Cadm1 Expression and Function in the Mouse Lens

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PURPOSE. The immunoglobulin superfamily member Cadm1 is a single-pass, type 1 membrane protein that mediates calcium-independent, cell-cell adhesion. Cadm1 has been implicated in tumor formation and synaptogenesis. A recent analysis of mouse lens cell membranes identified Cadm1 as a major constituent of the fiber cell membrane proteome. Here the authors examined the expression and function of Cadm1 in the mouse lens.

METHODS. Cadm1 expression was analyzed by Western blotting and immunofluorescence. The morphology of individual wild-type and *Cadm1*-null lens cells was visualized by confocal microscopy.

RESULTS. Cadm1 was present in epithelial and superficial fiber cells as a heavily glycosylated protein with an apparent molecular mass of ≈ 80 kDa. Analysis of proteins extracted from various strata of the lens indicated that Cadm1 was degraded during fiber cell differentiation, at approximately the same time as the lens organelles, an observation confirmed by confocal microscopy. In epithelial cells, Cadm1 was enriched in basolateral membranes, whereas, in fiber cells, expression was restricted to the lateral membranes. Lenses from *Cadm1*-null mice were of normal size and transparency. The three-dimensional morphology of the cells in the epithelial layer was unaltered in the absence of Cadm1. However, in contrast to wild-type lens fiber cells, Cadm1-null fiber cells had an irregular, highly undulating morphology.

CONCLUSIONS. Cadm1 is an abundant component of the lens fiber cell membrane. Although not essential for lens transparency, Cadm1 has an indispensable role in establishing and maintaining the characteristic three-dimensional architecture of the lens fiber cell mass. (*Invest Ophthalmol Vis Sci.* 2011;52:2293–2299) DOI:10.1167/iovs.10-6677

Cadm1 (Cell adhesion molecule 1; variously referred to as Nectin-like molecule-2 [Nec12],¹ RA175,² spermatogenic immunoglobulin superfamily member [SgIGSF],³ immunoglobulin superfamily member 4 [IGSF4],⁴ tumor suppressor in lung cancer 1 [TSLC1],⁵ and synaptic cell adhesion molecule 1

[Syncam1]⁶) is a member of the immunoglobulin superfamily (IgSF). Cadm1 is a single-pass, type 1 membrane protein composed of three extracellular immunoglobulin-like domains, a single transmembrane region, and a short carboxyl terminal intracellular tail. The intracellular region harbors two binding motifs: a protein 4.1-interaction sequence (which mediates binding to Dal-1⁷) immediately adjacent to the transmembrane domain and a PDZ-binding motif (through which Cadm1 binds the PDZ domain-containing proteins CASK, syntenin, and MPP3⁸) at the carboxyl terminus. Several isoforms (at least four) of Cadm1 have been detected, including soluble forms⁹ arising from alternative splicing. The protein is heavily glycosylated in many tissues and is also polysialylated.¹⁰

Cadm1 mediates both homotypic¹¹ and heterotypic^{1,12} calcium-independent cell-cell adhesion. The Cadm1 protein is widely expressed in the nervous system, where it is a prominent component of presynaptic and postsynaptic membranes.¹³ Cadm1 is also found in various epithelial tissues and may act as a tumor suppressor in cancers of epithelial origin.¹⁴ Two-hit inactivation (promoter methylation of one allele and loss of heterozygosity of the other) is observed in approximately 40% of primary non-small cell lung cancer.¹⁴

Perhaps surprisingly for an abundant and widely expressed protein, disruption of the *Cadm1* locus in mice by homologous recombination yields a relatively subtle phenotype. Homozygous null male mice are infertile^{15,16} because of disruption of the adhesive interaction between spermatocytes/spermatids and Sertoli cells in the testes, but few other overt physical defects have been reported. Alterations in social and emotional behaviors and motor performance in *Cadm1*-null mice hint at subtle alterations in neural connectivity.¹⁷ It has also been suggested that mutations in the *CADMI* gene in humans may be associated with autism spectrum disorder (a condition characterized by impaired social interaction and increased anxiety).¹⁸

We recently completed an analysis of the mouse lens fiber cell membrane proteome.¹⁹ Surprisingly, among numerous adhesion proteins identified in that study, Cadm1 was second only to N-cadherin in apparent abundance. Furthermore, published microarray data indicate that the level of *Cadm1* mRNA transcripts in the mouse lens exceeded that in 96 other tissue and cell types examined²⁰ and that, in human lenses, *CADMI* is an abundant transcript in both epithelial and fiber cells.²¹ These data suggest that Cadm1 is likely to be an important constituent of lens cell plasma membranes. In the present study, therefore, we analyzed the expression pattern of Cadm1 in the lens and assessed the lens phenotype in *Cadm1*-deficient mice.

METHODS

Animals

The generation of *Cadm1*-null mice has been described previously.¹⁶ Gene targeting was used to delete exon 9 of the *Cadm1* gene (encoding the single transmembrane domain) and to induce a frame shift to

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disrupt the cytoplasmic domain containing critical protein-protein interaction domains responsible for tumor suppressor activity.²² *Cadm1*-null mice are phenotypically indistinguishable from wild-type littermates, except that males are infertile because of the arrest of spermatogenesis at the spermatid stage.¹⁶ The null mutation was generated originally on a mixed 129S5/SvEvBRd-C57BL/6J background. The 129 mouse strain carries a mutation in *Bfsp2*, an intermediate filament protein and important component of the lens fiber cell cytoskeleton.²³ We verified by PCR of tail-clip DNA that the *Bfsp2* mutation was not present in the 129S5/SvEvBRd-C57BL/6J strain (data not shown). To image individual lens epithelial cells, wild-type or *Cadm1*-null mice were crossed with *TgN(GFPU)5Nagy* mice (obtained from the Jackson Laboratory, Bar Harbor, ME).

Mice (4–6 weeks old unless otherwise noted) were killed by CO₂ inhalation, and lenses were removed through an incision in the back of the eyeball. All procedures described herein were approved by the Washington University Animal Studies Committee and performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Photography

The clarity and optical quality of dissected lenses were assessed by photography against a fine hexagonal grid.

Western Blot Analysis

Proteins (50 µg) were separated on SDS-PAGE (NuPAGE 4%–12% or NuPAGE 10% Bis-Tris gels; Invitrogen, Carlsbad, CA) and were transferred to nitrocellulose membranes. In some experiments, proteins were isolated from different strata of the lens, as described previously,²⁴ in a modification of a method developed originally by Piercionek and Augusteyn.²⁵ Briefly, concentric layers of lens fiber cells were removed by controlled lysis. Pools of 8 to 10 decapsulated lenses from wild-type mice were dissolved progressively in 300 µL buffer (20 mM Tris-HCl, pH 7.4, 100 mM KCl, 5 mM EDTA, 4 mM dithiothreitol) containing protease inhibitor mixture (complete protease inhibitor cocktail; Roche Applied Science, Indianapolis, IN). Samples of the lysate were withdrawn at intervals, and protein content was determined by BCA assay (Bio-Rad, Hercules, CA) using immunoglobulin G as standard. Blots were incubated overnight at 4°C with anti-Cadm1 (catalog no. S4945; Sigma-Aldrich, St. Louis, MO) diluted 1:1000. To identify the border of the lens organelle-free zone (OFZ), blots were re-probed with an antibody against calnexin (catalog no. SPA-865; StressGen, Ann Arbor, MI), an integral membrane protein and marker for the endoplasmic reticulum. Primary antibodies were detected using horseradish peroxidase-conjugated secondary antibodies (Thermo Fisher Scientific, Rockford, IL) and chemiluminescence.

Immunofluorescence

The distribution of Cadm1 in vibratome sections of the young (postnatal day [P] 1–P5) mouse lens was determined, as described.²⁴ Lenses were fixed in 4% paraformaldehyde/PBS, embedded in agar, and sliced at 150 µm using a tissue processor (Vibratome series 1000 plus; TPI Inc., St. Louis, MO). Lens slices were permeabilized in 0.1% Triton X-100/PBS for 30 minutes and blocked in 3% bovine serum albumin in PBS overnight. Slices were incubated in primary antibody overnight at 4°C, washed in PBS, and incubated for 2 hours in secondary antibody at 37°C. Propidium iodide was used as a nuclear counterstain. Slices were coverslipped and examined using a confocal microscope (LSM 510-META; Carl Zeiss, Thornwood, NY). Lenses from older (P30) mice were cryosectioned. After fixation, lenses were cryoprotected by incubation in 5%, 10%, and 20% sucrose/PBS. Lenses were then mounted in optimum temperature cutting compound (Tissue-Tek OCT; Electron Microscopy Sciences, Hatfield, PA) and cut into 20 µm-thick sections in the equatorial plane.

Deglycosylation

Proteins were deglycosylated using a kit (Enzymatic Carboxylase Kit; QA-Bio, Palm Desert, CA) that utilizes peptide N-glycosidase F (PNGase F),

O-glycosidase, sialidase, β -galactosidase, and glucosaminidase to remove all N- and many O-linked oligosaccharides. The deglycosylation reaction was performed for 3 hours, in accordance with the manufacturer's instructions. Bovine fetuin was used as a positive control for the deglycosylation reaction.

Single-Cell Imaging

To evaluate epithelial cell morphology in wild-type or knockout animals, *Cadm1*^{-/-} mice were crossed with *TgN(GFPU)5Nagy* mice,²⁶ a strain in which a green fluorescent protein (GFP) transgene is expressed mosaically in lenses and other tissues.²⁷ GFP-positive F1 littermates were intercrossed, and resultant GFP-positive F2 littermates were genotyped to identify homozygous *Cadm1*-null animals. Lenses were dissected from the eye and placed, epithelium down, in warm DMEM/F12 medium (Invitrogen) in glass-bottomed microwell dishes (Mattek, Ashland, MA). Lenses were examined with a confocal microscope (LSM510; Zeiss). GFP fluorescence was visualized using the 488-nm line of an argon laser and a 505-nm long-pass emission filter. Images were collected using a 100× objective (Alpha Plan-Fluar, NA 1.45; Zeiss). Electronic zoom was set to 3.0, resulting in x,y pixel dimensions of 40 nm. A full-thickness stack of optical sections was collected across the epithelial layer with a z interval of 100 nm. Image stacks were deconvolved using deconvolution software (Huygens, version 3.4; Scientific Volume Imaging, Hilversum, The Netherlands) with a measured point spread function distilled from images of 170 nm-diameter fluorescent microspheres (PS-Speck; Invitrogen).

To evaluate the morphology of individual fiber cells, lenses were fixed (4% paraformaldehyde in PBS for 5 hours) and then teased apart manually with fine forceps. Fiber cells were dried onto polylysine-coated slides. Cells were permeabilized with 0.1% Triton X-100/PBS, blocked with 3% BSA for 30 minutes, and incubated overnight in 1:100 dilution of rabbit anti-MIP (catalog no. AQP01-A; Alpha Diagnostics, San Antonio, TX). MIP (aquaporin 0) is the most abundant integral membrane protein expressed in lens fiber cells²⁸ and is distributed throughout the fiber cell plasma membrane. Here, MIP immunofluorescence was used to visualize the three-dimensional structure of fiber cells from wild-type or *Cadm1*-null lenses. Cells were washed several times in PBS and then incubated in 1:200 dilution of Alexa 488-conjugated goat anti-rabbit antibody. Cells were washed, mounted in antifade reagent (Prolong; Invitrogen), and examined by confocal microscopy. Imaging parameters and postprocessing were as described for the epithelial cells.

RESULTS

Cadm1 belongs to a family of four genes (*Cadm1–4*) widely expressed in the vertebrate lineage.²⁹ We used end point PCR to screen cDNA prepared from mouse lens, retina, and brain for the expression of *Cadm1* to *Cadm4* transcripts. As reported previously,³⁰ *Cadm1* to *Cadm4* were each expressed in the retina and brain. In the lens, however, only *Cadm1* mRNA was detected (data not shown). Cadm1 protein expression was analyzed by Western blot in lysates prepared from brain, retina, and lens (Fig. 1).

Cadm1 protein was readily detected in each tissue. In brain, Cadm1 was predominantly present as a diffuse $\approx$ 60-kDa band, with a minor band at $\approx$ 45 kDa (the expected size of the Cadm1 protein). In retina, Cadm1 was present as a diffuse band of $\approx$ 75 kDa, with a band of lower abundance at $\approx$ 45 kDa. Cadm1 in the lens epithelium was present as a single band of $\approx$ 80 kDa. In samples from the lens cortex or core, a band of $\approx$ 65 kDa was predominant.

We next examined the time course of Cadm1 expression in lenses from 1- to 30-day-old (P1–P30) wild-type mice (Fig. 2). Age-matched samples from *Cadm1*-null mice were included as negative controls. In P1 lenses, Cadm1 was present as a single band of $\approx$ 80 kDa. As expected, no immunopositive band was

FIGURE 1. Expression of Cadm1 protein in brain (B), retina (R), lens epithelium (E), lens cortex (Cx), and lens core (Co) from a 1-month-old (P30) mouse.

detected in the *Cadm1*-null lens. The Cadm1 signal was reduced by P7 and had further declined by P30. Curiously, two strong immunopositive bands appeared in the P30 lens sample. These bands were also present in samples from P30 *Cadm1*-null lenses and, therefore, reflect nonspecific binding of antibody to unknown lens proteins. The background bands originated from the fiber cell mass because they were not evident in epithelial samples from lenses of this age (Fig. 1).

To better define the Cadm1 expression pattern within the lens and correlate it with other differentiation markers, a progressive lysis technique was used to fractionate the lens (Fig. 3). The lens grows by the continuous deposition of layers of fiber cells and, as a result, has an onion-like structure. Proteins were harvested from the various strata of the lens by gentle stirring of the fiber cell mass in lysis buffer until it dissolved, layer by layer. Aliquots were taken at intervals, yielding seven fractions; from the newly formed outer fiber layers (F1) to the oldest, innermost fibers (F7). Organelles are eliminated during the course of fiber cell differentiation.³¹ As a

FIGURE 2. Time course of *Cadm1* expression. Homogenates were prepared from P1, P7, and P30 wild-type lenses and age-matched *Cadm1*-null lenses. In the young (P1) lens, Cadm1 is present as a single, diffuse band of ≈ 80 kDa (arrow). With time, the 80-kDa band is diminished, and two lower molecular weight forms appear (arrowheads). However, the lower molecular weight bands are also present in P30 *Cadm1*-null lenses, indicating that they are caused by nonspecific interaction of the Cadm1 antibody with unknown lens proteins.

FIGURE 3. Concomitant disappearance of Cadm1 and lens fiber cell organelles. Fiber cell masses were solubilized to yield seven fractions from progressively deeper (and older) strata of the lens (F1–F7). The fractions were probed with antibodies to Cadm1 and calnexin (a marker for the endoplasmic reticulum). Cadm1 is prominent in the outer fiber cells but not detected beyond F2. Similarly, calnexin is detected in the outer two fractions (F1 and F2) only.

result, the center of the lens is an organelle-free zone (OFZ). The border of the OFZ can be identified by the abrupt disappearance of organelle-resident components, such as calnexin, an integral membrane protein of the endoplasmic reticulum. The progressive lysis technique confirmed that the calnexin signal was strongest in the outermost (F1) fraction, reflecting the relative abundance of organelles in the superficial layer of young fiber cells. Calnexin diminished in the F2 fraction and was undetectable in F3 to F7. These data suggest that in lenses of this age, the border of the OFZ is located at F2/F3. Reprob-ing the blot with anti-Cadm1 revealed that Cadm1 was also only present in the two outer fractions (F1 and F2). Thus, Cadm1 disappears during the course of fiber cell differentiation at or about the time of organelle breakdown.

The apparent molecular mass of Cadm1 on SDS-PAGE ranged from 45 to 80 kDa, depending on the age of the animal and the tissue type examined (Figs. 1, 2). This may reflect the expression of different Cadm1 splice variants or differential glycosylation patterns.¹¹ To explore the latter possibility, we treated samples from brain, retina, lens epithelium, and lens fiber cells with an enzymatic cocktail to release O- or N-linked oligosaccharides from Cadm1 (Fig. 4). Incubation with a combination of sialidase, β -galactosidase, O-glycosidase, and glucosaminidase was used to remove O-linked glycans. In each tissue

FIGURE 4. Differential glycosylation patterns of Cadm1 in retina (A), brain (B), lens epithelium (C), and lens fibers (D). Samples were treated with a combination of glycosidase enzymes to release O- or N-linked oligosaccharides. Lane 1, untreated lysate; lane 2, 3-hour enzymatic treatment to release O-linked sugars; lane 3, control lysate incubated for 3 hours without glycosidase treatment; lane 4, overnight incubation with PNGase-F (to release N-linked sugars); lane 5, overnight incubation with an enzymatic cocktail to remove both O- and N-linked sugars. The presence of nonspecific bands (arrowheads) in the lens fiber cell sample (D) obscures the decrease in molecular mass resulting from the removal of O- or N-linked sugars. Arrow: band corresponding to authentic Cadm1 in this sample (see also Fig. 2).

FIGURE 5. Cadm1 distribution in the membranes of epithelial and superficial fiber cells. Confocal microscopy was used to visualize the distribution of Cadm1 (red) in sections of P3 (A) or E15 (B) lenses. Blue: nuclei. Cadm1 is detected in the membranes of nucleated cortical fiber cells at P3 (A). Cadm1 is not present in the central fiber cells in the P3 lens but is detected in central fiber cells in the embryonic lens (B). Scale bars: 250 μ m (A); 100 μ m (B).

lysate, such treatment resulted in a small decrease in apparent molecular mass, indicating a modest degree of O-linked glycosylation. Incubation with PNGase-F, however, caused a significant reduction in the apparent molecular mass of Cadm1, suggesting that, in each of these tissues, the Cadm1 protein was heavily N-glycosylated. Interestingly, the apparent molecular mass of Cadm1 in the various tissue samples differed after removal of O- and N-linked oligosaccharides. In the brain sample, for example, deglycosylated Cadm1 had a molecular mass of $\approx$ 45 kDa (Fig. 4B). In contrast, deglycosylated Cadm1 from the lens epithelium (Fig. 4C) had a mass of $>$ 50 kDa. These differences may indicate the presence of Cadm1 isoforms in the various tissues.

The lens lysis technique (Fig. 3) provided some information on the spatial distribution of Cadm1 in the lens but, to investigate the cellular location of Cadm1 in detail, we used immunofluorescence. Vibratome sections of the lens were prepared from newborn (P3) animals or embryonic day (E) 15 embryos and were stained with anti-Cadm1. Low-magnification views of the P3 lens (Fig. 5A) revealed that Cadm1 was abundant in the outer layers of the lens (epithelium and superficial fibers) but absent from cells in the center of the lens. These observations suggest that either Cadm1 is degraded in the course of fiber differentiation or the central fiber cells never expressed Cadm1. To differentiate between these possibilities, we examined Cadm1 expression in the embryonic lens (Fig. 5B). At E15, Cadm1 was detected throughout the lens, including the membranes of the innermost fiber cells. In conjunction with the Western blot data shown in Figure 3, these observations indicate that Cadm1 is expressed initially by young fiber cells near the lens surface but is subsequently degraded.

High-magnification confocal images (Fig. 6A) revealed that, in the lens epithelium, Cadm1 was distributed throughout the basolateral membrane domain but was absent from the apical membrane (i.e., the membrane abutting the fiber cell mass). In contrast, in differentiating fiber cells, Cadm1 was abundant in the lateral membranes but excluded from the basal membrane (Fig. 6B). In cross-section, fiber cells have a flattened hexagonal appearance, with two broad faces (oriented parallel to the lens surface) and four narrow faces. Many membrane proteins are differentially distributed in these subdomains of the lateral membrane. For example, connexin proteins (Cx46 and Cx50) are most abundant on the broad faces,³² whereas the adhesive protein, N-cadherin, is concentrated at the narrow faces.³³ We examined lens slices cut in the equatorial plane (i.e., orthogonal to the optical axis) to determine whether Cadm1 was more abundant on one membrane face than another. The data indicated that Cadm1 was distributed equally between the broad

and the narrow faces of the fiber cell lateral membrane (Fig. 6C). Age-matched negative control samples from *Cadm1*-null animals showed only low background levels of fluorescence (Fig. 6D).

To analyze the function of Cadm1 in the lens, we obtained mice in which the *Cadm1* locus was disrupted. On gross examination, the lenses of *Cadm1*-null animals were indistinguishable from those of age-matched wild-type animals (Fig. 7). *Cadm1*-null lenses were completely transparent and of normal size and shape. The internal refractive properties of lenses can

FIGURE 6. Cadm1 distribution in specific membrane domains. In epithelial cells (A), Cadm1 (red) is located predominantly in the basolateral membrane domain (arrowhead) with little or no expression in the apical membrane (arrow). Cadm1 is absent from the apical membrane domain (arrow). In young fiber cells near the lens equator (B), Cadm1 is found in the lateral membrane but is absent from the basal membrane (arrowhead). In P30 lenses, sectioned orthogonal to the optical axis, fiber cells are seen in transverse section (C). In this orientation, the broad and narrow subdomains of the lateral membrane are revealed. Cadm1 is evenly expressed in the two subdomains. Samples from age-matched *Cadm1*-null animals are included as negative controls and show little or no membrane staining (D). Scale bars: 10 μ m (A, B, D); 20 μ m (C).

FIGURE 7. Optical properties of lenses from wild-type (A) and *Cadm1*-null (B) mice are indistinguishable.

be gauged by imaging a regular grid pattern through the isolated lens. Changes in the internal refractive properties of the lens cause characteristic distortions in the grid pattern.³⁴ However, the refractive properties of the *Cadm1*-null lenses were indistinguishable from those of the wild-type lenses (Fig. 7), suggesting that the internal refractive index gradient of the lens was undisturbed.

Cadm1 expression has been shown to influence epithelial cell morphology,³⁵ and, in neural tissue, *Cadm1* plays a role in regulating growth cone morphology.³⁶ To determine what role *Cadm1* might play in lens cell morphogenesis, we examined the detailed three-dimensional structure of individual cells in the lens epithelium and the fiber cell population. Immunofluorescence images suggested that the highest levels of *Cadm1* in the lens were found in the basolateral membranes of the epithelial cells (Fig. 6A). Living epithelial cells were imaged in intact lens tissue by crossing wild-type or *Cadm1*-null animals with a mouse strain (*TgN(GFPU)5Nagy*) in which GFP was expressed spontaneously by scattered cells in the lens epithelium. Side-by-side comparisons of wild-type and *Cadm1*-null epithelia failed to reveal any qualitative difference in epithelial morphologies between the two genotypes (Figs. 8A, 8B). In each case, epithelial cells had structurally complex basolateral membranes and relatively simple, polygonal, apical membranes. The GFP labeling technique was not suitable for visualizing lens fiber cell morphology because GFP is not well retained by expressing lens fiber cells.³⁷ Therefore, to visualize fiber cell morphology, we microdissected individual fiber cells from the lens and stained them with an antibody against MIP, an abundant lens membrane protein. Image stacks of wild-type or *Cadm1*-null fiber cells were deconvolved and volume rendered. A gallery of representative cells from each genotype is shown in Figure 8C. Previous scanning electron microscopy studies have revealed that fiber cells in the cortex of the mouse lens follow an undulating, sinusoidal course.³⁸ We confirmed this observation for wild-type lens fiber cells (Fig. 8C, right). However, the three-dimensional morphology of *Cadm1*-null fiber cells differed significantly from that of wild-type cells. Namely, in the *Cadm1*-null fiber cells, the undulations were irregular in period and exaggerated in amplitude (Fig. 8C, left). Thus, *Cadm1* plays an indispensable role in establishing and maintaining the characteristic three-dimensional architecture of the lens fiber cells.

DISCUSSION

A prerequisite for lens transparency is close association of neighboring cells. This ensures that light is not scattered at the interface between the highly refractive cells and the interven-

ing extracellular space. It is not surprising, therefore, that many adhesion proteins are expressed in the lens.¹⁹ Presumably, in those species capable of lenticular accommodation,

FIGURE 8. Deletion of *Cadm1* in the lens results in morphologic defects in lens fiber cells but not in epithelial cells. The three-dimensional structure of lens epithelial cells was visualized in the intact living lenses using intrinsic GFP fluorescence (A, B). In these en face maximum intensity projections, the apical membrane of the epithelium is shown in red and the basal membrane in blue. The basal membrane is complex and irregular in shape, with many lamellipodia-like extensions (arrows). In contrast, the apical membrane has a simple polygonal shape. No qualitative difference in morphology was noted between *Cadm1*-null epithelial cells (A) and wild-type cells (B). In contrast, fiber cells dissected from *Cadm1*-null lenses (C, left) differ markedly from wild-type cells (C, right). Wild-type cells follow a regular sinusoidal course, whereas the *Cadm1*-null fiber cells have an irregular, meandering form. The red lines in (C) are drawn by hand to help visualize the undulating cell morphology. Scale bars: 20 μ m (A, B); 5 μ m (C).

adhesion proteins and their associated cytoskeletal elements also serve to resist the physical stresses generated during the accommodation process. More than 20 proteins with demonstrated or suspected roles in cell-cell adhesion have been identified in the lens.¹⁹ These include members of the cadherin family and immunoglobulin superfamily (IgSF). Cadm1 is the most abundant IgSF member, second only to N-cadherin in apparent abundance in the fiber cell membranes. In the present study, we showed that Cadm1 is expressed by epithelial cells and early fiber cells. In epithelial cells, it is present in basolateral membranes; in fiber cells, it is located exclusively in the lateral membrane. A similar situation is found in other polarized epithelial cells such as MDCK and Caco-2 cells, where Cadm1 also localizes to lateral membranes.¹¹ The absence of Cadm1 from the apical membrane of the lens epithelial cells implies that Cadm1 does not mediate intercellular adhesion between the epithelial layer and the underlying fiber mass.

Cadm1 protein is degraded in the course of fiber cell differentiation at about the same time that the intracellular organelles of the lens fiber cells disappear. This appears to be a critical juncture in the differentiation process and is marked by a fundamental reorganization of cell-cell adhesion complexes,³⁹ spectrin,²⁴ and the intermediate filament cytoskeleton.⁴⁰ The cysteine protease calpain 3 is specifically activated in cells bordering the lens OFZ and contributes to proteolysis of both spectrin²⁴ and the gap junction protein connexin 50.⁴¹ It is not yet known whether Cadm1 serves as a calpain substrate in lens.

The apparent molecular mass of Cadm1 varies considerably between tissues. Much of this variability can be attributed to differential patterns of glycosylation. In lens, N-linked glycosylation predominates, as it appears to in other epithelial systems.¹¹ Molecular modeling has identified several potential N-glycosylation sites in the extracellular immunoglobulin-like domains of Cadm1.²⁹ Recent studies in neural tissue suggest that the degree of N-glycosylation may influence both the specificity¹² and the strength⁴² of Cadm1-mediated adhesive interactions.

Given that the Cadm1 protein is widely and strongly expressed, it is perhaps surprising that the phenotype of the *Cadm1*-null mouse is relatively mild (namely, male infertility in homozygous null animals). Similarly, lenses from *Cadm1*-null animals appear normal on gross observation. Despite this, a number of studies suggest that, at the cellular level, the presence or absence of Cadm1 can have marked effects on morphology and behavior. For example, Cadm1 expression is necessary for maintenance of the epithelial phenotype in HEK293 cells.³⁵ Suppression of Cadm1 expression by siRNA results in the flattening of the cells and the persistence of immature adhesion complexes. Cadm1 has also been shown to play a role in shaping neuronal growth cones, where its presence serves to restrict the number of active filopodia.³⁶ We used confocal microscopy to evaluate the cellular phenotypes of epithelial and fiber cells from lenses deficient in Cadm1. The structure of epithelial cells was similar to that reported previously²⁷ and appeared to be unaffected by the absence of Cadm1, despite the fact that the basolateral membranes of the epithelial cells contained the highest levels of Cadm1 in the lens. In contrast, fiber cell morphology was significantly disturbed in lenses from *Cadm1*-null mice. Scanning electron microscopic analysis has shown that fiber cells from the cortex of wild-type lenses have a regular, undulating appearance and that the sinusoidal form of the fibers appears relatively late in the differentiation process, shortly after the disappearance of cytoplasmic organelles.³⁸ We verified the earlier SEM findings on wild-type lenses using a novel microdissection approach. We confirmed that wild-type fibers have an undulating appearance of regular periodicity. Our analysis also revealed that fibers from *Cadm1*-

null lenses followed an irregular, meandering course. The undulations were irregular in period and exaggerated in amplitude. In wild-type lenses, Cadm1 disappeared from the cell membranes at the time of organelle breakdown, i.e., at approximately the same time that the cellular undulations were formed. We hypothesize that removal of Cadm1 from the membrane may be a prerequisite for the formation of cellular undulations and that the absence of Cadm1 might cause the undulations to form precociously or to a greater extent.

Changes in cell shape suggest that the organization of the lens fiber cell cytoskeleton might be altered in the *Cadm1*-null lens. Previous studies^{7,43} have shown that Cadm1 interacts with the actin cytoskeleton through linker proteins such as DAL-1 (erythrocyte protein band 4.1-like 3 [Eph4.113]) and 4.1G (Epb4112), two proteins that have also been identified in the membrane cytoskeleton of lens fiber cells.⁴³ Cadm1 is also known to interact with MPP3, a human homolog of the *Drosophila* tumor suppressor gene *Discs large* (*Dlg*).⁸ The PDZ-domain protein Dlg is abundantly expressed in the mouse lens, where it plays critical roles in epithelial cell structure and fiber cell morphogenesis.⁴⁴ The cytoplasmic domain of Cadm1 has also been shown to bind Tiam1, a guanine nucleotide exchange factor specific for Rac, a member of the Rho GTPase family. The interaction of Cadm1 with Tiam1 induces the formation of lamellipodia through Rac activation.⁴⁵ Any or all these molecular interactions could contribute to the unique, undulating morphology of wild-type lens fiber cells. Similarly, disruptions in Cadm1/cytoskeletal interactions could result in disturbances in fiber cell architecture. However, the binding partners for Cadm1 in the lens have yet to be positively identified.

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