Protocadherins control the modular assembly of neuronal columns in the zebrafish optic tectum

Sharon R. Cooper,1,2 Michelle R. Emond,1 Phan Q. Duy,1 Brandon G. Liebau,1 Marc A. Wolman,3 and James D. Jontes1,2

1Department of Neuroscience, Ohio State University Wexner Medical Center, Columbus, OH 43210
2Molecular, Cellular, and Developmental Biology Graduate Program, Ohio State University, Columbus, OH 43210
3Department of Zoology, University of Wisconsin–Madison, Madison, WI 53706

Cell–cell recognition guides the assembly of the vertebrate brain during development. δ-Protocadherins comprise a family of neural adhesion molecules that are differentially expressed and have been implicated in a range of neurodevelopmental disorders. Here we show that the expression of δ-protocadherins partitions the zebrafish optic tectum into radial columns of neurons. Using in vivo two-photon imaging of bacterial artificial chromosome transgenic zebrafish, we show that pcdh19 is expressed in discrete columns of neurons, and that these columnar modules are derived from proliferative pcdh19+ neuroepithelial precursors. Elimination of pcdh19 results in both a disruption of columnar organization and defects in visually guided behaviors. These results reveal a fundamental mechanism for organizing the developing nervous system: subdivision of the early neuroepithelium into precursors with distinct molecular identities guides the autonomous development of parallel neuronal units, organizing neural circuit formation and behavior.

Introduction

The vertebrate nervous system becomes progressively regionalized during development (Kiecker and Lumsden, 2005). These compartments may be large morphological subdivisions such as cerebellum, functional specializations such as visual cortex, or local regions such as laminae or nuclei. This partitioning of the nervous system into distinct domains enables each region to undergo a distinct developmental program. Despite the importance to nervous system development, the molecular and cellular mechanisms governing this modular assembly are not well understood.

The optic tectum, the largest subdivision in the zebrafish brain, processes retinal inputs to mediate vision (Portugues and Engert, 2009). Morphological studies in frogs (Lázár, 1973) and fish (Vanegas et al., 1974; Meek and Schellart, 1978) have identified multiple types of tectal neurons and have also revealed a conserved laminated structure. However, neither the cellular architecture of the optic tectum nor the mechanisms governing tectum development are known (Recher et al., 2013). δ-Protocadherins (δ-pcdhs) comprise a family of homophilic cell adhesion molecules (Wolverton and Lalande, 2001; Vanhalst et al., 2005), and prior work has shown that δ-pcdhs are strongly expressed in the zebrafish optic tectum (Biswas and Jontes, 2009; Emond et al., 2009; Liu et al., 2009, 2010; Blevins et al., 2011). Although the detailed function of these molecules is unclear, members of this family can participate in axon guidance (Leung et al., 2013), arborization (Biswas et al., 2014), and fasciculation (Hayashi et al., 2014). δ-Pcdhs are essential for neural development, as several have been implicated in neurodevelopmental disorders (Hirano and Takeichi, 2012; Redies et al., 2012). In particular, mutations in human PCDH19 result in a female-limited form of infant-onset epilepsy (Dibbens et al., 2008; Depienne et al., 2009), making PCDH19 the second most clinically relevant gene in epilepsy (Depienne and LeGuern, 2012). However, it is not known how loss of pcdh19 alters neural development or leads to epileptogenesis.

Here we show that δ-pcdhs are expressed in radial columns of neurons in the developing zebrafish optic tectum and that neurons within a column arise from a common progenitor cell. Elimination of pcdh19 degrades the columnar organization of the tectum because of reduced cell cohesion and increased cell proliferation. Moreover, pcdh19 mutants exhibit defects in visually guided behaviors. These data reveal a previously unknown columnar architecture of the optic tectum, suggesting that the tectum has an organization more similar to that of mammalian cortex than previously realized. In addition, the defects in visual processing suggest that the columnar organization is important for neural function. Thus, our results provide an initial link between δ-pcdhs, the development of neural architecture, and neural function.
Results and discussion

To better understand the expression of δ-pcdhs within the tectum, we imaged horizontal sections of zebrafish larvae at 4 d postfertilization (dpf) that were labeled with riboprobes against pcdh19, pcdh9, and pcdh10b (Fig. 1, B–D). Strikingly, larvae exhibited stripes of expression in the tectum, revealing that neurons expressing a particular δ-pcdh are organized as radial columns. These columns are not apparent in either whole-mount larvae or transverse sections. To investigate this phenomenon in more detail, we identified a bacterial artificial chromosome (BAC) clone harboring the complete gene and generated a BAC transgenic line, TgBAC(pcdh19:Gal4-VP16,UAS:Lifeact-GFP)129. The doubly modified BAC clone uses the regulatory elements of pcdh19 to express Gal4-VP16, which activates expression of Lifeact-GFP (Riedl et al., 2008) and labels F-actin in pcdh19-expressing cells. At 30 h postfertilization (hpf), the pcdh19 reporter generates a striped pattern in the midbrain neuroepithelium that will give rise to the optic tectum (Fig. 1 E). Consistent with the in situ hybridization data, cells that express pcdh19 in 3- to 4-dpf larvae are organized as radial columns (Fig. 1 F). Individual columns consist of clusters of neurons tightly associated with the radial fibers of one or more radial glia-like cells (Fig. 1, G and H). These cells are likely radial glia, as they express common glial markers including glutamine synthase (Fig. 1, I–L) and Her4.1 (Fig. 1, M–O).

The columnar organization of pcdh19-expressing neurons is reminiscent of the proliferative radial units observed in the developing mammalian cortex (Rakic, 1988; Mountcastle, 1997). To address whether the neurons within a pcdh19+ column are siblings (Noctor et al., 2001), we used two methods to mosaically label cells in the optic tectum. First, we transplanted 20–40 cells from transgenic [TgBAC(pcdh19:Gal4-VP16,UAS:Lifeact-GFP)129] blastula-stage embryos into unlabeled host embryos (Fig. 2 A). These cells disperse during gastrulation and contribute to different parts of the embryo; those cells fated to express pcdh19 will be labeled with Lifeact-GFP. Labeled tectal cells were predominantly organized in columns, even in the absence of other tectal expression, suggesting that these cells were derived from a common progenitor (Fig. 2, B and C). Additionally, we injected BAC DNA into embryos to transiently express Lifeact-GFP in pcdh19+ cells (Fig. 2 D). Transient expression of fluorescent reporters was largely restricted to radial columns (Fig. 2, E and F), again suggesting that the neurons within these columns arise from an initial pcdh19+ precursor. To further investigate the origins of tectal columns, we imaged
individual TgBAC(pcdh19:Gal4-VP16, UAS:Lifeact-GFP)$_{os49}$ embryos on consecutive days (Fig. 2, G–I). In fortuitous cases, isolated columns identified at 3–4 dpf (Fig. 2, H and I) could be unambiguously traced back to labeled neuroepithelial cells (Fig. 2 G). Our observations demonstrate that pcdh19 is expressed in radial columns within the optic tectum and that these columns assemble from the proliferation of one or a small number of pcdh19$^{+}$ precursors.

To show whether other δ-pcdhs define similar developmental units, as suggested by in situ hybridization (Fig. 1, A–D), we injected BAC clones of zebrafish pcdh18b and pcdh1a, modified as was done for pcdh19. In each case, we observed columns of labeled cells (Fig. S1, A and B), supporting the idea that the zebrafish optic tectum could be partitioned into distinct radial domains based on the expression of δ-pcdhs (Fig. S1 C). To test this, we performed double FISH with riboprobes directed against pcdh7a and pcdh19 (Fig. S1, D–I). Although some overlap of labeling can be observed (Fig. S1, F and I), the columnar distribution of pcdh7a and pcdh19 largely appears to be mutually exclusive, implying that the expression of each δ-pcdh labels distinct sets of columns.

To investigate the role of pcdh19 in the formation of tectal columns, we used transcriptional activator-like effector nucleases (TALENs; Cermak et al., 2011; Bedell et al., 2012; Dahlem et al., 2012) to generate germline lesions in zebrafish pcdh18b (Fig. 3 A). Homozygous mutants completely lack Pcdh19 (Fig. 3 B) but do not exhibit gross defects in neural organization (unpublished data). To investigate the effects of pcdh19 loss in detail, we injected BAC(pcdh19:Gal4-VP16, UAS:Lifeact-GFP) into one-cell–stage wild-type (WT) and mutant embryos (Fig. 3, C and D). At 4 dpf, 58% of labeled WT larvae exhibited columns (Fig. 3, C and E; n = 21/36 larvae). In contrast, only 31% of mutant larvae exhibited evidence of columnar organization (Fig. 3, D and E; n = 10/32 larvae, P = 0.028, Fisher exact test). Labeled neurons in mutants were more dispersed and arborized over a larger area within the synaptic neuropil (Fig. 3 D). To further assess the effects of Pcdh19 loss, we crossed the pcdh19$^{-c-c}$ mutants into the TgBAC(pcdh19:Gal4, UAS:Lifeact-GFP)$_{os49}$ line. Elimination of pcdh19 severely disrupted columnar organization in TgBAC(pcdh19:Gal4, UAS:Lifeact-GFP)$_{os49}$ larvae (Fig. 3, F–H; WT, 82%, n = 39/47; pcdh19$^{-c-c}$, 41%, n = 16/38; P = 0.0001, Fisher exact test). In addition, cell transplantation experiments indicated that pcdh19 is required in both host and donor cells for normal column development and normal neurite outgrowth and arborization (Fig. S2). Whereas groups of pcdh19$^{+}$ cells appear less tightly clustered in pcdh19$^{-c-c}$ mutants (Fig. 3 D), the loss of apparent columnar organization could also result from an increased number of labeled cells.

To determine whether there is an increase of cells expressing Lifeact-GFP in mutants, we identified and counted all labeled cells in image stacks collected from the optic tecta of six WT and five mutant larvae (Fig. 4, A–C). We found that there was an approximately twofold increase in the number of labeled cells in the mutants (WT, 181 ± 29, n = 6; pcdh19$^{-c-c}$, 328 ± 56, n = 5; P = 0.036), suggesting an increase in proliferation within this cell population. To directly assess levels of proliferation within the optic tectum, we sectioned and labeled 2-dpf embryos, with antibodies against phosphohistone H3 (pH3; Fig. 4, D–F). There was an increase in pH3-labeled nuclei in the pcdh19$^{-c-c}$ mutants compared with WT (pcdh19$^{-c-c}$, 20.3 ± 3.5, n = 4; WT, 9.3 ± 2.4, n = 3; P = 0.041, Student’s t test). Similarly, we injected 5-ethyl-2’-deoxyuridine (EdU) into 2-dpf embryos, fixed them at 4 dpf (Fig. 4, G–I). Again, we found evidence for increased proliferation in pcdh19$^{-c-c}$ mutants (pcdh19$^{-c-c}$, 84.5 ± 9.8, n = 4; WT, 63.8 ± 7.6, n = 4; P = 0.032, Student’s t test). Our data indicate that the loss of pcdh19 degrades the columnar organization of pcdh19$^{+}$ neurons through both an increase in neuron production and a reduction in cell cohesion.

Distinct tectal regions are known to differentially process visual input; therefore, disruption of tectal columnar organization may alter visually guided behaviors. To determine whether pcdh19 loss of function affects visual processing, we tested...
6-dpf larvae for positive phototaxis, a behavior in which larvae stereotypically turn and swim toward a target light. Compared with WT larvae, fewer pcdh19−/− larvae reached the target light area, and the pcdh19−/− larvae that did show positive phototaxis required more time to reach the target (Fig. 5, A and B; Burgees et al., 2010). Kinematic analyses of turning and swimming behaviors with millisecond resolution indicated that the reduced phototaxis was not caused by motor impairment (Fig. S3). To determine whether the reduced phototaxis in pcdh19−/− larvae was caused by a visual processing defect, we tested whether pcdh19−/− larvae initiated turns and swims that were biased toward the target light. As expected, WT larvae showed a strong
bias to swim or turn toward the target light based on their orientation (Burgess et al., 2010); however, pcdh19−/− larvae showed no directional bias, indicating that a visual impairment underlies the phototaxis defect (Fig. 5, C and D). Consistent with these results, we also found that pcdh19−/− larvae showed a reduced likelihood of initiating the O-bend maneuver (Fig. 5 E), a stereotyped visuomotor behavior to a sudden extinguishment of light (Burgess and Granato, 2007). Again, once initiated, the performance of O-bend maneuvers was indistinguishable between WT and pcdh19−/− larvae (Fig. 5 F–H), suggesting that the behavior initiation defect is caused by a visual, not motor, impairment. In contrast, the acoustic startle response was normal in the pcdh19−/− mutants (unpublished data). Thus, visual processing is impaired in pcdh19−/− larvae, potentially because of disrupted tectal neuronal columns.

Retroviral lineage tracing has suggested the radial development of the optic tectum in chickens (Gray and Sanes, 1991, 1992) and medaka fish (Nguyen et al., 1999). Here we show that this radial development results in neuronal columns that may be analogous to the microcolumns found in mammalian cortex (Peters and Walsh, 1972; Peters and Sethares, 1996). However, it remains to be shown if the columns defined by δ-pcdhs exhibit comparable defects, and how this family of molecules collaborates to guide both circuit assembly and the development of neural function.

Materials and methods

Fish maintenance

Adult zebrafish (Danio rerio) and embryos of the Tübingen longfin and AB strains were maintained at ∼28.5°C and staged according to Westerfield (1995). Embryos were raised in E3 embryo medium (Westerfield, 1995) with 0.003% phenylthiourea (Sigma-Aldrich) to inhibit pigment formation.

Whole-mount in situ hybridization

Riboprobes directed against δ-pcdhs were amplified by PCR as described previously (Biswas and Jontes, 2009; Emond et al., 2009; Biswas et al., 2014). A T7 RNA polymerase binding site was included in each of the reverse primers, and these PCR products were used as templates for in vitro transcription (Promega). Antisense riboprobes were labeled with digoxigenin-dUTP (Roche). Whole-mount in situ hybridizations were performed using standard methods (Westerfield, 1995). In brief, embryos were fixed at 4°C overnight in 4% paraformaldehyde in PBS, dehydrated in a methanol series, and stored in 100% methanol overnight at −20°C. They were rehydrated in decreasing concentrations of methanol, and embryos ≥24 hpf were permeabilized using proteinase K (10 µg/ml; Roche). Embryos were refixed in 4% paraformaldehyde before hybridization. Digoxigenin-dUTP–labeled riboprobe was added to the hybridization buffer at a final concentration of 200 ng/ml, and hybridization was performed at 65°C overnight. AP-conjugated anti-digoxigenin Fab fragments (Roche) were used at 1:5,000 dilution. Nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Roche) was used for the coloration reaction. Labeled embryos were equilibrated in...
30% sucrose in PBS overnight. They were then embedded in OCT (Ted Pella), sectioned on a cryostat at 14 μm, and placed on gelatin-coated glass slides. Images were captured on an AxioStar (Carl Zeiss).

For double FISH, 3- and 4-dpf fish were fixed and hybridized with riboprobe against pcdh7a (labeled with digoxigenin-dUTP) and pcdh19 (labeled with fluorescein-dUTP). The digoxigenin-labeled probe was detected using antidigoxigenin Fab–HRP (Roche) and developed using the tetramethylrhodamine substrate from the TSA Plus kit (Perkin-Elmer). Subsequently, the fluorescein probe was detected with an antifluorescein primary antibody (Roche) and a goat anti-mouse HRP secondary antibody (Invitrogen) and developed using the fluorescein substrate from the TSA Plus kit.

The PCR primers used to generate DNA template for riboprobe synthesis are as follows: pcdh19-F: 5'-ATGCAATCCAAGGACATGATTTC-3'; pcdh19-T7R: 5'-GTAAATACGCTCACTATAGGGCAAGACTTGGCTGCTTCTGTCG-3'; pcdh10b-F: 5'-ATGTTTGTTTTCCTGCTCTG-3'; pcdh10b-T7R: 5'-GTAAATACGACTCACTATAGGGCCAAGACTTGGCTTCTGTCGG-3'; pcds9-F: 5'-GCACATTTGTTCTGCTTCTGG-3'; pcds9-T7R: 5'-CTGAAATACGCTCACTATAGGGCCAAGACTTGGCTTCTGTCG-3'; pcds7a-T7R: 5'-ATGAACAGAAGACCGCCACCCTGGCG-3'; pcds9-T7R: 5'-GTAAATACGCTCACTATAGGGCCAAGACTTGGCTTCTGTCG-3'; and her4.1-T7R: 5'-GTAAATACGCTCACTATAGGGCCAAGACTTGGCTTCTGTCG-3'.

Cryosectioning and immunocytochemistry
Zebrafish larvae were fixed for 1 h in 4% paraformaldehyde in PBS, equilibrated in 30% sucrose in PBS overnight, embedded in OCT (Ted Pella, Inc.), sectioned on a cryostat at 16–20 μm, and placed on gelatin-coated glass slides. Sections were permeabilized and blocked in PBS, 0.5% Triton X-100, 1% DMSO, and 10% normal goat serum. For immunocytochemistry, sections were labeled with chicken anti-GFP (1:200; Jackson ImmunoResearch). Images were obtained using a TCS-SL confocal microscope (Leica) and either a Leica 20×/NA 0.7 HC Plan Apochromat oil microscope (Leica) and either a Leica 20×/NA 0.7 HC Plan Apochromat oil microscope (Leica) or a Leica 63×/NA 0.75–1.25 (glutamine synthase).

BAC recombinering and transgenesis
BAC clone CH211-156N5 was obtained from BACPAC Resources, Children’s Hospital Oakland. CH211-156N5 includes 21 kb upstream of the start codon and 15 kb downstream of the stop codon. Purified BAC DNA was introduced into EL250 cells (Lee et al., 2001) obtained from transgenesis. We first introduced an inverted Tol2 cassette (iTol2) into the backbone (Suster et al., 2011). The iTol2 reagents were provided by M. Suster (Uni Research AS High Technology Center, Bergen, Norway). We then inserted a Gal4-VP16-FRT-Kanamycin-FRT cassette into exon 1 of the pcdh19 gene. The kanamycin marker was excised by arabinose induction of Flpe recombinase. Finally, a 5xUAS:Lifeact-GFP cassette was introduced into exon 2, which is ~45 kb downstream of exon 1. The same procedure was performed for BAC clones harboring genes for pcdh1a (DKEY-225G6; Source BioScience) and pcdh18b (CH211-154P8; BACPAC Resources). The CHO RI-211 library (CH211) was built in the pTARBAC2.1 vector, whereas the DanioKey library (DKEY) was built in the pfnidoBac-536 vector.

Each of the BAC clones contained a complete protocadherin gene. To generate germline BAC transgenic zebrafish, we co-injected one-cell–stage embryos with 1 nl of 100 ng/µl purified BAC DNA and 100 ng/µl mRNA encoding the Tol2 transposase. At 2 dpf, embryos were screened for Lifeact-GFP expression, and fluorescent embryos were grown to adulthood. To establish stable lines, founders were outcrossed with WT zebrafish, and fluorescent embryos were pooled and grown to adulthood. For transient expression, injections were performed as described, but embryos were kept in 0.01% propylthiouracil and imaged at 3-4 dpf.

TALEN construction and production of germline lesions
We used the online tool ZfFt (Sander et al., 2010; Zinc Finger Consortium) to search for a TALEN target site in exon 1 of zebrafish pcdh19. We identified an appropriate target site downstream of the signal peptide, 5'-TTTCTGTCAAAATTGTGGTTGCTCCTCCTGCGAC-3' (upper case indicates TAL left and right binding sites). The TALEN arrays (left, NG-NG-HD-NN-NG-NI-HD-NI-NI-NG-NN; right, HD-NN-NI-HD-HD-NI-NI-NN-NN-NN) were assembled in RCIScript-GoldyTALEN (Bedell et al., 2012) using the TAL Effector kit 1.0 (Addgene; Cermak et al., 2011). Plasmid encoding assembled TALENS was linearized with ScaI and used as template for mRNA synthesis with a T3 Message Machine kit (Ambion). To generate germline lesions in zebrafish pcdh19, we injected one-cell–stage embryos with 50 pg mRNA encoding the left and right nucleases. Injected embryos were grown to adulthood and screened for germline lesions. For screening, adult F0 fish were outcrossed with WT, and genomic DNA was prepared from eight embryos of each cross. High-resolution melting analysis (HRMA) was used to identify putative founders (Dahlem et al., 2012). PCR products exhibiting aberrant melting curves were cloned and sequenced. F0 adults exhibiting frameshift mutations were outcrossed, and the F1 embryos were grown to adulthood. To screen the adult F1 fish, genomic DNA was prepared from caudal fin clips screened by HRMA. We obtained three mutants: 1-bp deletion (Δ-1), 10-bp deletion (Δ-10), and 13-bp deletion (Δ-13). These heterozygote F1 founders were outcrossed again, and the F2 offspring were raised and screened to establish mutant lines pcdh19Δ-1, pcdh19Δ-10, and pcdh19Δ-13. To obtain homozygous pcdh19 mutants, which are viable and fertile, heterozygotes for each allele were intercrossed, and embryos were grown to adulthood and screened by HRMA.

To establish mutant/transgenic lines, each of the three homozygous mutant lines was crossed with TgBAC(pcdh19-Gal4VP16: UAS:Lifeact)Δ-1. Fluorescent embryos were selected and grown to adulthood. These transgenic-pcdh19 mice were intercrossed, and the offspring were raised to adulthood and screened for homozygous mutants.

Western blotting
Protein lysates were prepared by homogenizing 40 deyolked embryos at 3 dpf in lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 1 mM PMSF, and complete protease inhibitor cocktail [Roche]). Lysates were microcentrifuged at 4°C for 10 min, and identical volumes were loaded onto NuPAGE 10% Bis-Tris SDS-PAGE gels (Life Technologies) for each condition. Proteins were subjected to electrophoresis and transferred (Bio-Rad Laboratories) to PVDF (GE Healthcare), blocked with 5% nonfat milk in TBST (10 mM Tris-HCL, pH 7.6, 100 mM NaCl, and 0.1% Tween-20), and incubated overnight with...
primary antibody (1:1,000 anti-Pcdh19, custom polyclonal [Covance]; 1:5,000 mouse monoclonal anti-β-actin [Santa Cruz Biotechnology]). HRP-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) were used at 1:5,000, and the chemiluminescent signal was amplified using Western Lightning Ultra (PerkinElmer). Blots were imaged on a molecular imaging system (Omega 12Ci; UltraLum). Custom polyclonal antibodies against zebrafish Pcdh19 were generated in rabbit, directed against the Pcdh19 intracellular domain (aa 702–1083).

Two-photon imaging

Two-photon imaging of live embryos and larvae was performed at RT on a custom-built microscope controlled by ScanImage (Polgroto et al., 2003). Excitation was provided by a Chameleon-XR Ti: Sapphire laser (Coherent) tuned to 890 nm. Fluorescence was detected using a Multiphoton Detection Unit mounted on a SliceScope (Scientifica). We used water immersion objectives from Olympus, either 60×/NA1.0 (LUMPLFLN60X/W) or 20×/NA1.0 (XLUMPLFLN20XW). For imaging, larvae were embedded in 1% low melting point agarose, covered with embryo medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, and 0.33 mM MgCl₂), and imaged at RT.

For the imaging of transgenic/mutant larvae, TgBAC/pcdh19−/−-fish were crossed and embryos were kept in individual wells of a 24-well plate. After imaging, fish were genotyped by HRMA to identify homzygous mutants. As homzygous mutants are viable and fertile, some of the experiments were performed on incrossed TgBAC/pcdh19−/− adults.

Image analysis

All image analysis was performed with Fiji (Schindelin et al., 2012). For cell identification and modeling, the TrakEM2 plugin (Cardona et al., 2012) in Fiji was used. Image stacks of ~100 optical sections were collected from the transgenically labeled tecta of WT or pcdh19−/− larvae. Each detected cell body was modeled as a sphere, and primary projections were traced from cell bodies to the synaptic neuropil when possible. Multipanel images were assembled in Adobe Photoshop, and figures were made in Adobe Illustrator.

Cell transplantation

Transplantations were performed as described previously (Biswas et al., 2014). Host and donor embryos were dechorionated with pronase, and then arranged in an agar injection tray. At 3–3.5 hpf, 20–40 cells from transgenic donor embryos (WT or pcdh19−/−) were transplanted into unlabeled host embryos (WT or pcdh19−/−). Embryos were maintained in embryo medium supplemented with penicillin/streptomycin, and then arranged in an agar injection tray. At 3–3.5 hpf, 20–40 cells from transgenic donor embryos (WT or pcdh19−/−) were transplanted into unlabeled host embryos (WT or pcdh19−/−). Embryos were maintained in embryo medium supplemented with penicillin/streptomycin. At 24 hpf, embryos were screened for health, normal swimming; see Video S1. Embryos were maintained in embryo medium supplemented with penicillin/streptomycin, and then arranged in an agar injection tray. At 3–3.5 hpf, 20–40 cells from transgenic donor embryos (WT or pcdh19−/−) were transplanted into unlabeled host embryos (WT or pcdh19−/−). Embryos were maintained in embryo medium supplemented with penicillin/streptomycin, and then arranged in an agar injection tray. At 3–3.5 hpf, 20–40 cells from transgenic donor embryos (WT or pcdh19−/−) were transplanted into unlabeled host embryos (WT or pcdh19−/−). Embryos were maintained in embryo medium supplemented with penicillin/streptomycin. At 24 hpf, embryos were screened for health, normal swimming; see Video S1.

Visual behavior analysis

Behavioral experiments were performed at 6 dpf and analyzed with the FLOTE software package as previously described (Burgess and Granato, 2007; Burgess et al., 2010). Video was captured at 1,000 frames per second with an IDT MotionPro Y4 camera. 20 larvae of identical genotype were grouped in a 6-cm-wide Petri dish with 9 ml of E3 embryo medium and preadapted to the uniform light intensity for 3 h before testing. 3 dishes of each genotype were tested for positive phototaxis and a response to a sudden removal of preadapted light (termed a “dark flash”). The phototaxis assay was performed as described previously to elicit positive phototaxis (Burgess et al., 2010). Larvae were given 30 s to reach the target light, and only larvae initially positioned on the opposite side of the dish from the target light were included in our analyses. To determine directionality of turns and swims (Fig. 4, C and D), the 30-s video was partitioned into thirty 1-s events. For each event, larvae oriented within 30° of the target light were considered “facing” the target and used for swim direction analyses (Fig. 4 C). Larvae oriented 75°–104° away from the target light were used for turn direction analyses (Fig. 4 D). The visual dark flash assay was performed and analyzed as previously described (Burgess and Granato, 2007). In brief, larvae are adapted to uniform light, and then exposed to ten 1-s periods of darkness separated by 30 s of light. Larvae are expected to perform an O-bend, which has stereotyped kinematic parameters.

Online supplemental material

Fig. S1 shows transgene expression by injection of recombinant BAC clones into zebrafish embryos and double FISH with riboprobes against pcdh7a and pcdh19. In Fig. S2, cell transplantation reveals that pcdh19 function is required in cells and their environment. Fig. S3 shows that the kinematics of swimming behaviors is normal in pcdh19 mutants. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201507108/DC1.

Acknowledgments

We thank Min An for technical assistance. We thank Koichi Kawakami and Maximilliano Suster for tToI2 plasmids. The reagents used for BAC recombineering were a gift of Dr. Neal Copeland. The plasmid kit used for the generation of TALENs was a gift of Daniel Voytas and Adam Bogdanove [Addgene kit #1000000016].

This work was supported by a National Institute of Mental Health grant to J.D. Jontes (MH099453), a grant from the National Science Foundation (IOS 1457126), and a Neurosciences Core Grant (P30 NS045758).

The authors declare no competing financial interests.

Submitted: 28 July 2015
Accepted: 13 October 2015

References

Bedell, V.M., Y. Wang, J.M. Campbell, T.L. Poshusta, C.G. Starker, R.G. Krug II, W. Tan, S.G. Penheiter, A.C. Ma, A.Y. Leung, et al. 2012. In vivo genome editing using a high-efficiency TALEN system. Nature. 491:114–118.

Biswas, S., and J.D. Jontes. 2009. Cloning and characterization of zebrafish protocadherin-17. Dev. Genes Evol. 219:265–271. http://dx.doi.org/10.1007/s00427-009-0288-6

Biswas, S., M.R. Emond, P.Q. Duy, T. Hao, C.E. Beattie, and J.D. Jontes. 2014. Protocadherin-18b interacts with Nap1 to control motor axon growth and arborization in zebrafish. Mol. Cell. 25:633–642. http://dx.doi.org/10.1016/j.mcb.2013.08.047

Blevins, C.J., M.R. Emond, S. Biswas, and J.D. Jontes. 2011. Differential expression, alternative splicing, and adhesive properties of the zebrafish 61-protocadherins. Neuroscience. 199:523–534. http://dx.doi.org/10.1016/j.neuroscience.2011.09.061

Burgess, H.A., and M. Granato. 2007. Modulation of locomotor activity in larval zebrafish during light adaptation. J. Exp. Biol. 210:2526–2539. http://dx.doi.org/10.1242/jeb.003939

Burgess, H.A., H. Schoch, and M. Granato. 2010. Distinct retinal pathways drive spatial orientation behaviors in zebrafish navigation. Curr. Biol. 20:381–386. http://dx.doi.org/10.1016/j.cub.2010.01.022

Cardona, A., S. Saalfeld, J. Schindelin, I. Arganda-Carreras, S. Preibisch, M. Longair, P. Tomancak, V. Hartenstein, and R.J. Douglas. 2012. TrakEM2 software for neural circuit reconstruction. PLoS One. 7:e38011. http://dx.doi.org/10.1371/journal.pone.0038011

Cermak, T., E.L. Doyle, M. Christian, L. Wang, Y. Zhang, C. Schmidt, J.A. Baller, N.V. Somaia, A.J. Bogdanove, and D.F. Voytas. 2011. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. 39:e82. http://dx.doi.org/10.1093/nar/gkr218

δ-Protocadherins partition the optic tectum • Cooper et al. 813
