals the arrangement of longitudinal (CM-l, long white arrows) and circumferential (CM-c, white arrowheads) muscle fibers. In the iris (Iris), constrictor (direction of constriction shown by the arrowheads) and dilator (by the arrow) muscles are visible near the posterior margin. Elastin surrounds the posterior superficial layers of the CM and distributes on the process of a ciliary body (CB-p) and the lens (Lens) capsule (wide short arrows). B (a): Double immunostaining of α-elastin (green) with α-smooth muscle actin (red). Elastin distributes at the CM margins (long arrows), protruding trunks (small arrows) and the superficial layer of the CB-p (arrowhead). B (b): Double immunostaining of elastin (red) with fibrillin (green). Fibrillin-stained filaments are tethered to the uneven epithelial surface of CB-p (arrowheads). The filaments unite to form a fibril (boxed area), which, in turn, forms bundles (zonule). Nuclei of the epithelial cells of the processes appear as dots. B (c): Immunostaining of fibrillin (green) of zonules. Zonular fascicles in a flaccid fiber are arranged in a horizontal striped pattern (paired arrows) in the muscle belly. In contrast, the tensile fiber is uniform (single arrows). C: Conventional staining of zonular fascicles (Aldehyde-fuchsin). The zonule diverges into a bidirectional arrangement of mainly longitudinal (zonule-l, arrows) and fewer circumferential (zonule-c, arrowheads)-branches in the belly of the fascicle in the extension toward the lens.
bodies cover the FBN mantles, which sprout from CB-p (ciliary body process) to the lens capsule (boxed b). The main body of the CM and basal ciliary body process (CB-P) are marked faintly visible in background for orientation (Toluidine blue stain). B: Immunofluorescent staining of the lens capsule and epithelial cell layer. B (a): A bundle of zonules (fibrillin; green, opposing thin arrows) attaches to the lens capsule and ramifies gradually into fine filaments at its point of termination (large arrows). Fibronectin (red) distributes in the internal layer of the capsule (small arrow) and epithelial cell layer (opposing arrowheads). B (b): The hexagonal epithelial cells (margin as E) bind with surrounding fibronectin (green, arrows). B (c): Collagen-4 (green) connects epithelial cells (small circle). Each epithelial cell has a nucleus (N, arrowhead) and is surrounded by a collagenous net (large circle). C: The structure of the capsule, lens epithelium and lens fibers lamellae in the C (a); antero-axial, C (b); mid-equatorial and C (c); postero-axial regions (Aldehyde-fuchsin stain). Lens fibers and the epithelial cell layer are clearly differentiated by the density of purple lamellae and arrangement of nuclei. C (a): The outermost capsular layer (bidirectional black arrow) is double-layered with an intra-capsular zonular layer only in the anterior capsule. The epithelium has cells in the anterior [C(a)] and equatorial [C(b)] regions (bidirectional short white arrows), but not in the posterior [C(c)]. The posterior capsule, attaches to the vitreous membrane [C(c), arrowhead]. The most prominent feature is the nucleus of newly produced lens fibers aligned obliquely and anteriorly one-by-one, [C(b), arrowheads next to long bidirectional white arrow]. Inside the capsule, the zone of light-purple lamellae contains more elastic ECM than the inner dark red zone [*: C(a), C(b), C(c)].
paucity of microfibril development in the anterior and posterior lens capsules (Traboulsi et al., 2000). This elas- tomeric macromolecular structure of zonules has the fundamental function of transmitting the CM contractility to the lens capsule.

The most prominent feature of the zonule is that the ramified fibrils (Fig. 4Db) penetrate deeply and connect with the epithelial cell layer through the mediation of fibronectin (Fig. 3Ba, Bb) and collagen (Fig. 3Bc). The capsule is attached to the lens fiber zone inside by the adhesive molecule laminin (Hiraoka et al., 2013). The final point of linkage between zonules and the capsule is the mesh-like basement membrane of collagen surrounding the epithelial cells (Fig. 3Bc, Muiznieks and Keeley, 2013). Life-long growth of the lens fiber occurs mainly in the anterior half and accumulates in a concentric encircled direction (Figs. 1B and 3Cb, Hiraoka et al., 2006). As a result, the ECM between lens fibers in lamellae is wider in the anterior lens cortex than the posterior cortex (Fig. 3Ca:Cc). Differences between the zonular distribution (Fig. 1Ab) and lens matrix composition account for the shape of the lens being more convex in the posterior than the anterior on radii of curvature in the fixed preparation (Fig. 1Aa). In accommodation the convex curvature of the surface becomes steeper, particularly in the anterior (Croft et al., 2006a). In vivo observations in humans have demonstrated that the width of the lens increases during the focusing distance of accommodation, resulting in a decrease of the anterior chamber depth as recorded by three-dimensional Optical Coherence Tomography. Chamber depth is a good index of the accommodative change of the anterior radius of lens curvature. The posterior radius changes slightly (Gambarra et al., 2013). This is a well-coordinated manifestation of the in vivo fluctuation of the lens configuration.

The posterior vitreous zonules have been suggested to control the magnitude of centripetal lens movement (Lütjen-Drecoll et al., 2010; Croft et al., 2013). We noted that zonules were distributed mainly in the anterior centripetal directions (Fig. 1Aa); however, zonules at the equator and in the posterior vitreous region were shorter and sparser (Figs. 1Aa and 4C).

We have previously described the development of the lens fiber from a series of specimens in monkey (Hiraoka et al., 2006). Those images suggest that the nuclei of the epithelial cells may have elongated, diverting from the row of epithelium one by one at the same point in the posterior to sagittal midline. Then the cell could have changed direction toward the fiber zone underneath, converted into a lens fiber cell and accumulated in a layer. These cells have a distinct large nucleus in the center, with the cytoplasm elongated bidirectionally, longer toward the anterior than the posterior arc. These nuclei are lined obliquely in a row on the inward extension in the new fiber zone and they disappear as the lamellar organization develops. Newly produced fibers are more elastic and gradual accumulation into compact lamella might cause them to lose their elasticity, as revealed by the difference in the density of the histological staining (Hiraoka et al., 2006).

The accumulation of lens fibers would make the shape of the lens protrude posteriorly as shown in a sagittal section (Fig. 1A). Compaction of lamellae would result in loss of the connective tissue interspaces, resulting in decreased plasticity in the anteroposterior axis during accommodation. In addition to changes in CM function with age (Croft et al., 2006a), the amplitude of the flexi- bility of the anteroposterior axis may decrease with age due to the successive accumulation of lens fibers (Hiraoka et al., 2006). As a result, the intralenticular plasticity may bring about age-related loss of accommoda- tion, “presbyopia.”

**Relationship Between the Morphology and Physiological Studies**

Accommodation is a highly organized sensory-motor interaction between the brain and the eye. The circumferential CMs work with iris constrictor muscles concomitantly to provide the “near reflex.” Our study in cats obtained evoked electromyograms (EMG) from both muscles with electrical stimulation of the Edinger-Westphal (E-W) and anteromedian nuclei. The responses were spike waves followed by long refractory periods on both muscles. The EMG did not follow repetitive stimulation for frequencies over 1 Hz. (Hiraoka and Shimamura, 1989). Physiological studies have been carried out on the accommodative refractive changes and the move- ments of the accommodative apparatus in monkey (Glasser and Kaufman, 1999, 2003; Glasser et al., 2006; Croft et al., 2006a, 2009). A stimulating train of pulses (72 Hz: Glasser et al., 2006, Ostrin and Glasser, 2007 and 100Hz: Croft et al., 2006a) applied to the Edinger-Westphal (E-W) nucleus caused a refractive change of maximum amplitude 20 (Croft et al., 2006a) and 7 (Ostrin and Glasser, 2007) dioptries as recorded by refractometry (Glasser et al., 2006; Ostrin and Glasser, 2007). In addition, gonioscopic observation of the ciliary processes detected centripetal movement during stimula- tion (Glasser et al., 2006; Ostrin and Glasser, 2007; Croft et al., 2006a, 2009).

Discrepancies of results from repetitive electrical stimula- tion may depend on intrinsic differences in the nature of the muscle between the two species, and also on the method of detection, namely direct observation versus EMG. The contractile dynamics are further con- fused by the pharmacological reaction to the different receptor subtypes (Kaufman PL, 1992). In vitro experi- ments with isolated muscle strips of the ciliary body from a human eye demonstrated a dual sympathetic and parasympathetic nerve supply (van Alphen, 1976). We also found α-1 adrenergic receptors of the circumferential CM in cats (Hiraoka et al., 2002). Nevertheless, dur- ing accommodation, linkage of autonomic innervation with smooth muscle contraction causes deformation of the lens, which itself lacks muscle or nerve, and it does so in a graded manner.

Therefore the organization and content of the connec- tive tissue components play fundamental roles in the production of zonular elasticity and lens plasticity. This is a departure from the traditional lens or capsule theo- ries of accommodation. Therefore, we propose a new theory related to the dynamism of connective tissue ele- ments working together to link the ciliary process- zonule-lens complex as a mechanism of accommodation.

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LITERATURE CITED


