



Celsr1–3 Cadherins in PCP and Brain Development

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Abstract

Cadherin EGF LAG seven-pass G-type receptors 1, 2, and 3 (Celsr1–3) form a family of three atypical cadherins with multiple functions in epithelia and in the nervous system. During the past decade, evidence has accumulated for important and distinct roles of Celsr1–3 in planar cell polarity (PCP) and brain development and maintenance. Although the role of Celsr in PCP is conserved from flies to mammals, other functions may be more distantly related, with Celsr working only with one or a subset of the classical PCP partners. Here, we review the literature on Celsr in PCP and neural development, point to several remaining questions, and consider future challenges and possible research trends.

Celsr1–3 genes encode atypical cadherins of more than 3000 amino acids (Fig. 7.1). Their large ectodomain is composed of nine N-terminal cadherin repeats (typical cadherins have five repeats), six epidermal growth factor (EGF)-like domains, two laminin G repeats, one hormone receptor motif (HRM), and a G-protein-coupled receptor proteolytic site (GPS). This is followed by seven transmembrane domains (classic cadherins are single-pass type I proteins) and a cytoplasmic tail (CT) that varies in size and, in contrast to the ectodomain, is poorly conserved among the three members. Celsr1–3 belong to the “adhesion receptor” family of G-protein-coupled receptors (GPCRs), also referred to as long N-terminal group B (LNB) GPCRs.

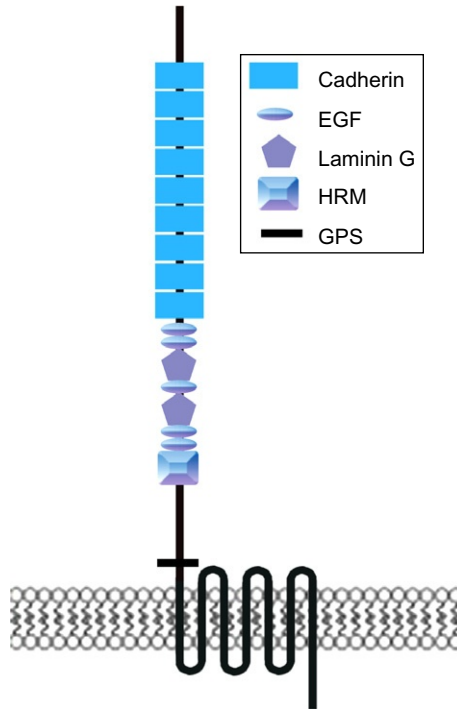


Figure 7.1 Schematic representation of the Celsr1 protein. The extracellular domain includes nine cadherin repeats, six epidermal growth factor (EGF)-like domains, two laminin G repeats, one hormone receptor motif (HRM), and a G-protein-coupled receptor proteolytic site (GPS). In contrast to typical cadherins which are single-pass proteins, Celsr1–3 cadherins are anchored to the plasma membrane by seven transmembrane domains. The cytoplasmic tail varies in size and is poorly conserved among the three Celsrs.

These receptors are thought to be natural chimeras of cell adhesion proteins and signaling receptors, able to convert cell–cell communication cues into intracellular signals. Studies of the latrotoxin receptor latrophilin, a member of LNB–GPCR, showed that cleavage at the GPS occurs intracellularly. However, the N-terminal (cell adhesion domain) and C-terminal (GPCR) fragments remain associated by noncovalent bonds at the plasma membrane ([Krasnoperov et al., 2002](#); [Volynski et al., 2004](#)). It is not clear yet whether cleavage at the GPS occurs in Celsr1–3 proteins (see below). In addition to cadherin and EGF-like repeats which confer adhesive properties and facilitate cell–cell communication, the extracellular domain of Celsr1–3

contains a HRM which may bind putative ligands, although none has been identified thus far.

Celsr genes were first identified in mice ([Hadjantonakis et al., 1997](#)). They form a highly conserved family with homologs in ascidians, worms, flies, and vertebrates. The fruit fly *Celsr* ortholog was cloned independently by two groups and is known as *Flamingo* (*Fmi*) or *Starry night* (*Stan*) ([Chae et al., 1999](#); [Usui et al., 1999](#)). Genetic studies have established a critical role for *Fmi/Stan* in planar cell polarity (PCP) events, particularly in the stereotypic organization of wing hairs (trichomes) and sensory bristles ([Chae et al., 1999](#); [Usui et al., 1999](#)), the alignment of ommatidia in the eye ([Mlodzik, 1999](#)), and the asymmetric division of sensory organ precursors ([Bellaiche, Gho, Kaltschmidt, Brand, & Schweisguth, 2001](#); [Segalen & Bellaiche, 2009](#)). In these processes, *Fmi/Stan* interacts genetically and functionally with so-called core PCP proteins. *Drosophila* core PCP proteins include serpentine receptors Frizzled (*fz* and *fz2* are partially redundant in PCP); the tetraspannin Van Gogh (*vang*, also named strabismus); and the three cytoplasmic proteins Disheveled (*dsh*), Prickle (*pk*), and Diego (*dgo*) ([Adler, 2002](#); [Feiguin, Hannus, Mlodzik, & Eaton, 2001](#); [Gubb et al., 1999](#); [Strutt, Johnson, Cooper, & Bray, 2002](#); [Taylor, Abramova, Charlton, & Adler, 1998](#); [Wolff & Rubin, 1998](#)). In the insect wing, the distribution of core PCP proteins is tightly and dynamically regulated. Initially distributed in all apical junctions, they adopt a transient polarized partition along the proximal–distal axis shortly before the growth of wing hairs, and this partition is essential to the establishment of polarity and the proper orientation of hairs. *fmi/stan*, *fzd*, *dsh*, and *dgo* are enriched at the distal border of wing cells, whereas *fmi/stan*, *vang*, and *pk* are enriched at the proximal border ([Bastock, Strutt, & Strutt, 2003](#); [Jenny, Darken, Wilson, & Mlodzik, 2003](#); [Usui et al., 1999](#)). Given that *fmi/stan* localizes to both proximal and distal junctions, it was considered a permissive molecule with no active effect on polarity: *fmi/stan* homodimers would act as a scaffold, promoting cell adhesion and bridging the distal (*fz*-expressing) side of a cell and the proximal (*vang*-expressing) side of the adjacent cell. However, more recent data provide evidence that *fmi/stan* plays an instructive role: its central portion containing the HRM and TM domains interacts physically with *fz*. Furthermore, *fmi/stan* selectively recruits *fz* and *vang* to opposite cell boundaries, thereby initiating bidirectional polarity signals ([Chen et al., 2008](#)). In line with this, genetic analyses in flies demonstrated a mutual requirement for *fmi/stan*, *fz*, and *dsh* to achieve a

correct partition of polarity complexes (Das, Reynolds-Kenneally, & Mlodzik, 2002). In zebrafish, a serine acidic amino acid-rich domain (SE/D) in the CT of *Celsr* is required for membrane localization of the Frizzled–Disheveled complex, PCP signaling, and convergent extension (Carreira-Barbosa et al., 2009).



1. CELSR1–3 EXPRESSION PATTERNS

In mammals, the *Celsr* family is composed of three members, whereas birds apparently lack *Celsr2* (Formstone, 2010) and fish have two, *Celsr1a* and *1b* (Wada, Tanaka, Nakayama, Iwasaki, & Okamoto, 2006). In the mouse, *Celsr1–3* genes have similar genomic organizations, with 35 (*Celsr1* & *Celsr3*) or 34 exons (*Celsr2*). Apart from 3' alternative exons in *Celsr2*, alternative splicing has not been described. *Celsr1–3* expression is regulated spatially and temporally, indicating that they are important for development. A striking feature is the complementary pattern of *Celsr1* and *Celsr3* expressions in different developing systems (Formstone & Little, 2001; Shima et al., 2002; Tissir, De Backer, Goffinet, & Lambert de Rouvroit, 2002). In the nervous system, *Celsr1* mRNA is heavily expressed in zones of neural stem cell (NSC) proliferation, namely, all ventricular zones during embryonic and early postnatal development, and telencephalic ependymal zones as well as subgranular layer of the dentate gyrus in the mature brain. In contrast, *Celsr3* mRNA is absent from NSC and associated with most postmitotic neural cells, whereas *Celsr2* mRNA is found in both NSC and postmitotic cells. Expression of *Celsr1* abates during early postnatal development, in parallel to decreasing numbers of NSC. That of *Celsr3* is downregulated postnatally but persists in the cerebellar granular layer, the hilus of the dentate gyrus, the rostral migratory stream, and the central region of the olfactory bulb. By contrast, *Celsr2* expression remains stable throughout life. These expression patterns hint to functions of *Celsr1* in NSC, *Celsr3* in neural cell maturation, and *Celsr2* in development and maintenance of the nervous system. *Celsr1–3* mRNAs are also variably expressed in nonneural tissue, such as the skin, lungs, kidney, and digestive and reproductive systems. In the rodent testis, the spatiotemporal pattern of *Celsr1–3* expression is somewhat reminiscent of that in the brain. *Celsr3* is expressed exclusively in postmeiotic germ cells, and *Celsr1* and *Celsr2* in Sertoli cells, with a postnatal downregulation of *Celsr1* and persistence of

Celsr2 in the adult (Beall, Boekelheide, & Johnson, 2005; Hadjantonakis, Formstone, & Little, 1998; Johnson, Patel, & Boekelheide, 2000).

Polyclonal antibodies against the extra- and intracellular segments of *Celsr1* allowed detection of two forms, full length (p400) and cleaved (p85 kD), generated by as yet unidentified proteolysis events not involving the GPS (Formstone, Moxon, Murdoch, Little, & Mason, 2010). In the hindbrain and spinal cord, *Celsr1* protein immunoreactivity was detected in the floor and roof plates, as well as in radial neuroepithelial progenitors, not only in the apical but also in their basolateral domain, particularly in end-feet abutting the pial surface (Formstone et al., 2010). In the embryonic skin, *Celsr1* is asymmetrically expressed in hair germ cells and in basal layer epidermal cells, a pattern evocative of that of *fini/stan* in the *Drosophila* wing (Devenport & Fuchs, 2008). The cellular localization of *Celsr2* and *Celsr3* could not be investigated thus far due to lack of antibodies suitable in immunohistochemistry.



2. CELSR1: A MAJOR PLAYER IN VERTEBRATE PCP

Two *Celsr1* mutant alleles, *Crash* (*Celsr1^{Crsh}*) and *Spin Cycle* (*Celsr1^{Scy}*), were identified in an ENU screen (Curtin et al., 2003). In *Celsr1^{Crsh}*, a G-to-A mutation at nucleotide 3126 results in an aspartate-to-glycine substitution in codon 1040, within the eighth cadherin repeat. In *Celsr1^{Scy}*, a T-to-A point mutation at nucleotide 3337 results in an asparagine-to-lysine substitution in codon 1110, in a region connecting cadherin repeats. Heterozygous animals show abnormal head-shaking behavior. Both heterozygous and homozygous mice have defective organization of stereocilia bundles in inner ear hair cells. Normally, the apical surface of each cochlear hair cell is decorated with actin-filled stereocilia forming a “V” centered on a microtubular kinocilium, with all “Vs” pointing to the external aspect of the cochlear canal. In *Celsr^{Crsh}* and *Celsr1^{Scy}* mutant mice, this typical organization is altered. Stereociliary bundles are randomly oriented, some displaying up to 180° rotation relative to the normal orientation (Curtin et al., 2003). The precise orientation of hair bundles in the cochlea is a classical hallmark of PCP and parallels the polarized distribution of core PCP proteins such as *Fzd3*, *Fzd6*, *Vangl2*, and *Prickle2* (Deans et al., 2007; Montcouquiol et al., 2006; Wang, Guo, & Nathans, 2006). PCP proteins localize asymmetrically to one edge of the apical cortex of the cells by mechanisms involving selective targeting and protein stabilization and degradation. Intriguingly, studies of the distribution of PCP proteins

were carried out in late embryogenesis, starting from embryonic day 18 when the kinocilium has already migrated from the center to the lateral edge of the cell, raising the question whether the asymmetric partition of PCP proteins is the initial event that sets up the polarity and organizes the epithelium, or simply a molecular readout of polarity signals induced by as yet unidentified cues.

Celsr1^{Crsh} and *Celsr1*^{Scy} homozygotes as well as compound heterozygotes *Celsr1*^{Crsh/Scy} exhibit craniorachischisis, a severe neural tube defect due to a failure to initiate neural tube closure in the cervical region (Curtin et al., 2003). A role for *Celsr1* in neural tube closure was recently confirmed by identification of six *Celsr1* mutations in human fetuses with craniorachischisis. In *in vitro* assays, those mutations all impair trafficking of Celsr1 protein, reducing its membrane localization (Robinson et al., 2011).

As *Celsr1*^{Crsh} and *Celsr1*^{Scy} homozygous mutants are embryonic lethal, a conditional allele (*Celsr1*^f) was generated to allow tissue-specific inactivation upon expression of Cre recombinase (Ravni, Qu, Goffinet, & Tissir, 2009), a null allele *Celsr1*^{ko} was obtained by crosses with germline-expressing Cre mice, and Western blot with an antibody against the N-terminal region of Celsr1 confirmed absence of Celsr1 protein in embryonic mouse brain extracts. Unlike heterozygous *Celsr1*^{Crsh} and *Celsr1*^{Scy}, heterozygous *Celsr1*^{ko/+} mice have no perceptible phenotype. In contrast, homozygous *Celsr1*^{ko/ko} mutants display abnormal behavior such as circling and hyperactivity. About 20% of them die at embryonic stages with various degrees of neural tube closure defects, and many have a looping tail. Some have striking skin hair patterning defects with whorls and crests instead of regular caudally and distally pointing hairs on the body and limbs (Fig. 7.2) (Ravni et al., 2009). When *Celsr1* is inactivated in crosses with *Emx1-Cre* mice, inactivation of the gene in the apical ectodermal ridge induces a whorl in distal hindlimb, showing that the action of Celsr1 is ectodermal cell autonomous. The mechanism of Celsr1 action in the hair bulb has been scrutinized by the Fuchs lab (Devenport & Fuchs, 2008). They found that, prior to hair growth, Celsr1 becomes asymmetrically localized along the anterior/posterior (A/P) axis in basal epidermal cells in hair follicles and in interfollicular epithelium. This asymmetric localization is essential for A/P orientation of skin hair. Hair follicles fail to adopt the A/P orientation in skin explants isolated from E13.5 embryos, before the polarization of Celsr1. In contrast, when explants are isolated from E14.5 embryos, when Celsr1 was fully polarized, epidermis keeps the Celsr1 polarization established *in vivo*, and the A/P polarity. Consistent with this, hair follicles are misaligned in E18.5 embryos with

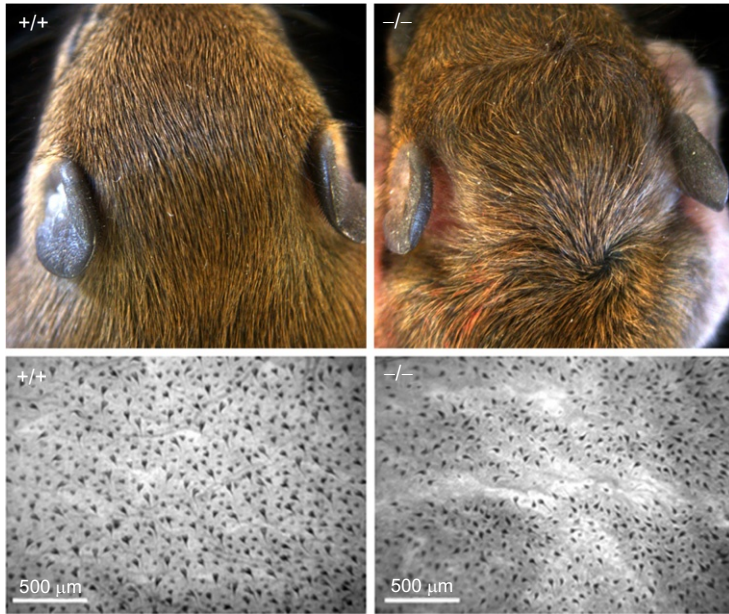


Figure 7.2 Hair patterning defects in *Celsr1* knockout mice. In normal mice (+/+), all body hairs point caudally and limb hairs point distally. *Celsr1* mutant mice (-/-, right) have fur whorls and crests variably located on the head, body, and on lower dorsal hindlimbs, where the trait is fully penetrant. Lower panels show whole mount preparations of skin at P1, stained with hematoxylin eosin to visualize disoriented hair follicles in the mutant (-/-, right) as compared to the wild type (+/+, right).

homozygous *Crsh* mutation. In mutant embryos, *Celsr1* protein is produced as in the wild type, but no longer asymmetrically distributed. Moreover, the membrane recruitment of *Fzd6* and the asymmetric localization of *Vangl2* along the A/P axis are compromised, with some *Vangl2* immunoreactivity forming intracellular puncta. Taken together, these results demonstrate that *Celsr1* plays a critical role for PCP establishment in the developing skin and hair follicles (Devenport & Fuchs, 2008).

Importantly, PCP phenotypes in *Celsr1* mutants were also seen in mice with mutations in other core PCP genes (Table 7.1). The inner ear phenotype was reported in mice with mutated *Vangl2* (Montcouquiol et al., 2003, 2006), and *Fzd3* and *Fzd6* (Wang et al., 2006). Neural tube closure defects were observed in *Vangl2*, double *Dvl1* and 2, and *Fzd3* and 6 (Kibar et al., 2001; Wang et al., 2006; Ybot-Gonzalez et al., 2007). Skin hair patterning defects were described in *Fzd6* and *Vangl2* (Devenport & Fuchs, 2008; Devenport,

Table 7.1 Summary of phenotypes in *Celsr1–3*, with comparison of some other PCP mutations

Gene	Allele	Phenotype
<i>Celsr1</i>	<i>Celsr1 Crsh</i> and <i>Scy</i>	Heterozygotes have inner ear PCP abnormalities, head shaking, looptail, and abnormal FBM neuron migration. Homozygotes are embryonic lethal with open neural tube.
	<i>Celsr1 KO</i>	Heterozygotes are normal and fertile. Homozygotes are viable but mostly sterile. About 20% die at embryonic stages with neural tube closure defects. Adults have abnormal behavioral traits, a looping tail, and abnormal skin hair patterning. The direction of FBM neuron migration is affected.
<i>Celsr2</i>	<i>Celsr2 Dgen</i>	Heterozygotes are normal and fertile. Homozygotes are fertile, except for some females with vaginal atresia. There is abnormal trajectory of FBM neuron migration and hydrocephalus due to defective ependymal ciliogenesis.
	<i>Celsr2 Dgen</i>	Heterozygotes are normal and fertile. Homozygotes are fertile and have abnormal FBM neuron migration.
<i>Celsr3</i>	<i>Celsr3 KO</i> and <i>Celsr3 F</i>	Heterozygotes are normal and fertile. Homozygotes die in few hours after birth and have severe axonal defects.
<i>Fzd3</i>	<i>Fzd3 KO</i>	Heterozygotes are normal and fertile. Homozygotes die in few hours after birth and have axonal defects similar to those in <i>Celsr3</i> mutants. Some have loop tail or open neural tube and PCP phenotype in the inner ear. The trajectory of FBM neuron migration is affected and mimics <i>Celsr2</i> and <i>3</i> double KO.
<i>Fzd6</i>	<i>Fzd6 KO</i>	Homozygous mutants survive and breed, and have abnormal skin hair patterning.
<i>Vangl2</i>	<i>Vangl2 Lp (Looptail)</i>	Some heterozygotes have kinked or looped tails. Homozygotes do not survive due to open neural tube, loop tail, inner ear PCP defects, FBM neuron migration defects, and other defects in cardiovascular, skeletal, and respiratory systems.
	<i>Vangl2 KO</i> and <i>Vangl2 F</i>	Heterozygotes are normal and fertile. Homozygote nulls have open neural tube and other PCP defects in the inner ear.
<i>Vangl1</i>	<i>Vangl1 gt</i>	Homozygotes are normal and fertile

Oristian, Heller, & Fuchs, 2011; Guo, Hawkins, & Nathans, 2004). These data provide strong evidence that *Celsr1* is involved in classical PCP together with *Fzd3* and 6, *Dvl1* and 2, and *Vangl2*.



3. CELSR2 AND 3 IN CILIOGENESIS

Cilia are appendages that extend from the cell surface to the extracellular environment. Based on their structure and motility, cilia are classified into three types: motile, primary, and nodal cilia (Davenport & Yoder, 2005). Motile cilia garnish the apical surface of epithelial cell lining airways, reproductive tracts, and cerebral ventricles. They have a central pair of microtubules surrounded by nine doublet of microtubules, an organization known as 9 + 2, and are anchored to a basal body in the apical cortex. Motile cilia form tufts and their concerted beats generate directional flow at the surface of epithelial sheets. In the airways, they are crucial for clearing mucus and debris, and in genital tracts, they assist in the transit of sperm and eggs (Salathe, 2007; Voronina et al., 2009). In the mouse brain at late embryonic stage and during the first postnatal week, neuroepithelial cells that line the cerebral ventricles differentiate into a monolayer of ependymal cells. At birth, ependymal cells are not multiciliated yet (Fig. 7.3). Motile cilia develop progressively and reach their mature shape by the end of brain maturation (Fig. 7.3B). The basal body of each cilium has a lateral extension called “basal foot” that can be detected by transmission electron microscopy. Basal feet point in the effective beat direction and are used as a hallmark of cilia polarity (Gibbons, 1961; Hagiwara, Ohwada, Aoki, Suzuki, & Takata, 2008; Wallingford, 2010). To generate an efficient directional flow, cilia coordinate their beats within the same cell (each cell has dozens of motile cilia) and in all cells in the epithelial sheet. Thus, basal feet are aligned in the same orientation with respect to the tissue polarity axis, a process referred to as “rotational polarity” (Mirzadeh, Han, Soriano-Navarro, Garcia-Verdugo, & Alvarez-Buylla, 2010). Elegant studies in *Xenopus* ciliated epidermal explants isolated at different developmental stages showed that motile cilia acquire their rotational polarity in two steps. Early in development, genetic cues specify a rough planar axis that allows cells to produce a directional flow, thereby inducing a positive feedback loop that tunes basal feet polarity (Mitchell, Jacobs, Li, Chien, & Kintner, 2007). In mice, *Celsr1–3* are expressed in the developing ependymal layer. Mutation of *Celsr2* and *Celsr3* impairs ependymal ciliogenesis (Tissir et al., 2010). Although differentiation of ependymal cells occurs normally, their motile cilia never

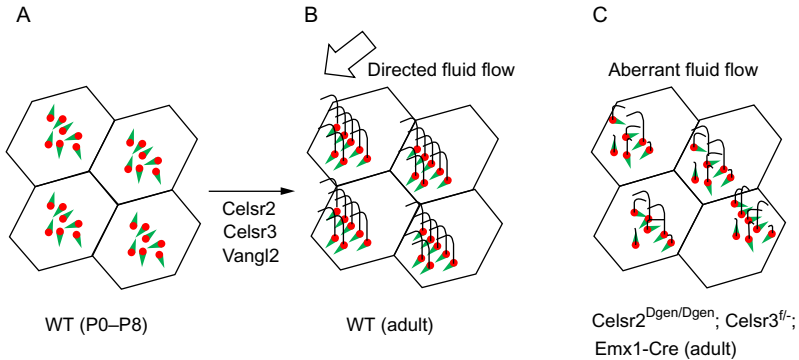


Figure 7.3 Celsr2 and Celsr3 regulate rotational polarity of ependymal cilia. Representation of motile cilia anchored to basal bodies (red dots) on the apical surface of ependymal cells. Cilia develop progressively according to ventral to dorsal, medial to lateral, and caudal to rostral gradients. During the first postnatal week (P0–P8), basal feet (green arrowheads) point in different directions (A). In normal animals, they rotate and adopt a more uniform orientation between P8 and P21, in response to planar cell polarity signals and hydrodynamic forces. This uniform orientation—also referred to as rotational polarity—enables cilia to beat in a coordinated manner and to generate a directed fluid flow (B). In *Celsr2* and *Celsr3* mutant ependyma (C), basal feet exhibit divergent orientation and fail to generate a directed fluid flow.

develop in normal numbers and those that develop display abnormalities in position and planar organization. At the cellular level, basal feet are misoriented, a rotational polarity defect, and some basal bodies assemble deep in the cytoplasm. At the tissue level, ciliary tufts from neighboring cells point aberrantly in divergent directions, generating a translational polarity defect (Fig. 7.3C). Mutant cilia display the typical “9 + 2” structure. They are still able to beat with the same frequency as the controls. Yet, because of their abnormal orientation, they fail to generate a robust and directed flow (Fig. 7.3C). The membrane localization of *Vangl2* and *Fzd3* is disrupted in mutant ependymal cells, providing strong evidence that *Celsr2* and *Celsr3* regulate ciliogenesis via PCP signaling. In accord with this, downregulation of core PCP genes *Disheveled1–3*, and PCP effectors *Inturned* and *Fuzzy* affects the orientation of multicilia in *Xenopus* (Park, Haigo, & Wallingford, 2006; Park, Mitchell, Abitua, Kintner, & Wallingford, 2008). Furthermore, ependymal cells with the “looptail” mutation in *Vangl2* fail to align their motile cilia in response to hydrodynamic forces in *in vitro* assays (Guirao et al., 2010).



4. CELSR1–3 IN NEURONAL MIGRATION

The migration of facial branchiomotor (FBM) neurons in the developing rhombencephalon is an intriguing case that combines tangential and radial migration modes. FBM neurons, which innervate muscles responsible for facial expression (Chandrasekhar, 2004; Garel, Garcia-Dominguez, & Charnay, 2000; Guthrie, 2007), are generated in medial rhombomere4 (r4) at E10.5 and immediately extend their axons laterally toward muscle targets. At E11.5, their cell bodies initiate a tangential caudal migration from r4 to r6. They migrate in the subventricular region, pass medial to the nucleus abducens (nVI) in r5 (Song et al., 2006), and then move laterally and dorsally in r6. Finally, they undergo a radial migration in r6 to reach their subpial location where they form the motor nucleus of the facial (nVII) nerve (Fig. 7.4A) (Chandrasekhar, 2004). The caudal soma translocation of FBM neurons, with looping of their axons (so-called *genu* of facial nerve) is conserved from fish to mammals, with important species differences. For example, it is blunted in chick (Gilland & Baker, 2005). The first indication that *Celsr* genes are involved in FBM neurons migration came from an ENU mutagenesis screen in zebrafish that identified four point mutations in the *Celsr2/off road* locus (Wada et al., 2006). In these mutants, FBM neurons fail to migrate caudally to r6, moving instead prematurely into lateral r4–r5. Morpholino knockdown experiments showed that *Celsr1a* and *1b* have adjunct functions in FBM neuron migration. Whereas combined downregulation of *Celsr1a* and *Celsr1b* has little effect on its own, it worsens the *off road* mutant phenotype, with more cells stacked in r4 in *Celsr1a; Celsr1b; Celsr2* triple mutants than in *Celsr2* mutants. In mice, FBM neuron migration was studied using *in situ* hybridization for *Islet1* or *Tbx20*, two established markers. In wild-type embryos, FBM neurons form streams from medial r4 to lateral r6, with a sharp rostral edge. In *Celsr1^{ko/ko}* mice, FBM neurons are still able to move out of r4, but a subset migrate rostrally into r3 and r2 rather than caudally, a phenotype never seen in any other vertebrate. This rostral migration phenotype is fully penetrant, although with variable expressivity. Caudally directed *Celsr1^{ko/ko}* neurons move through r5, medially to the abducens nucleus (nVI), before moving laterally in r6 like their wild-type counterparts. Hence, a facial (nVII) nucleus forms in its normal location in lateral r6 by E13.5. In addition, rostrally migrating FBM neurons form an ectopic nucleus adjacent or

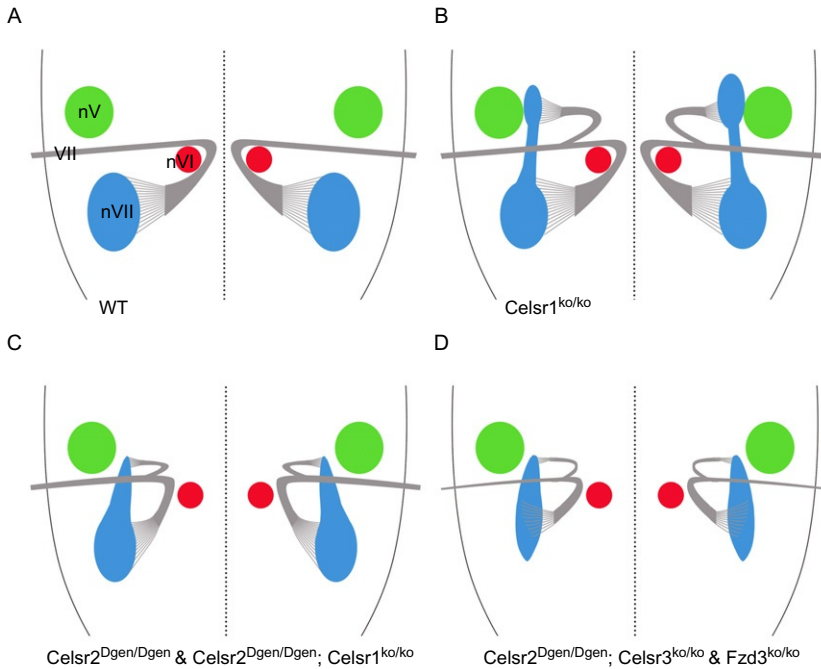


Figure 7.4 Migration of facial branchiomotor (FBM) neurons in normal and *Celsr1*–3 knockout mice. Drawings summarize the phenotype at P0. In wild-type animals (A), FBM neurons form a single nucleus (nVII) in a region that derives from r6. Their axons loop around the abducens nucleus (nVI) before exiting the rhombencephalon dorsally, caudal to the trigeminal nucleus (nV). In *Celsr1*^{ko/ko} mutants (B), in addition to the normal nVII, FBM neurons form another ectopic nucleus at the level of nV, but their axons leave the rhombencephalon at the right position. This is due to aberrant migration of FBM neurons in lateral r2 during embryogenesis. In *Celsr2*^{Dgen/Dgen} and *Celsr2*^{Dgen/Dgen}; *Celsr1*^{ko/ko} (C), the facial nerve *genu* is completely abnormal and axons do not loop around nVI because FBM neurons migrate prematurely in lateral r4–r5, forming lateral heterotopias. In *Celsr2*^{Dgen/Dgen}; *Celsr3*^{ko/ko} and in *Fzd3*^{ko/ko}, in addition to the absence of the *genu* of the facial nerve, the size of nVII is reduced because of embryonic cell death (D). “r,” rhombomere; nV, motor trigeminal nucleus; nVI, abducens nucleus; nVII, facial nerve nucleus.

even rostral to the trigeminal (nV) nucleus but send their axons normally in the facial nerve (Fig. 7.4B). In the hindbrain, *Celsr1* is expressed in progenitors and in the floor plate, but not in postmitotic FBM neurons. Consistent with this, conditional inactivation of *Celsr1* in progenitors under *Nk6.2-Cre* recombination induces abnormal rostral migration, whereas its deletion in FBM neurons using *Isl1-Cre* does not.

In *Celsr2* mutant mice (*Celsr2*^{Dgen/Dgen}), like in *off road* mutant fishes, the caudal FBM neuron migration stream is severely truncated. Cells turn laterally, rostral to the abducens nucleus, so that axons do not loop around nVI and an elongated facial nucleus forms in r4 and r5 instead of r6 (Fig. 7.4C). When *Celsr2* is specifically inactivated in FBM neurons by crossing with *Isl1-Cre* mice, a similar phenotype is generated, suggesting that *Celsr2* is required cell autonomously in FBM neurons for their caudal migration (Qu et al., unpublished). This does not rule out the possibility that *Celsr2* may also be necessary in neuroepithelial cells along the migration path, as suggested in zebrafish (Wada et al., 2006). The aberrant migration of FBM neurons in *Celsr2* mutant mice is reminiscent of the situation in birds, which have no facial nerve *genu* and, intriguingly, lack a *Celsr2* gene (Formstone, 2010). Whether there is a causal link between the absence of *Celsr2* and blunted FBM neuron migration remains an open question. In zebrafish, *Celsr2*, *Celsr1a*, and *Celsr1b* redundantly regulate FBMN caudal migration. In mouse, double inactivation of *Celsr1* and *Celsr2* phenocopies single *Celsr2* mutation, with no obvious rostral migration of FBM neurons, suggesting that *Celsr2* is epistatic to *Celsr1* (Fig. 7.4C). It looks as if *Celsr2* deficiency hampers or slows down neuronal migration, thus masking the *Celsr1* mutant phenotype. Although *Celsr3* is required for tangential migration of calretinin-positive interneurons and radial migration of calbindin-positive interneurons in the forebrain (Ying et al., 2009), its deficiency does not affect the migration of FBM neurons. It does, however, exacerbate the *Celsr2* phenotype: the facial nucleus is greatly reduced in size in *Celsr2*^{Dgen/Dgen}; *Celsr3*^{ko/ko} and *Celsr2*^{Dgen/Dgen}; *Celsr3*^{Isl1cko} as compared to *Celsr2*^{Dgen/Dgen} mutant (Fig. 7.4C and D). *Celsr3* deficiency increases apoptosis in a cell-autonomous manner. The phenotype of *Celsr2*^{Dgen/Dgen}; *Celsr3*^{ko/ko} double mutants is similar to that of *Fzd3* mutants, and other PCP-related genes such as *Vangl2* and *Wnt5a* have been implicated in FBM neuron migration, suggesting that *Celsr1–3* regulate neuronal migration along the rostrocaudal axis by PCP-dependent mechanisms (Qu et al., 2010; Vivancos et al., 2009) (Table 7.1). In line with this, normal function of PCP genes such as *van gogh-like 2* (*vangl2*), *fzd3a*, *celsr2*, *prickle1a*, and *prickle1b* is required for caudal migration of FBM neurons in fish (Bingham, Higashijima, Okamoto, & Chandrasekhar, 2002; Carreira-Barbosa et al., 2003; Jessen et al., 2002; Mapp, Wanner, Rohrschneider, & Prince, 2010; Rohrschneider, Elsen, & Prince, 2007; Wada et al., 2006).



5. CELSR2 AND CELSR3 IN BRAIN WIRING

Functional neuronal networks are crucial for brain function. Network formation is finely orchestrated at the cellular and molecular levels by genetic programs and interactions with the environment. After reaching their location, postmitotic neurons extend axons that are guided to their targets by intrinsic programs, guidepost cells, and attractive and repulsive molecular cues. They ramify receptive dendritic fields according to tiling rules. In addition to their role in neuronal migration, *Celsrs* are implicated in dendrite development and axon guidance, from *Caenorhabditis elegans* and *Drosophila* to mammals (Berger-Muller & Suzuki, 2011; Gao, Kohwi, Brenman, Jan, & Jan, 2000; Matsubara, Horiuchi, Shimono, Usui, & Uemura, 2011).

In flies, sensory neurons extend dendrites dorsally toward the midline. Dendrites from homologous neurons in the two opposite hemisegments avoid each other, leading to a dendrite-free zone near the dorsal midline. This reciprocal inhibition of dendrite growth, akin to tiling, is defective in *fmi/stan* mutants, where dendrites do grow across the midline to occupy fields overlapping those of homologous neurons (Gao et al., 2000). A role for *fmi/stan* in the tiling of dendrites is further supported by the identification of *fmi/stan* mutants in genetic screens for aberrant dendritic extension of mushroom body neurons (Reuter et al., 2003). The function of *fmi/stan* in dendrite growth does not rely solely on adhesion mediated by the extracellular domain because overexpression of an N-terminally truncated *fmi* that lacks cadherin, EGF-like, and laminin G motifs rescues the dendritic phenotype partially (Kimura, Usui, Tsubouchi, & Uemura, 2006). Intriguingly, neither loss or gain of function of *frizzled* nor overexpression of *dsh* phenocopy the *fmi/stan* dendritic phenotype. Moreover, to our knowledge, no such phenotype has been described in other PCP mutants. In the mammalian nervous system, *Celsr2* is required for the maintenance of dendritic arbors: RNAi-induced downregulation of *Celsr2* in brain slices reduces the length of dendrites in cortical pyramidal neurons, and the complexity of dendritic trees of Purkinje cells (Shima, Kengaku, Hirano, Takeichi, & Uemura, 2004). By contrast, the silencing of *Celsr3* results in dendrite overextension. The opposite effects of *Celsr2* and *Celsr3* are related to a single amino acid change in the first intracellular loop: like *fmi/stan*, mammalian *Celsr3* has a histidine at position 2413, and both repress growth and/or induce retraction of dendrites. That histidine residue is replaced with an arginine in *Celsr2*, which promotes dendrite growth and maintenance (Shima et al., 2007).

Early observations showed that *fmi/stan* is essential for the development of axonal tracts. Null mutations *fmi*^{E45} and *fmi*^{E59} are embryonic lethal, and in addition to PCP defects, mutant embryos display abnormalities in longitudinal axonal tracts. Rescue of axonal, but not PCP defects, by brain specific expression of the *fmi* cDNA restores viability (Usui et al., 1999). Subsequent elegant studies in the visual system showed that *fmi/stan* mediates axon–axon and axon–target interactions required for guidance of photoreceptor axons (Lee et al., 2003; Senti et al., 2003). The *fmi*-mediated choice of postsynaptic target is dose dependent and non-cell autonomous. It looks as if growth cones “compare” their *fmi/stan* levels with that of their neighbors to “decide” whether to establish contact or not (Chen & Clandinin, 2008). In mice, *Celsr3* is crucial for axon guidance. *Celsr3* mutant mice display marked defects in major tracts such as the anterior commissure, internal capsule, medial lemniscus, and corticospinal tract (Table 7.1). *Celsr3* is also essential for the anterior–posterior organization of monoaminergic axon in the brainstem (Fenstermaker et al., 2010) and for the rostral turning of commissural axons after midline crossing in the spinal cord (Price et al., 2006; Wang & Nathans, 2007). *Celsr3* deficiency does not affect axonal growth *per se*, but rather guidance, resulting in stalling at intermediate targets or misrouting of axons (Tissir, Bar, Jossin, De Backer, & Goffinet, 2005). For instance, during development of corticothalamic reciprocal projections, mutant corticofugal axons travel normally in the subcortical layer but stall underneath the pallial–subpallial boundary. Reciprocally, thalamic axons never reach their cortical targets in layer 4; instead, they course ventrally along the hypothalamus and then turn externally toward the cortical marginal zone. Conditional removal of *Celsr3* in a stream that extends in the basal forebrain and ventral diencephalon precludes the entry of corticosubcortical and thalamocortical fibers in the “corridor” and results in defective internal capsule. This result demonstrates that *Celsr3* is required in intermediate targets to connect the cortex with subcortical structures. Target cells are molecularly defined by expression of *Dlx5/6* and qualify as “guidepost cells” (Zhou et al., 2008). To test whether guidance of corticofugal axon is mediated by homophilic interactions of *Celsr3* in navigating growth cones and guidepost cells, *Celsr3* was specifically deleted in corticofugal axons by crossing the *Celsr3*^f allele with *Emx1-Cre*. *Celsr3*-defective corticothalamic axons develop normally, strongly suggesting that those fibers use guidance mechanisms independent of *Celsr3*–*Celsr3* homophilic interactions. Consistent with this finding, a stall phenotype of

axons at intermediate target cells was reported for *fmi*-defective sensory neurons in flies (Steinel & Whittington, 2009). These phenotypes can be rescued by *fmi* constructs that lack most of the extracellular domains, indicating that the advance of sensory axons in flies does not depend on *fmi*–*fmi* homophilic interactions (Steinel & Whittington, 2009). Contrary to corticothalamic axons, corticospinal axons do not develop in *Celsr3*^{*Emx1cKO*}, suggesting that *Celsr3* mediates homophilic interactions between corticospinal growth cones and guidepost cells. In support of this, in the fly visual system, *fmi* mediates interactions between the growth cones of photoreceptor axons and their targets in the medulla (Hakeda-Suzuki et al., 2011). Like in flies and mammals, FMI-1, the sole *C. elegans* *fmi*/*stan* ortholog, enables navigation of both pioneer and follower axons in the worm's ventral nerve cord. Rescue of FMI-1 mutant phenotype with different portions of FMI-1 revealed that the C- and N-termini are required for guidance of pioneer and follower axons, respectively (Steinmetz et al., 2010). Taken together, these data show that *flamingo* and its mammalian ortholog *Celsr3* are major players in axon guidance and that their mechanisms of action are context dependent and involve both homophilic and heterophilic interactions.

5.1. Motifs of Celsr important for their functions

As mentioned in the introduction, *Celsr* proteins contain a large extracellular N-terminus with nine cadherin repeats, EGF-like and laminin G-like motifs, a HRM, a G-proteolysis site, followed with seven transmembrane domains and a variable intracellular C-terminal tail. Although the functional relevance of these motifs remains poorly understood, some studies have revealed a few hints. In *Drosophila* S2 cells—which have no self aggregation properties—expression of full-length *fmi* induces cell aggregation, whereas expression of a form lacking most of the ectodomain does not, showing that the extracellular domain indeed promotes homophilic cell adhesion (Usui et al., 1999). Expression of mutant forms of mammalian *Celsr2* in S2 cells assigned adhesive properties to the cadherin repeats (Shima et al., 2004).

Dendritic overgrowth and tiling defects of *fmi* mutants as well as fly viability can be rescued by expression of full-length *fmi*, although the rescue can be ascribed to two different functions of the protein. In transgenic rescue experiments, Δ N*fmi*—lacking all extracellular domains except the HRM—can rescue the dendrite overgrowth but not the tiling phenotype. Unlike full-length *fmi*, Δ N*fmi* does not promote S2 cell aggregation *in vitro*.

By contrast, C-terminally deleted fmi (Δ CFmi) retains the ability to mediate homophilic binding *in vitro* and rescues the tiling phenotype, but not the dendritic overgrowth or fly viability, pointing to a key role of the C-terminus. Thus, a dual molecular function of Fmi plays pivotal roles in dendrite morphogenesis. In the initial growing phase, Fmi might function as a receptor for an unidentified ligand, and this hypothetical heterophilic interaction would be responsible for limiting branch elongation. At a later stage, homophilic Fmi-binding at dendro-dendritic interfaces would elicit avoidance between dendritic terminals from opposing neurons (Kimura et al., 2006). Likewise, studies in worms, flies, and mice show that the function of fmi/Celsr in axon guidance does not rely only on its ability to mediate homophilic binding of cadherin repeats but also requires interactions of the C-terminus with unknown molecules (Steimel et al., 2010; Steinel & Whittington, 2009; Zhou et al., 2008).

The concept of distinct roles of the extra- and intracellular domains of fmi/Celsr is supported by mutation analysis in vertebrates. In rodents, Celsr2 and Celsr3 have opposing roles on dendrites in brain slice in culture, with Celsr2 promoting and Celsr3 restricting dendrite growth (Shima et al., 2004, 2007). Studies of chimeric constructs in which the ectodomains of Celsr2 and Celsr3 are swapped show that, whereas homophilic interactions (Celsr2–Celsr2 and Celsr3–Celsr3) are important, the transmembrane domains and C-terminus determine the dendrite enhancing or suppressing action. Celsr2 and Celsr3 have different effects on calcium release and activate, respectively, CamKII and Calcineurin signaling, and an amino acid change (R2413H) in the first intracellular loop is crucial to these distinct functions.

Another evidence for the importance of the C-terminus comes from a study in zebrafish (Carreira-Barbosa et al., 2009). Injection of a C-terminally truncated form of Celsr (Δ C-celsr; lacking 6 TM and tail) in wild-type embryos generates epiboly defects. This is accompanied by sequestration of Δ C-Celsr in the Golgi, where the protein behaves as a dominant negative, dimerizing with and precluding trafficking of the wild-type protein. Dimerization is thought to involve a conserved arginine-rich sequence N-terminal to the first cadherin repeat. Injection of the CT of Celsr2 fused to the membrane localization signal from the Lyn tyrosine kinase (Lyn-celsr) generates convergent extension defects without affecting epiboly. Lyn-celsr perturbs the Frizzled-induced membrane localization of Disheveled and thus PCP signaling. In mammals, knock-in of the mouse Celsr1 C-tail fused to membrane localization signal Lyn in the ubiquitous Rosa26 locus also perturbs

the trafficking of Dvl2, and Vangl2 as a well as the subcellular of F-actin (Trichas et al., 2011). Interestingly, when a conserved serine acidic amino acid-rich domain (SE/D) is deleted from the fish Lyn-Celsr, the membrane localization of Disheveled is restored (Carreira-Barbosa et al., 2009). Whereas the extracellular and TM domains are important for the distribution of Frizzled–Disheveled complexes at the membrane, this process depends critically on the Cter SE/D domain. The ability of Celsr to regulate epiboly is closely associated with its ability to modulate cell cohesive property, whereas its ability to interact with the PCP pathway to regulate convergent extension may not require cell adhesion mediated by the cadherin repeats.

In the mouse skin, Celsr1 is internalized during epidermal basal progenitor division, a process which is crucial for balanced distribution of PCP proteins Fzd6 and Vangl2 in daughter cells and requires a cytoplasmic dileucine motif. Whereas E-Cadherin is normally not internalized, its fusion with the Celsr1 cytoplasmic domain induces internalization during mitosis and confers to the chimeric protein the ability to recruit Fzd6 and Vangl2. When the two leucines (2748–2749) are mutated to alanines, chimeric E-Cad-Celsr1, like mutated Celsr1, no longer translocates to endosomes (Devenport et al., 2011).

The Celsr1–3 CTs display much less similarity than their extracellular and TM regions. As Celsr1 is directly implicated in PCP, and Celsr2–3 in more distantly related processes, could differences in the C-terminus account for functional differences? In *Drosophila*, fmi, fz, and dsh depend on each other for their membrane localization (Das et al., 2002). Fmi interacts physically with fz via a region encompassing its HRM and TM domains, and selectively recruits fz and vang (strabismus) to opposing cell boundaries (Chen et al., 2008). In mice, the membrane localization of Fzd3 and Vangl2 in ependymal cells, Fzd6 and Vangl2 in skin epithelial cells, and Dvl2 and Vangl2 in the visceral endoderm cells depends on Celsr cadherins (Devenport & Fuchs, 2008; Tissir et al., 2010; Trichas et al., 2011). In *Celsr1*^{Crsh/Crsh} mutant mice, a single amino acid substitution results in failure of the Celsr1 protein to reach the apical membrane (Devenport & Fuchs, 2008; Formstone et al., 2010; Ravni et al., 2009). Furthermore, all six Celsr1 mutations recently identified in human fetuses with craniorachischisis impair membrane trafficking of Celsr1 in *in vitro* assays (Robinson et al., 2011). Although not directly useful for definition of structure–function relationships, this exquisite sensitivity to minor sequence changes underscores the importance of fmi/Celsr conformation

for their membrane insertion as well as for proper targeting of partner proteins. This raises the question of the role of putative proteins assisting in folding, like receptor-activating protein RAP does for the lipoprotein receptor family (Herz, 2006), or the beta-2 microglobulin for major histocompatibility complexes (Hansen, Connolly, Gould, & Fremont, 2010). Would Celsr, Fzd, and Vangl help each other's folding and complex formation in the endoplasmic reticulum and traffic as a complex to the Golgi and to the membrane, or are they assisted by other unidentified partners, and what would be the role of chaperonins in the process?

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