NIH Public Access
Author Manuscript
J Comp Neurol. Author manuscript; available in PMC 2011 July 27.

Published in final edited form as:
J Comp Neurol. 2007 November 10; 505(2): 166–176. doi:10.1002/cne.21489.

Novel Distribution of Junctional Adhesion Molecule-C in the Neural Retina and Retinal Pigment Epithelium

LAUREN L. DANIELE[1], **RALF H. ADAMS**[2], **DIANE E. DURANTE**[3], **EDWARD N. PUGH Jr.**[1], and **NANCY J. PHILP**[3,*]

[1]F.M. Kirby Center for Molecular Ophthalmology, Department of Ophthalmology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

[2]Vascular Development Laboratory, Cancer Research UK London Research Institute, London WC2A3PX, United Kingdom

[3]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Abstract

Junction adhesion molecules-A, -B, and -C (Jams) are cell surface glycoproteins that have been shown to play an important role in the assembly and maintenance of tight junctions and in the establishment of epithelial cell polarity. Recent studies reported that Jam-C mRNA was increased threefold in the all-cone retina of the *Nrl*$^{-/-}$ mouse, suggesting that Jam-C is required for maturation and polarization of cone photoreceptors cells. We examined the expression of Jams in the mouse retina by using confocal immunofluorescence localization. Jam-C was detected in tight junctions of retinal pigment epithelium (RPE) and at the outer limiting membrane (OLM) in the specialized adherens junctions between Müller and photoreceptor cells. Additionally, Jam-C labeling was observed in the long apical processes of Müller and RPE cells that extend between the inner segments and outer segments of photoreceptors, respectively. Jam-B was also detected at the OLM. In the developing retina, Jam-B and -C were detected at the apical junctions of embryonic retinal neuroepithelia, suggesting a role for Jams in retinogenesis. In eyes from *Jam-C*$^{-/-}$ mice, retinal lamination, polarity, and photoreceptor morphology appeared normal. Although Jam-A was not detected at the OLM in wild-type retinas, it was present at the OLM in retinas of *Jam-C*$^{-/-}$ mice. These findings indicate that up-regulation of Jam-A in the retina compensates for the loss of *Jam-C*. The nonclassical distribution of Jam-C in the apical membranes of Müller cells and RPE suggests that Jam-C has a novel function in the retina.

Indexing terms

Jam-C; Jam-B; retina; cell polarity; adherens junction; outer limiting membrane

---

The retina has been used as a model system for investigating proteins involved in the establishment of polarity and lamination of neural tissues. It is derived from the optic vesicle, an evagination of the embryonic forebrain, which invaginates to form the double-layered optic cup. The outer layer differentiates into the retinal pigmented epithelium (RPE)

© 2007 Wiley-Liss, Inc.

*Correspondence to: Nancy J. Philp, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107. nancy.philp@jefferson.edu.

This article includes Supplementary Material available via the Internet at http://www.interscience.wiley.com/jpages/0021-9967/suppmat.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

and the inner layer into the neural retina. As with the cerebral cortex, the neural retina develops from a single layer of pseudostratified neuroepithelia into a highly organized tissue made up of distinct lamina. Neuroepithelial cells of the embryonic vertebrate nervous system are polarized cells (Chenn et al., 1998) and, like epithelial cells, have distinct apical and basal membrane domains that are separated by adherens junctions. The specification of distinct apical and basal domains in neuroepithelial cells is the foundation for proper cell fate specification and lamination of neural tissue (Zhadanov et al., 1999; Pujic and Malicki, 2001; Kosodo et al., 2004; Junghans et al., 2005; Koike et al., 2005; Imai et al., 2006; Afonso and Henrique, 2006). Proteins belonging to evolutionarily conserved cell polarity complexes such as the Par3 (partitioning defective)/Par6/aPKC (atypical protein kinase C) complex (Ohno, 2001) are found at apical junctional complexes of neuroepithelia in developing retina and cortex (Manabe et al., 2002; Takekuni et al., 2003). Genetic mutations or deletions of adherens junction proteins, such as N-cadherin (Pujic and Malicki, 2001; Erdmann et al., 2003; Masai et al., 2003; Babb et al., 2005; Fu et al., 2006), or of associated cell polarity proteins (Malicki and Driever, 1999; Wei and Malicki, 2002; Wei et al., 2004; van de Pavert et al., 2004; Koike et al., 2005) result in retinas with disordered lamination and cell polarity defects.

In the mature retina, adherens junctions are maintained between photoreceptors and Müller glia. Photoreceptors of the vertebrate retina are part of a unique class of neurons, which, like auditory hair cells of the cochlea and olfactory epithelium, have both neural and epithelial characteristics (Koike et al., 2005; Omori and Malicki, 2006). The outer segments (OS) and inner segments (IS) of photoreceptors are divided from the soma and axon by adherens junctions (Williams et al., 1990; Koike et al., 2005). The adherens junctions between photoreceptors and Müller glia appear in histological sections as a line of demarcation between the outer nuclear layer and the sub-retinal space (SRS) and were thus collectively termed the “outer limiting membrane” (OLM). Proteins associated with both adherens junctions, e.g., N-cadherin, and tight junctions, e.g., zonule occludens 1 (ZO-1), are found at the OLM (Paffenholz et al., 1999; van de Pavert et al., 2004; Koike et al., 2005), leading some to refer to these complexes as “specialized adherens junctions” (Paffenholz et al., 1999). Occludin, an integral membrane protein known to bind ZO-1 at tight junctions, was not detected at the OLM (Paffenholz et al., 1999). Normal elaboration of photoreceptor OS, IS, and synaptic specializations depends on the assembly of adherens junctions (Fu et al., 2006) and recruitment of aPKC (Koike et al., 2005).

Jams are members of the immunoglobulin superfamily that play a role in the assembly and maintenance of tight junctions and in the establishment of epithelial polarity. Members of this family have also been shown to mediate interactions of leukocytes and cancer cells with endothelial cells (Bazzoni, 2003; Ebnet et al., 2004; Santoso et al., 2005). Jams mediate cell– cell interaction through the formation of homophilic and heterophilic *trans* dimers. There are three closely related Jam isoforms (A, B, and C), which all have a similar structure, consisting of an extracellular domain with two Ig-like folds that participate in cell– cell adhesion, a transmembrane segment, and a short cytoplasmic tail (Arrate et al., 2001; Aurrand-Lions et al., 2001b; Ebnet et al., 2004). The cytoplasmic tail of all Jams have a PDZ binding domain that mediates the association of Jams with the Par3/Par6/aPKC complex (Ebnet et al., 2001, 2003; Bazzoni, 2003; Gliki et al., 2004). Jams also associate with another PDZ protein, ZO-1 (Bazzoni et al., 2000; Ebnet et al., 2003). A recent study of mice with a targeted deletion of *Jam-C* has demonstrated that Jam-C is required for maturation and polarization of spermatids in the seminiferous tubules (Gliki et al., 2004). In the absence of Jam-C, the Par3/Par6/aPKC complex is not properly localized to junctions made between spermatids and supportive Sertoli cells. Spermatids of *Jam-C*$^{-/-}$ mice fail to develop the membrane specializations characteristic of mature polarized sperm, such as the acrosomes and flagella (Gliki et al., 2004).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Despite the implication of Jams in epithelial and endothelial cell polarity, their expression in neural tissues and their roles in neuroepithelial and sensory cell polarity have not been investigated. Jam-C mRNA levels were found to be increased threefold in the retinas of mice null for the neural retina leucine zipper protein (*Nrl*$^{-/-}$; Yoshida et al., 2004), whose photoreceptors have been shown to be exclusively cones (Nikonov et al., 2005; Daniele et al., 2005). In this study, we examined the expression of Jam proteins in the mouse retina and investigated whether Jam-C is required for normal lamination of the retina and polarity of RPE and photoreceptor cells.

MATERIALS AND METHODS

Animals

All experiments were performed in compliance with National Institutes of Health guidelines, as approved by the Institutional Animal Care and Use Committees of Thomas Jefferson University and the University of Pennsylvania.

Chemicals and antibodies

Goat anti-mouse Jam-A (AF1077; Kang et al., 2007) and Jam-C (AF1213) and rat anti-mouse Jam-B (MAB9882) antibodies were obtained from R&D Systems (Minneapolis, MN). Other antibodies used in this study were obtained from the following sources: anti-β-actin (A5441; Sigma, St. Louis, MO); anti-β-catenin (6F9; Sigma); anti-carbonic anhydrase II, which was a gift of Dr. Paul Linser (University of Florida, Jacksonville; Linser et al., 1984; Ochrietor et al., 2005); anti-carbonic anhydrase XIV, which was a gift of Dr. William Sly (Washington University, St. Louis; Nagelhus et al., 2005); anti-M cone opsin (AB5405; Chemicon, Temecula, CA); anti-cone transducin (sc-390; Santa Cruz Biotechnology, Santa Cruz, CA); antiezrin (E8897; Sigma); anti-MCT3 (Philp et al., 2003); anti-rhodopsin (4D2; Laird and Molday, 1988); and anti-ZO-1 (40-2200; Zymed, San Francisco, CA). Antibodies to Jam-B and Jam-C reacted with retinal tissue from wild-type (wt) but not *Jam-B*$^{-/-}$ or *Jam-C*$^{-/-}$ mice, respectively. All other antibodies labeled eye tissues with an expected distribution based on previously reported studies. (See Supplementary Table 1 for the methods used to determine the specificity of each antibody and the immunogens used to generate them.) TO-PRO-3 iodide and Alexa-Fluor conjugates of phalloidin, avidin, and secondary antibodies were obtained from Molecular Probes (Eugene, OR). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Bio-Rad Laboratories (Hercules, CA) and Molecular Probes.

Immunohistochemical analysis

Mice were killed by overdose of anesthetic and eyes removed with a fine surgical blade and forceps directly or after cardiac perfusion and fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4). Eyes were cryoprotected by equilibration with graded concentrations of sucrose (10%, 20%, and 30%) in PBS and then embedded in Tissue Freezing Medium (Triangle Biomedical Sciences, Durham, NC) and frozen in liquid nitrogen. Cryosections 10 –20 μm thick were made and collected on Superfrost Plus slides (Fisher Scientific, Waltham, MA). For histochemical analyses of retinas from mice 3 days old and younger, the whole head was subjected to the fixation, embedding, and cryosectioning procedure described above. For antibody labeling, cryosections were blocked with 5% bovine serum albumin (BSA) in PBS with 0.1% Tween 20 (PBST) for 1 hour and incubated overnight at 4°C with primary antibodies. Cryosections were then washed with PBST, incubated in secondary antibody for 30 minutes, washed, and mounted with Gelvatol. Both primary and secondary antibodies were diluted in 1% BSA in PBST. Immunofluorescence labeling of tissue was visualized with a Zeiss LSM 510 confocal microscope (Zeiss Microscope Imaging, Inc., Thornwood, NY). Images were exported in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TIF format with LSM Image Browser software (version 3,5,0,376; Carl Zeiss GmbH Jena 1997–2005; Zeiss Microscope Imaging, Inc., Thornwood, NY), and adjustments were made to brightness and contrast only. Figures 1, 3, 6 and 7 show optical projections of 3.5– 6 μm thickness. All other figures show single optical sections.

Western blot analysis

Neural retinas were isolated from eyes of 4 – 6-week-old wt and $Nrl^{-/-}$ mice, and proteins were solubilized with ice-cold lysis buffer (25 mM HEPES buffer, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100) containing protease inhibitors (Complete Mini; Roche, Indianapolis, IN) for 30 minutes, then centrifuged at 14,000*g* (4°C) for 30 minutes. Protein concentration of cleared lysates was determined using the BCA Reagent (Pierce, Rockford, IL) and SOFTmax Pro software (Molecular Devices Corporation, Sunnyvale, CA). The lysates used for immunoblot analysis were diluted in 2× LDS sample buffer (Invitrogen, Carlsbad, CA). Samples of equal protein mass (15 μg) from wt and $Nrl^{-/-}$ mouse retinas were electrophoretically separated on 4 –12% NuPAGE gels (Invitrogen) and then transferred to Immobilon-P membranes (Millipore, Bedford, MA). Membranes were incubated for 1 hour at room temperature in blocking buffer (20 mM Tris, 137 mM NaCl, pH 7.5, 5% dry skim milk), followed by a 1-hour incubation with primary antibodies and a 30-minute incubation with HRP-conjugated secondary antibodies diluted 1:5,000. Blots were probed with the following antibodies and dilutions: Jam-C (1:500) and carbonic anhydrase XIV (1:5,000). β-Actin (1:20,000) was used as an internal loading control. Reactive bands were visualized with chemiluminescence detection reagents (ECL; Amersham, GE Healthcare Bio-Sciences, Corp., Piscataway, NJ).

Retinal tissue dissociation

Retinal cells were enzymatically dissociated with papain (Papain Dissociation Kit; Worthington Biochemical Corporation, Lakewood, NJ) using a published procedure (Wahlin et al., 2004). Eyes were enucleated from 6 –10-week-old C57BL/6J mice immediately after death. The anterior portion of the eye was removed with a sharp razor, and the retina was separated from the RPE in 1× PBS without Ca^{2+} and Mg^{2+} at room temperature. Each retina was gently teased into ~10 pieces using fine needles, then incubated in papain solution for 1–5 minutes. The papain was then washed out with three changes of PBS, and the neural retina was dissociated further by trituration with glass-fired pipettes. Cells were fixed in 2% paraformaldehyde with 1.5% sucrose in PBS. After ~10 minutes, the cell suspension was pipetted onto poly-L-lysine slides (Erie Scientific Company, Portsmouth, NH) and the slides placed at 4°C for 1 hour. The unattached cells were gently removed with a transfer pipette, and the attached cells were washed with three changes of PBS. Cells were then immunolabeled with the same procedure used for cryosections, as described above.

RESULTS

Jam-C is polarized to the apical processes of Müller cells and RPE cells

Jams have been localized to tight junctional complexes of endothelia and epithelia (Aurrand-Lions et al., 2001a,b; Ebnet et al., 2003; Zen et al., 2004); however, the expression of these proteins in neuroepithelia has not previously been investigated. Expression of Jam-A–C in the adult mouse retina was examined by immunofluorescence localization on fixed frozen sections. We were surprised to find that Jam-C expression was not limited to the apical junctional complexes but was also detected in the long apical processes of both Müller and RPE cells. As shown in Figure 1A, Jam-C localized to the actin-containing microvilli (Müller cells) and lamellapodia (RPE) that extend into the subretinal space (SRS) and ensheath photoreceptors. Punctate labeling with the Jam-C antibody was detected at the OLM, where phalloidin labeled the circumferential bundles of actin filaments associated

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

with the adherens junctions (Fig. 1A). A similar localization pattern was found in cryosections of human retina (data not shown). In cryosections labeled with anti-Jam-C and anti-ZO-1, the two proteins were found to colocalize at the OLM (Fig. 2A). Double labeling with antibodies to Jam-C and β-catenin, a marker for adherens junctions, revealed that Jam-C was localized just apical to the adherens junctions (Fig. 2B). Jam-C and ZO-1 were also found to colocalize at the tight junctions of RPE cells (Fig. 2C,D).

Jam-A and Jam-B immunoreactivity was also detected in the retina, but the distributions were more limited. Jam-B labeling was detected at the OLM but not on Müller cell or RPE apical processes (Fig. 1B). Jam-A labeled microglia found in the inner layers of the retina but was not detected at the OLM. In RPE cells, Jam-A was found at tight junctional plaques (data not shown), as recently reported in chick (Luo et al., 2006a) and human RPE (Luo et al., 2006b).

Jam-C protein is increased in the all-cone *Nrl*$^{-/-}$

The levels of *Jam-C* mRNA in retinas isolated from *Nrl*$^{-/-}$ mice were found to be increased by about threefold compared with wt (Mears et al., 2001; Yoshida et al., 2004). We confirmed this finding with semiquantitative RT-PCR (data not shown). In wt retinas, rods outnumber cones by a ratio of 30:1. The retinas of *Nrl*$^{-/-}$ mice do not develop rods but instead develop an increased number of cones (Mears et al., 2001; Daniele et al., 2005). To determine whether the increase in Jam-C mRNA was accompanied by an increased expression of protein, immunoblot analysis was performed on protein samples of equivalent concentration extracted from *Nrl*$^{-/-}$ and wt mouse eyes (Fig. 3A). The blot was divided and probed for Jam-C and carbonic anhydrase XIV (CA-XIV). The Jam-C antibody detected a band that migrated with a mobility of ~43 kDa in lanes loaded with samples from the *Nrl*$^{-/-}$ and wt retinas as well as from RPE (Fig. 3A, and data not shown). The intensity of signal for Jam-C was greater in protein samples prepared from *Nrl*$^{-/-}$ retinas than from wt retinas. CA-XIV is expressed throughout the retina but is not specifically enriched in cones (Nagelhus et al., 2005). Consistent with this, the level of CA-XIV was similar in samples prepared from Nrl$^{-/-}$ and wt retinas. As a control, the Jam-C blot was reprobed with an antibody to β-actin, and similar levels of protein were detected in both retinal samples.

To confirm that the increase in Jam-C protein levels in the *Nrl*$^{-/-}$ retina was not due to an altered expression pattern, the distribution of Jam-C was investigated with immunofluorescence (Fig. 3B). The Jam-C antibody labeled the apical processes of Müller cells and RPE cells of the *Nrl*$^{-/-}$ retina, consistent with our findings in the wt retina. The width of the SRS was noticeably smaller in the *Nrl*$^{-/-}$, mainly because of the absence of rods (which have ~24-μm long outer segments) and the shorter *Nrl*$^{-/-}$ outer segments compared with those of wt cones (~7 vs. ~13 μm; Mears et al., 2001; Daniele et al., 2005). As observed in retinal sections from wt mice, Jam-C was also localized to the OLM and the tight junctions of the RPE.

Jam-C is expressed in Müller cells and photoreceptor cells

It was evident from the immunolabeling shown in Figure 1 that both Jam-B and Jam-C were present in the OLM, but it was not clear whether both Müller cells and photoreceptors express Jam-B and/or Jam-C. The up-regulation of expression of Jam-C in the *Nrl*$^{-/-}$ mouse retina suggested that Jam-C may be expressed in cones but not in rods. To determine the cell type-specific localization of Jam-B and Jam-C, fixed isolated cells were immunolabeled for these Jams and other proteins exclusive to Müller cells, rods, or cones. The profile in Figure 4A was identified as a cone based on the intense labeling with an antibody against mouse cone transducin. Jam-C is localized to the narrow region between the inner segment and soma with a granular or punctate appearance and to the cone process extending from the

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

base of the soma. All cells identified with cone transducin were positive for Jam-C (n = 35). Jam-C was also localized to isolated rods. The profile shown in Figure 4B was intensely labeled with an anti-rhodopsin antibody, and Jam-C immunoreactivity was detected at the base of the inner segment where it joins the process connecting to the soma. Jam-C labeling was observed in the same location as shown in Figure 4B in all rhodopsin-positive cells identified as rods (n = 26). The profile in Figure 4C was identified as a Müller cell based on its morphology and labeling with an antibody to carbonic anhydrase II (CA-II), a protein expressed in Müller cells (Ochrietor et al., 2005). Jam-C was detected on all Müller cells, and the labeling was intense over the whole Müller cell membrane (Fig. 4C). The Müller cell microvillus processes were not evident and appeared to be lost in our preparations. Expression of Jam-B appeared to be rod specific. Jam-B immunolabel was frequently detected on isolated rods (12 of 15 cells) and similarly to Jam-C was localized to the base of the inner segment (Suppl. Fig. 1). Jam-B was not detected on cones (n = 9) or Müller cells.

Expression of Jam-C during retinogenesis

We examined Jam-C expression during retinogenesis, a period when it could play a role in cell– cell adhesion or as a cell polarity cue within the retinal neuroepithelial sheet. Cryosections from embryonic and early postnatal stages of mouse retinas were immunolabeled with an anti-Jam-C antibody. At embryonic day 10.5 (E10.5), Jam-C was detected in all cells of the neuroblast layer, with the most intense labeling at the apical (ventricular) margin (Fig. 5B). The focal points of Jam-C label at the ventricular surface of the neuroepithelium overlapped with the phalloidin label for f-actin (Fig. 5C,D). By E17, discrete lamina develop in the retina, the presumptive ganglion cell layer, and the neuroblast layer. Jam-C was still detected in all cells of the neuroblast layer, with particularly strong localization at the apical margin, but cell processes within the ganglion cell layer were labeled as well (Fig. 5F). The more intense phalloidin label at the apical (ventricular) margin of the neuroepithelia (Fig. 5C,G) has been shown to correspond to the actin bundles associated with the adherens type cell– cell junctions between neuroepithelial progenitors in the developing brain and retina (Chenn et al., 1998; Malicki and Driever, 1999; Manabe et al., 2002; Koike et al., 2005). Expression of Jam-A was detected in the surface ectoderm of the embryonic eye, nascent choroidal vessels, blood vessels present at the inner surface of the retina, and nascent blood vessels in the zone between the ganglion cell and neuroblast layers (Fig. 6A–C). The localization pattern of Jam-A in the embryonic retina is consistent with its role in angiogenesis (Cooke et al., 2006; Kang et al., 2007). Jam-B was detected in a discrete zone corresponding to the apical junctions of retinal neuroepithelia (Fig. 6D–F).

Retinal lamination and photoreceptor cell maturation is not disrupted in *Jam-C*$^{-/-}$ mice

The expression of *Jam-C* at the apical junctions of the optic cup suggested that it may play a role in the lamination of the neural retina and polarization of photoreceptor and RPE cells. Initial characterization of the *Jam-C*$^{-/-}$ mouse revealed that the animals are sterile because of impaired maturation of sperm, but an ocular phenotype was not described (Gliki et al., 2004). At the macroscopic level, the eyes of the *Jam-C*$^{-/-}$ were about 50% smaller than wt eyes, and the lenses were opaque (data not shown). Examination of the lenses in frozen sections revealed the presence of a nuclear cataract. However, despite these more obvious anomalies, the thickness of the outer and inner nuclear layers and the lamination of the *Jam-C*$^{-/-}$ retina were normal (Fig. 7A).

To determine whether protein trafficking was disrupted in the *Jam-C*$^{-/-}$, cryosections were labeled with antibodies to proteins polarized to distinct membrane domains in RPE and photoreceptor cells. In *Jam-C*$^{-/-}$ retinas, there were no differences in the localization of rhodopsin in rods or cone transducin in cones compared with wt (Fig. 7B, and data not shown). Additionally, there was no change in the morphology of the rod and cone

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

photoreceptor cells. The distribution of ezrin, a protein expressed at the apical microvilli of RPE cells, and MCT3, a basolateral membrane monocarboxylate transporter, was normal (data not shown). Thus, the immunohistochemistry demonstrates proper polarized distribution of RPE and photoreceptor-specific proteins and also highlights the normal elaboration of polarized membrane structures in these cells.

Increased expression of Jam-A at the OLM of *Jam-C*$^{-/-}$ mice

As described above, no disruption of cell polarity or lamination occurred in the absence of Jam-C. To test the possibility that the loss of Jam-C is compensated for by a developmentally regulated increase in another Jam isoform, we investigated the expression pattern of Jam-A and Jam-B in the retinas of the *Jam-C*$^{-/-}$ mouse. Cryosections of the *Jam-C*$^{-/-}$ mouse retina were immunolabeled for Jam-A and Jam-B. As in the wt retina, Jam-B was detected only at the OLM in the *Jam-C*$^{-/-}$ retina. Although Jam-A was not detected at the OLM of the wt retina, it was detected in the OLM of retinas from the *Jam-C*$^{-/-}$ mouse (Fig. 7C). Figure 7C shows that Jam-A colocalized with ZO-1 at the OLM of the *Jam-C*$^{-/-}$ retina, indicating that junctions between Müller cells and photoreceptors were assembled and in the proper location. Jam-A was not detected in the apical processes of Müller cells or RPE.

DISCUSSION

Members of the Jam family participate in the biogenesis of junctional complexes, establishment of cell polarity, and regulation of tight junction permeability. In these studies, we show for the first time that Jam-C not only is a component of the junctional complexes in the retina but is also expressed on the apical processes of Müller glia and RPE. This unique distribution of Jam-C suggests that this adhesion molecule might have a functional role in maintaining a close association of RPE and Müller cells with photoreceptors required to facilitate the exchange of metabolites necessary for photoreceptor function.

Two *tight* junction-associated proteins, Jam-B and Jam-C, are localized to “specialized” *adherens* junctions, forming the OLM of the retina

The subretinal space (SRS) is a privileged extracellular milieu into which the apical domains of photoreceptors project. Two permeability barriers circumscribe the SRS, an inner barrier formed by the “specialized” adherens junctions between Müller cells and photoreceptors (Williams et al., 1990) and an outer barrier formed by the tight junctions of the RPE. Though not as restrictive as RPE tight junctions, the OLM barrier is impermeable to proteins with Stokes radii greater than 30 –36 Å (Bunt-Milam et al., 1985). Our studies show for the first time that Jam-B and Jam-C localize at the OLM just apical to the adherens junctions and colocalize with ZO-1 (Fig. 2, and data not shown). ZO-1 is a scaffolding protein that is known to bind to the cytoplasmic domains of Jams. Although ZO-1 was previously detected at the OLM apical to cadherin, a binding partner was not identified (Paffenholz et al., 1999; van de Pavert et al., 2004). Given that a direct binding of Jam-C to ZO-1 has been demonstrated (Bazzoni et al., 2000; Ebnet et al., 2003), their colocalization at the OLM suggests a direct interaction.

Recent in vitro studies have shown that Jams can regulate the paracellular permeability at tight junctions (Martin-Padura et al., 1998; Aurrand-Lions et al., 2001a,b; Mandell et al., 2005; Orlova et al., 2006; Mandicourt et al., 2007) and are important for the resealing of epithelial tight junctions in a model of tight junction disruption (Liu et al., 2000; Liang et al., 2000). The presence of Jams at the SRS boundaries suggests that Jams may be involved in formation and regulation of the permeability barriers at the OLM (Jam-C and -B) as well as at the RPE layer (Jam-A and -C). The importance for the regulation of the permeability

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

barrier at the OLM was demonstrated for IRPB, a soluble protein found in the interphotoreceptor matrix that shuttles retinoids between the RPE and photoreceptor cell outer segments (for review see Lamb and Pugh, 2004). Thus, the OLM barrier and the tight junctions of the RPE prevent the diffusion of IRBP out of the SRS, where it is specifically needed (Bunt-Milam et al., 1985).

Isolated retinal cell preparations clearly show that Jam-C is expressed in both rods and cones. Jam-C has a more widespread distribution in isolated cones compared with rods, which could explain the increased expression of Jam-C in *Nrl*$^{-/-}$ mouse. In cones, Jam-C was localized to the inner segment base and the apex of the soma as well as in processes. In rods, Jam-C localized to a very narrow band at the inner segment base (Fig. 4A,B). Furthermore, cone inner segments are wider than rod inner segments (Carter-Dawson and Lavail, 1979). Thus, the increased expression of Jam-C in the *Nrl*$^{-/-}$ could reflect a higher expression and more widespread membrane distribution of Jam-C in cones vs. rods.

Unexpected localization of Jam-C to apical processes of RPE and Müller cells facing the SRS

Our data show a striking polarization of Jam-C to the apical Müller cell and RPE processes (Fig. 1), suggesting an additional role for this protein beyond its involvement in apical junctions. Photoreceptors depend on close contact with the apposing RPE layer, a critical source for metabolites and neurotrophic factors and the site for much of the visual cycle. The apical regions of the RPE, Müller cells, and photoreceptors all project into an extracellular matrix, called the “interphotoreceptor matrix” (IPM) composed of hyaluronan, proteoglycans, and glycoproteins. Interaction of these cells with the IPM is thought to be critical for maintaining the apposition of RPE and retina. Chemical disruption of components of the IPM leads to retinal detachment (Lazarus and Hageman, 1992; Hageman et al., 1995). Some cell surface adhesion molecules found at the apical domains of the cells facing the SRS participate in cell-IPM adhesion. For example, CD44 a transmembrane glycoprotein expressed on Müller cell microvilli has binding domains for IPM components hyaluronan as well as chondroitin sulfate (Chaitin et al., 1994). Thus, the pattern of expression of Jam-C in the SRS presents an intriguing possibility that Jam-C may participate in RPE-IPM and/or Müller cell-IPM adhesion.

Another possibility suggested by the apical polarization of Jam-C in Müller glia and RPE processes is that it is acting as a chaperone regulating membrane targeting of another protein. Another Ig superfamily protein, CD147, has been shown to be necessary for the proper targeting of proton-coupled transporters to the cell surface (Philp et al., 2003; Halestrap and Meredith, 2004).

The presence of Jam-C in the apical processes of the RPE and Müller glia may be regulated by post-translational modification. It was found that the tight junctional localization of Jam-C is negatively regulated by phosphorylation of C-terminal serine 281 (Ebnet et al., 2003; Mandicourt et al., 2007). Thus the dual localization of Jam-C in the retina suggests that two forms (phosphorylated and nonphosphorylated) are present.

Embryonic expression of Jam-C in the retina indicates possible early involvement in cell polarity and retinal lamination

In the embryonic retina, Jam-C is enriched at the apical surface of pseudostratified neuroepithelia (Fig. 5). Jam-C binds to Par3, which has recently been shown to act as an upstream organizer coordinating the recruitment of the Par3/Par6/aPKC complex as well as other junctional complex proteins to the apical membrane of neuroepithelial cells (Afonso and Henrique, 2006). Jams heterologously expressed in CHO cells can lead to the assembly

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

of the Par3/Par6/aPKC complex otherwise absent at apical membrane sites. Furthermore, ablation of Jam-C in mice led to improper recruitment of Par6 and aPKC to the proximity of adherens junctions in spermatids, resulting in defects in spermatid polarization (Gliki et al., 2004). Soluble Jam-C applied to spermatids was sufficient to recruit members of the cell polarity complex and to activate the GTPase cdc42 (Gliki et al., 2004). Therfore, the localization of Jam-C to the apical junctional complexes in retinal neuroepithelia suggested to us that its expression may be critical for normal lamination and polarity of the retina. Although it was surprising that we did not detect any lamination or polarity defects in the *Jam-C*$^{-/-}$ retina (Fig. 7A), our detection of Jam-A at the OLM colocalizing with ZO-1 (Fig. 7C) suggests that it may compensate for the loss of Jam-C at the OLM.

Jam-A, -B, and -C share a relatively high degree of sequence homology (Arrate et al., 2001). Thus, it is likely that some of the functions of Jam-C have been subsumed by Jam-A in the *Jam-C*$^{-/-}$ mouse. Functional redundancy has recently been identified for ZO-1 and ZO-2 in tight junction strand formation (Umeda et al., 2006), for aPKCλ and aPKCζ in vertebrate retinogenesis (Cui et al., 2007), and for crumbs homologues Crb-1, -2, and -3 in specification of apical membrane domains in cilia-containing cells (Omori and Malicki, 2006).

Although the thicknesses of the retinal layers of the *Jam-C*$^{-/-}$ mouse were comparable to wt, the eyes were much smaller and the lenses opaque. It is likely that the microphthalmia observed in the *Jam-C*$^{-/-}$ mouse results from the disruption of normal development and growth of the lens. It is well established that the lens regulates growth of the eye, insofar as ablation of the lens or mutation of genes critical for lens development results in microphthalmia (Coulombre and Coulombre, 1964). Previous reports have shown that deletion of the Cx50 gene results in a small eye and the appearance of a zonular pulverulent nuclear cataract, without changes in lamination or cell thickness of the neural retinal layers (White et al., 1998). Examination of the lens in sections of the eye from the *Jam-C*$^{-/-}$ mouse revealed a nuclear cataract, indicating that Jam-C is required for growth and clarity of the lens. Further studies are underway to test for the existence of more subtle visual function deficits in *Jam-C*$^{-/-}$ mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant sponsor: National Institutes of Health; Grant number: EY-012042; Grant number: EY-02660; Grant sponsor: The Foundation Fighting Blindness; Grant sponsor: Research to Prevent Blindness Foundation.

LITERATURE CITED

Afonso C, Henrique D. PAR3 acts as a molecular organizer to define the apical domain of chick neuroepithelial cells. J Cell Sci. 2006; 119:4293–4304. [PubMed: 17003110]

Arrate MP, Rodriguez JM, Tran TM, Brock TA, Cunningham SA. Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2 counterreceptor. J Biol Chem. 2001; 276:45826–45832. [PubMed: 11590146]

Aurrand-Lions M, Duncan L, Ballestrem C, Imhof BA. JAM-2, a novel immunoglobulin superfamily molecule, expressed by endothelial and lymphatic cells. J Biol Chem. 2001a; 276:2733–2741. [PubMed: 11053409]

Aurrand-Lions M, Johnson-Leger C, Wong C, Du Pasquier L, Imhof BA. Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members. Blood. 2001b; 98:3699–3707. [PubMed: 11739175]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Babb SG, Kotradi SM, Shah B, Chiappini-Williamson C, Bell LN, Schmeiser G, Chen E, Liu Q, Marrs JA. Zebrafish R-cadherin (Cdh4) controls visual system development and differentiation. Dev Dyn. 2005; 233:930–945. [PubMed: 15918170]

Bazzoni G. The JAM family of junctional adhesion molecules. Curr Opin Cell Biol. 2003; 15:525–530. [PubMed: 14519386]

Bazzoni G, Martinez-Estrada OM, Orsenigo F, Cordenonsi M, Citi S, Dejana E. Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. J Biol Chem. 2000; 275:20520–20526. [PubMed: 10877843]

Bunt-Milam AH, Saari JC, Klock IB, Garwin GG. Zonulae adherentes pore size in the external limiting membrane of the rabbit retina. Invest Ophthalmol Vis Sci. 1985; 26:1377–1380. [PubMed: 4044165]

Carter-Dawson LD, Lavail MM. Rods and cones in the mouse retina. 1. structural analysis using light and electron microscopy. J Comp Neurol. 1979; 188:245–262. [PubMed: 500858]

Chaitin MH, Wortham HS, Brunzinkernagel AM. Immunocytochemical localization of CD44 in the mouse retina. Exp Eye Res. 1994; 58:359–365. [PubMed: 7513650]

Chenn A, Zhang YA, Chang BT, McConnell SK. Intrinsic polarity of mammalian neuroepithelial cells. Mol Cell Neurosci. 1998; 11:183–193. [PubMed: 9675050]

Cooke VG, Naik MU, Naik UP. Fibroblast growth factor-2 failed to induce angiogenesis in junctional adhesion molecule-A-deficient mice. Arterioscler Thromb Vasc Biol. 2006; 26:2005–2011. [PubMed: 16809549]

Coulombre AJ, Coulombre JL. Lens development. I. Role of lens in eye growth. J Exp Zool. 1964; 156:39–47. [PubMed: 14189921]

Cui S, Otten C, Rohr S, Abdelilah-Seyfried S, Link BA. Analysis of aPKClambda and aPKCzeta reveals multiple and redundant functions during vertebrate retinogenesis. Mol Cell Neurosci. 2007; 34:431–444. [PubMed: 17223574]

Daniele LL, Lillo C, Lyubarsky AL, Nikonov SS, Philp N, Mears AJ, Swaroop A, Williams DS, Pugh EN. Cone-like morphological, molecular, and electrophysiological features of the photoreceptors of the Nrl knockout mouse. Invest Ophthalmol Vis Sci. 2005; 46:2156–2167. [PubMed: 15914637]

Ebnet K, Suzuki A, Horikoshi Y, Hirose T, Meyer zu Brickwedde MK, Ohno S, Vestweber D. The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). EMBO J. 2001; 20:3738–3748. [PubMed: 11447115]

Ebnet K, Aurrand-Lions M, Kuhn A, Kiefer F, Butz S, Zander K, Meyer zu Brickwedde MK, Suzuki A, Imhof BA, Vestweber D. The junctional adhesion molecule (JAM) family members JAM-2 and JAM-3 associate with the cell polarity protein PAR-3: a possible role for JAMs in endothelial cell polarity. J Cell Sci. 2003; 116:3879–3891. [PubMed: 12953056]

Ebnet K, Suzuki A, Ohno S, Vestweber D. Junctional adhesion molecules (JAMs): more molecules with dual functions? J Cell Sci. 2004; 117:19–29. [PubMed: 14657270]

Erdmann B, Kirsch FP, Rathjen FG, More MI. N-cadherin is essential for retinal lamination in the zebrafish. Dev Dyn. 2003; 226:570–577. [PubMed: 12619142]

Fu XY, Sun HX, Klein WH, Mu XQ. Beta-catenin is essential for lamination but not neurogenesis in mouse retinal development. Dev Biol. 2006; 299:424–437. [PubMed: 16959241]

Gliki G, Ebnet K, Aurrand-Lions M, Imhof BA, Adams RH. Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional adhesion molecule-C. Nature. 2004; 431:320–324. [PubMed: 15372036]

Hageman GS, Marmor MF, Yao XY, Johnson LV. The interphotoreceptor matrix mediates primate retinal adhesion. Arch Ophthalmol. 1995; 113:655–660. [PubMed: 7748138]

Halestrap AP, Meredith D. The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch Eur J Physiol. 2004; 447:619–628. [PubMed: 12739169]

Imai F, Hirai S, Akimoto K, Koyama H, Miyata T, Ogawa M, Noguchi S, Sasaoka T, Noda T, Ohno S. Inactivation of aPKC lambda results in the loss of adherens junctions in neuroepithelial cells without affecting neurogenesis in mouse neocortex. Development. 2006; 133:1735–1744. [PubMed: 16571631]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Junghans D, Hack I, Frotscher M, Taylor V, Kemler R. Beta-catenin-mediated cell-adhesion is vital for embryonic forebrain development. Dev Dyn. 2005; 233:528–539. [PubMed: 15844200]

Kang LI, Wang Y, Suckow AT, Czymmek KJ, Cooke VG, Naik UP, Duncan MK. Deletion of JAM-A causes morphological defects in the corneal epithelium. Int J Biochem Cell Biol. 2007; 39:576–585. [PubMed: 17118692]

Koike C, Nishida A, Akimoto K, Nakaya MA, Noda T, Ohno S, Furukawa T. Function of atypical protein kinase C lambda in differentiating photoreceptors is required for proper lamination of mouse retina. J Neurosci. 2005; 25:10290–10298. [PubMed: 16267237]

Kosodo Y, Roper K, Haubensak W, Marzesco AM, Corbeil D, Huttner WB. Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. EMBO J. 2004; 23:2314–2324. [PubMed: 15141162]

Laird DW, Molday RS. Evidence against the role of rhodopsin in rod outer segment binding to RPE cells. Invest Ophthalmol Vis Sci. 1988; 29:419–428. [PubMed: 3343097]

Lamb TD, Pugh EN Jr. Dark adaptation and the retinoid cycle of vision. Prog Ret Eye Res. 2004; 23:307–380.

Lazarus HS, Hageman GS. Xyloside-induced disruption of interphotoreceptor matrix proteoglycans results in retinal detachment. Invest Ophthalmol Vis Sci. 1992; 33:364–376. [PubMed: 1740367]

Liang TW, Demarco RA, Mrsny RJ, Gurney A, Gray A, Hooley J, Aaron HL, Huang A, Klassen T, Tumas DB, Fong S. Characterization of huJAM: evidence for involvement in cell–cell contact and tight junction regulation. Am J Physiol Cell Physiol. 2000; 279:C1733–C1743. [PubMed: 11078687]

Linser PJ, Sorrentino M, Moscona AA. Cellular compartmentalization of carbonic anhydrase-C and glutamine synthetase in developing and mature mouse neural retina. Brain Res. 1984; 315:65–71. [PubMed: 6144368]

Liu Y, Nusrat A, Schnell FJ, Reaves TA, Walsh S, Pochet M, Parkos CA. Human junction adhesion molecule regulates tight junction resealing in epithelia. J Cell Sci. 2000; 113:2363–2374. [PubMed: 10852816]

Luo Y, Fukuhara M, Weitzman M, Rizzolo LJ. Expression of JAM-A, AF-6, PAR-3 and PAR-6 during the assembly and remodeling of RPE tight junctions. Brain Res. 2006a; 1110:55–63. [PubMed: 16859655]

Luo Y, Zhuo YZ, Fukuhara M, Rizzolo LJ. Effects of culture conditions on heterogeneity and the apical junctional complex of the ARPE-19 cell line. Invest Ophthalmol Vis Sci. 2006b; 47:3644–3655. [PubMed: 16877439]

Malicki J, Driever W. oko meduzy Mutations affect neuronal patterning in the zebrafish retina and reveal cell–cell interactions of the retinal neuroepithelial sheet. Development. 1999; 126:1235–1246. [PubMed: 10021342]

Manabe N, Hirai S, Imai F, Nakanishi H, Takai Y, Ohno S. Association of ASIP/mPAR-3 with adherens junctions of mouse neuroepithelial cells. Dev Dyn. 2002; 225:61–69. [PubMed: 12203721]

Mandell KJ, Babbin BA, Nusrat A, Parkos CA. Junctional adhesion molecule 1 regulates epithelial cell morphology through effects on beta 1 Integrins and Rap1 activity. J Biol Chem. 2005; 280:11665–11674. [PubMed: 15677455]

Mandicourt G, Iden S, Ebnet K, Aurrand-Lions M, Imhof BA. JAM-C regulates tight junctions and integrin-mediated cell adhesion and migration. J Biol Chem. 2007; 282:1830–1837. [PubMed: 17099249]

Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, Simmons D, Dejana E. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J Cell Biol. 1998; 142:117–127. [PubMed: 9660867]

Masai I, Lele Z, Yamaguchi M, Komori A, Nakata A, Nishiwaki Y, Wada H, Tanaka H, Nojima Y, Hammerschmidt M, Wilson SW, Okamoto H. N-cadherin mediates retinal lamination, maintenance of fore-brain compartments and patterning of retinal neurites. Development. 2003; 130:2479–2494. [PubMed: 12702661]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Mears AJ, Kondo M, Swain PK, Takada Y, Bush RA, Saunders TL, Sieving PA, Swaroop A. Nrl is required for rod photoreceptor development. Nat Genet. 2001; 29:447–452. [PubMed: 11694879]

Nagelhus EA, Mathiisen TM, Bateman AC, Haug FM, Ottersen OP, Grubb JH, Waheed A, Sly WS. Carbonic anhydrase XIV is enriched in specific membrane domains of retinal pigment epithelium, Muller cells, astrocytes. Proc Natl Acad Sci U S A. 2005; 102:8030–8035. [PubMed: 15901897]

Nikonov SS, Daniele LL, Zhu XM, Craft CM, Swaroop A, Pugh EN. Photoreceptors of Nrl−/− mice coexpress functional S- and M-cone opsins having distinct inactivation mechanisms. J Gen Physiol. 2005; 125:287–304. [PubMed: 15738050]

Ochrietor JD, Clamp MF, Moroz TP, Grubb JH, Shah GN, Waheed A, Sly WS, Linser PJ. Carbonic anhydrase XIV identified as the membrane CA in mouse retina: strong expression in Muller cells and the RPE. Exp Eye Res. 2005; 81:492–500. [PubMed: 16126196]

Ohno S. Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. Curr Opin Cell Biol. 2001; 13:641–648. [PubMed: 11544035]

Omori Y, Malicki J. oko meduzy And related crumbs genes are determinants of apical cell features in the vertebrate embryo. Curr Biol. 2006; 16:945–957. [PubMed: 16713951]

Orlova VV, Economopoulou M, Lupu F, Santoso S, Chavakis T. Junctional adhesion molecule-C regulates vascular endothelial permeability by modulating VE-cadherin-mediated cell–cell contacts. J Exp Med. 2006; 203:2703–2714. [PubMed: 17116731]

Paffenholz R, Kuhn C, Grund C, Stehr S, Franke WW. The arm-repeat protein NPRAP (neurojungin) is a constituent of the plaques of the outer limiting zone in the retina, defining a novel type of adhering junction. Exp Cell Res. 1999; 250:452–464. [PubMed: 10413599]

Philp NJ, Ochrietor JD, Rudoy C, Muramatsu T, Linser PJ. Loss of MCT1, MCT3, and MCT4 expression in the retinal pigment epithelium and neural retina of the 5A11/basigin-null mouse. Invest Ophthalmol Vis Sci. 2003; 44:1305–1311. [PubMed: 12601063]

Pujic Z, Malicki J. Mutation of the zebrafish glass onion locus causes early cell-nonautonomous loss of neuroepithelial integrity followed by severe neuronal patterning defects in the retina. Dev Biol. 2001; 234:454–469. [PubMed: 11397013]

Santoso S, Orlova VV, Song KM, Sachs UJ, Andrei-Selmer CL, Chavakis T. The homophilic binding of junctional adhesion molecule-C mediates tumor cell-endothelial cell interactions. J Biol Chem. 2005; 280:36326–36333. [PubMed: 16118203]

Takekuni K, Ikeda W, Fujito T, Morimoto K, Takeuchi M, Monden M, Takai Y. Direct binding of cell polarity protein PAR-3 to cell–cell adhesion molecule nectin at neuroepithelial cells of developing mouse. J Biol Chem. 2003; 278:5497–5500. [PubMed: 12515806]

Umeda K, Ikenouchi J, Katahira-Tayama S, Furuse K, Sasaki H, Nakayama M, Matsui T, Tsukita S, Furuse M, Tsukita S. ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. Cell. 2006; 126:741–754. [PubMed: 16923393]

van de Pavert SA, Kantardzhieva A, Malysheva A, Meuleman J, Versteeg I, Levelt C, Klooster J, Geiger S, Seeliger MW, Rashbass P, Le Bivic A, Wijnholds J. Crumbs homologue 1 is required for maintenance of photoreceptor cell polarization and adhesion during light exposure. J Cell Sci. 2004; 117:4169–4177. [PubMed: 15316081]

Wahlin K, Lim L, Grice EA, Campochiaro PA, Zack DJ, Adler R. A method for analysis of gene expression in isolated mouse photoreceptor and Muller cells. Mol Vis. 2004; 10:366–375. [PubMed: 15205663]

Wei X, Malicki J. nagie oko, Encoding a MAGUK-family protein, is essential for cellular patterning of the retina. Nat Genet. 2002; 31:150–157. [PubMed: 11992120]

Wei XY, Cheng Y, Luo YY, Shi XH, Nelson S, Hyde DR. The zebrafish Pard3 ortholog is required for separation of the eye fields and retinal lamination. Dev Biol. 2004; 269:286–301. [PubMed: 15081374]

White TW, Goodenough DA, Paul DL. Targeted ablation of connexin50 in mice results in microphthalmia and zonular pulverulent cataracts. J Cell Biol. 1998; 143:815–825. [PubMed: 9813099]

Williams DS, Arikawa K, Paallysaho T. Cytoskeletal components of the adherens junctions between the photoreceptors and the supportive Muller cells. J Comp Neurol. 1990; 295:155–164. [PubMed: 2341633]

Yoshida S, Mears AJ, Friedman JS, Carter T, He S, Oh E, Jing Y, Farjo R, Fleury G, Barlow C, Hero AO, Swaroop A. Expression profiling of the developing and mature Nrl−/− mouse retina: identification of retinal disease candidates and transcriptional regulatory targets of Nrl. Hum Mol Genet. 2004; 13:1487–1503. [PubMed: 15163632]

Zen K, Babbin BA, Liu Y, Whelan JB, Nusrat A, Parkos CA. JAM-C is a component of desmosomes and a ligand for CD11b/CD18-mediated neutrophil transepithelial migration. Mol Biol Cell. 2004; 15:3926–3937. [PubMed: 15194813]

Zhadanov AB, Provance DW, Speer CA, Coffin JD, Goss D, Blixt JA, Reichert CM, Mercer JA. Absence of the tight junctional protein AF-6 disrupts epithelial cell-cell junctions and cell polarity during mouse development. Curr Biol. 1999; 9:880–888. [PubMed: 10469590]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

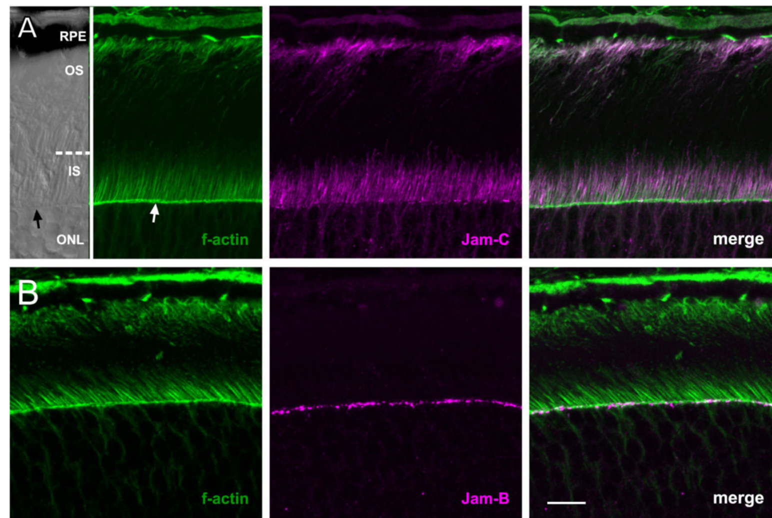

Fig. 1.

Immunofluorescence localization of Jams in the retina. In **A** and **B**, confocal images of the same region of retina are shown with either f-actin staining (left panel with adjacent DIC view in A), immunolabeling with polyclonal antibodies to Jam-C or -B (middle), or a merge of the two labels (right). Arrows in A (left) point to the outer limiting membrane (OLM). RPE, retinal pigment epithelium; OS, outer segment layer; IS, inner segment layer; ONL, outer nuclear layer. Jam-C localizes to fine filamentous processes in the subretinal space, conventionally defined as the region between the OLM and RPE. Scale bar = 10 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

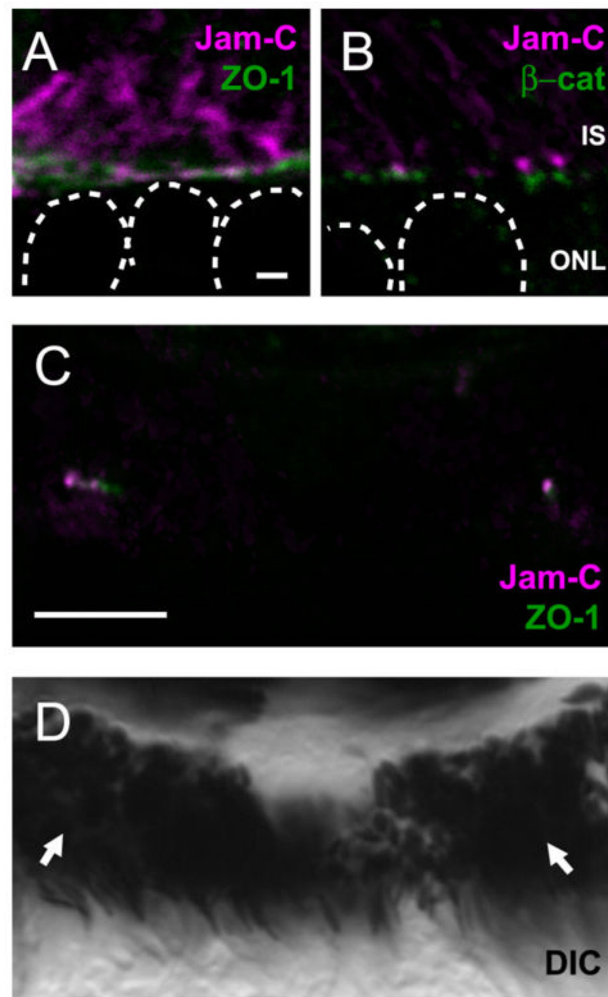

Fig. 2.

Colocalization of Jam-C and adherens junction component ZO-1. Immunolabeling for Jam-C and ZO-1 at the OLM (**A**) and RPE layer (**C**) shows colocalization. Coimmunolabeling for Jam-C and β-catenin at the OLM (**B**). The dashed lines in A and B trace rough outlines of photoreceptor somata obtained from a companion DIC view (not shown). The arrows in **D** represent the boundary of junctional zones of the single RPE cell shown in C. IS, inner segment layer; ONL, outer nuclear layer. Scale bars = 1 μm in A (applies to A,B); 10 μm in C (applies to C,D).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

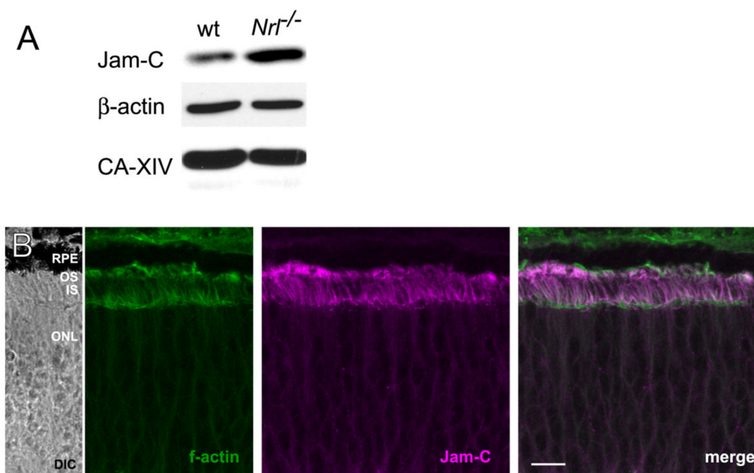

Fig. 3.

Expression and localization of Jam-C in the *Nrl*$^{-/-}$ retina. Western blot analysis reveals Jam-C expression in both wt and *Nrl*$^{-/-}$ retinas (**A**). Equivalent amounts of protein were loaded from wt or *Nrl*$^{-/-}$ retina lysates. Antibodies used to blot are indicated at left (CA-XIV, anti-carbonic anhydrase XIV). Localization of Jam-C in the *Nrl*$^{-/-}$ retina (**B**). The same region of retina is shown stained for f-actin (left panel with adjacent DIC view), immunolabeled for Jam-C (middle), or a merge of the two labels (right). RPE, retinal pigment epithelium; OS, outer segments; IS, inner segments; ONL, outer nuclear layer. Scale bar = 10 μm.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

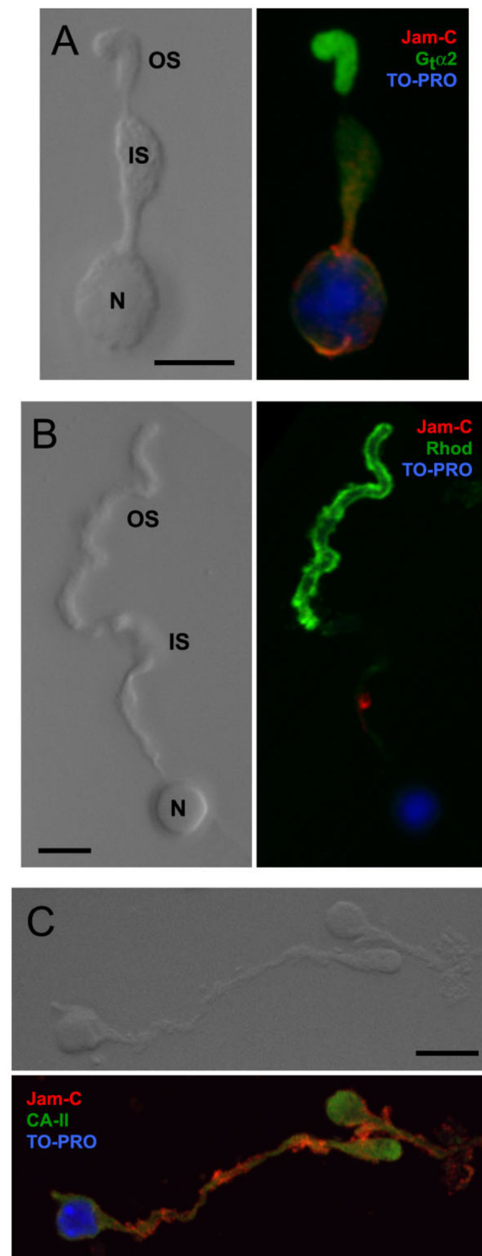

Fig. 4.

Jam-C expression in isolated retinal cells. **A–C** show DIC and immunofluorescence views of the same cell. The cells were classified as a cone (A), a rod (B), and a Müller cell (C). OS, outer segment; IS, inner segment; N, nucleus; $G_t\alpha2$, cone transducin; Rhod, rhodopsin; CA-II, carbonic anhydrase II; TO-PRO: TO-PRO-3 iodide nuclear stain. Scale bars = 5 μm in A,B; 10 μm in C.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

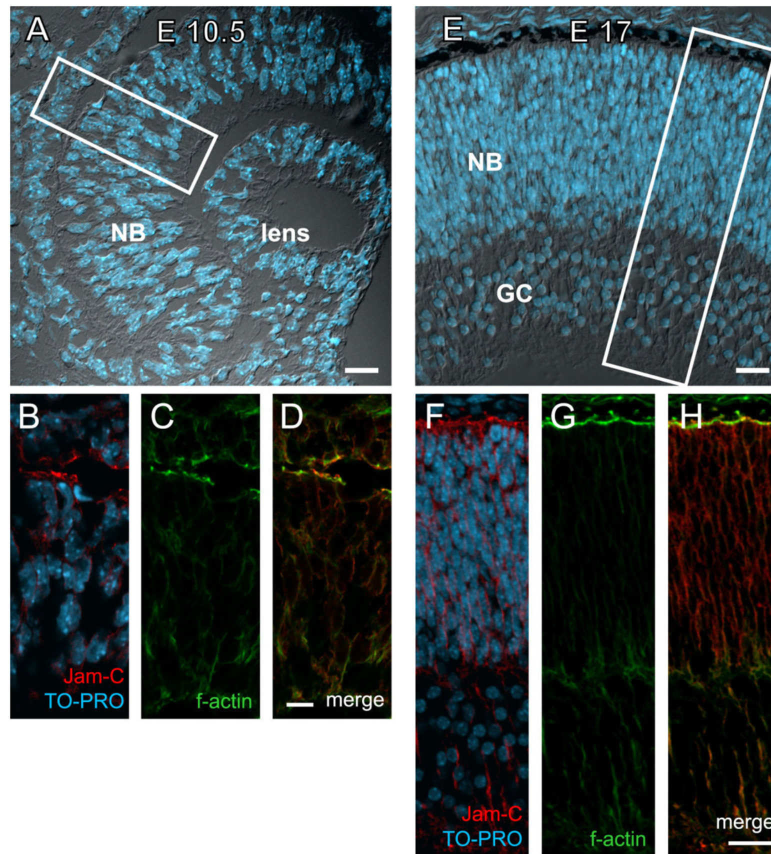

Fig. 5.

Expression of Jam-C during retinogenesis. Cryosections of E10.5 (**A–D**) and E17 (**E–H**) mouse eyes were immunolabeled with a polyclonal antibody to Jam-C. Higher magnification images of the sections in A and E (with overlapping DIC and TO-PRO-3 views) are shown with immunolabeling for Jam-C and TO-PRO-3 nuclear staining (B,F), phalloidin staining for f-actin (C,G), and merge of Jam-C and f-actin (D,H). NB, neuroblast layer; GC, presumptive ganglion cell layer. Scale bars = 20 μm in A,E; 20 μm in H (applies to F–H); 10 μm in D (applies to B–D).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

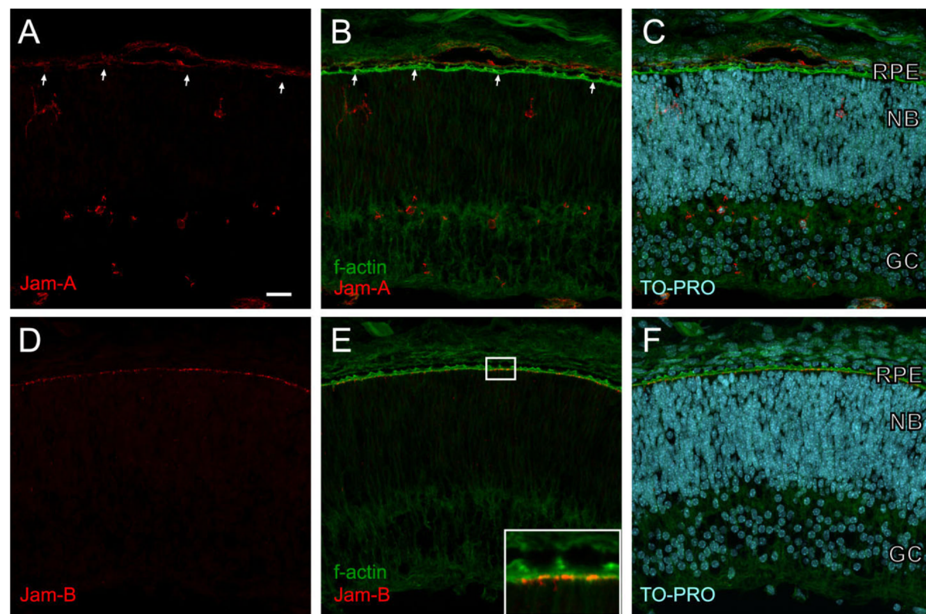

Fig. 6.

Expression of Jam-A and Jam-B during retinogenesis. Confocal images of the retina at embryonic day 17 (E17) are shown with immunolabeling for either Jam-A (**A–C**) or Jam-B (**D–F**), merged with phalloidin stain (B,E) and TOPRO-3 (C,F). For reference, arrows in A and B point to the apex of the neuroepithelial layer. **Inset** in E shows higher magnification view of boxed area. RPE, retinal pigmented epithelium; NB, neuroblast layer; GC, presumptive ganglion cell layer. Scale bar = 20 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

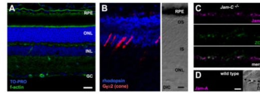

Fig. 7.

Retinal lamination and photoreceptor morphology are unaffected in the *Jam-C*$^{-/-}$. Cryosections of *Jam-C*$^{-/-}$ mouse retina labeled with phalloidin and the nuclear stain TO-PRO-3 (**A**). Higher magnification view of the *Jam-C*$^{-/-}$ retina at the photoreceptor layer (**B**). Immunolabeling for rhodopsin (blue) and cone transducin (red) reveals proper polarized distribution of proteins of the photoreceptor outer segment. Partial DIC view shows normal photoreceptor morphology. Jam-A is immunolocalized to the outer limiting membrane in the *Jam-C*$^{-/-}$ (**C**), whereas none is detected in the wild type (**D**). Dashed line in D traces the OLM on an adjacent DIC view. RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer; GC, ganglion cell nuclei; OS, outer segment layer; IS, inner segment layer; $G_t\alpha2$, anti-cone transducin antibody. Scale bar = 20 μm in A; 5 μm in B; 2 μm in D (applies to C,D).