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Review

# Seven pass Cadherins CELSR1-3

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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 a key role of CELSR1 in epithelial planar cell polarity (PCP), and for CELSR2 and CELSR3 in ciliogenesis and neural development, especially neuron migration and axon guidance in the central, peripheral and enteric nervous systems. Phenotypes in mutant mice indicate that CELSR proteins work in concert with FZD3 and FZD6, but several questions remain. Apart from PCP signaling pathways implicating CELSR1 that begin to be unraveled, little is known about other signals generated by CELSR2 and CELSR3. A crucial question concerns the putative ligands that trigger signaling, in particular what is the role of WNT factors. Another critical issue is the identification of novel intracellular pathways and effectors that relay and transmit signals in receptive cells? Answers to those questions should further our understanding of the role of those important molecules not only in development but also in regeneration and disease.



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## 1. Introduction

Among the large cadherin superfamily, vertebrate CELSR1-3 proteins ("cadherin, EGF LAG seven-pass G-type receptor 1, 2 and 3") and their orthologs Flamingo (Fmi)/Starry night (Stan) in *Drosophila*, and FMI-1 in *C. elegans*, form a small group character-

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ized by the presence of seven transmembrane segments, whereas classic cadherins are type I membrane proteins. This specific feature is at the origin of the recent terminology ADGRC1-3 for "Adhesion G protein-coupled receptors, subfamily C" [1]. Mammalian *CELSR1-3* genes have similar genomic organizations, with 35 (*CELSR1* & *CELSR3*) or 34 exons (*CELSR2*). There is 3′ alternative exon splicing in *CELSR2* [2] and developmentally regulated neuronal inclusion of a 15nt micro-exon 16b in the mouse *CELSR3* (unpublished).

CELSR proteins have a large ectodomain (~2700 amino acids) composed of nine N-terminal cadherin repeats (typical cadherins have five repeats), several epidermal growth factor (EGF)-like domains, two laminin G-type repeats, one hormone receptor (HRM) motif, and a G-protein-coupled receptor (GPCR) proteolytic site (GPS) embedded in a GAIN (GPCR Autoproteolysis INducing) domain [3]. This is followed by seven transmembrane segments and a cytoplasmic tail that varies in size from 300 to 600 residues which, in contrast to the ectodomain, is poorly conserved between the three members. Cadherin, EGF-like and laminin repeats presumably confer adhesive properties and facilitate cell–cell communication, and the GAIN and laminin domains might bind unidentified ligands. Contrary to what the term ADGRC conveys, no coupling with G-proteins has been demonstrated thus far. Whether autoproteolytic cleavage at the GPS occurs in CELSR1-3 proteins also remains unclear. Antibodies to CELSR1 allowed detection of full length (400 kD) and cleaved (85 kD) forms generated by as yet unidentified proteolysis events not involving the GPS [4].

CELSR1 and its invertebrate orthologs regulate epithelial planar cell polarity (PCP) in several organs and species, and is thus a "core PCP" gene/protein [5–8]. In contrast, in most cases, CELSR2-3 interact closely with FRIZZLED (FZD), especially FZD3, but not with VANGL and other PCP members, and therefore do not qualify as bona fide PCP genes.

## 2. Expression of CELSR1-3

In the nervous system, *CELSR1-3* expression is regulated spatially and temporally, in complementary patterns [9–11]. *CELSR1* mRNA is heavily expressed in zones of neural stem cell (NSC) proliferation and abates postnatally in parallel to decreasing numbers of NSC. *CELSR3* mRNA is absent from NSC, but expressed at low levels in intermediate progenitors (IP) and very highly in neurons. It is sharply downregulated at the end of neuron maturation and persists only in the adult cerebellar granular layer, the hilus of the dentate gyrus and the rostral migratory stream (RMS). *CELSR2* mRNA is found in NSC, IP and postmitotic cells and remains stable in the adult. This hints at roles of *CELSR1* in NSC, of *CELSR3* in neuronal maturation, and of *CELSR2* in development and maintenance of the nervous system. *CELSR1-3* mRNAs are also variably expressed in non-neural epithelia, such as the skin [12,13], lungs [14], kidney [15], endothelial cells [16], gastrointestinal [17] and reproductive systems [18].

## 3. Roles of CELSR1-3

### 3.1. CELSR1 and epithelial PCP

*CELSR1* mutant mice have a complex phenotype, with looping tail, defective neural tube closure (craniorachischisis), head shaking behavior, abnormal body hair whorls. Mutant inner ear cells have defective organization of stereocilia bundles. Normally, the apical surface of receptor cells is decorated with a "V" of stereocilia centered on a kinocilium, pointing to the external aspect of the cochlear canal. This orientation is a classical hallmark of PCP and parallels the polarized distribution of PCP proteins such as FZD3, FZD6, VANGL2, and PRICKLE2 [19–21]. PCP proteins localize asymmetrically to one edge of the apical cell cortex, presumably by selective targeting and protein stabilization or degradation, and this is thought to be crucial for vectorial PCP signaling [22].

Remarkably, PCP phenotypes similar to those in *CELSR1* mutants are found in mice with mutations in other PCP genes. The inner ear phenotype was reported in mice with mutated *VANGL2* [19,23], and *FZD3* and *FZD6* [20]. Neural tube closure defects were observed in *VANGL2*, double Disheveled *DVL1* and *2*, and *FZD3* and *−6* [20,24,25]. Skin hair patterning defects were described in *FZD6* and *VANGL2* mutants [12,26,27]. This provides strong evidence that CELSR1 is involved in classical epithelial PCP, together with FZD3 and 6, DVL1 and 2, and VANGL2 (Fig. 1).

### 3.2. CELSR1 and ectoderm patterning

Inactivation of CELSR1 in embryonic ectoderm generates striking skin hair patterning defects [13,27], and a similar phenotype results from inactivation of FZD6 [28]. Prior to hair growth, CELSR1 becomes asymmetrically localized along the anterior/posterior (A/P) axis in basal epidermal cells, and this is essential for A/P orientation of skin hairs. In *CELSR1* mutant embryos, the membrane recruitment of FZD6 and the asymmetric localization of VANGL2 along the A/P axis are compromised. Thus, CELSR1 plays a critical role for PCP establishment in the developing skin and hair follicles. Like that of skin hairs, the orientation of ectodermal lingual papillae is under the control of PCP genes VANGL1, VANGL2, and CELSR1 [29].

### 3.3. CELSR1 in the reproductive system

Homozygous male and female *CELSR1* mutant mice are sterile. In males, sterility is due to anatomical defects and obstructions of the rete testis, which blocks sperm entry in the epididymus (Fig. 2A–E). Similar defects result from inactivation of FZD3 and FZD6, further indicating that a PCP-like mechanism is involved. Females have anomalies of the oviduct with abnormal cilia orientation and beats [18,30], as well as spectacular cystic dilatations of the oviduct or uterus (Fig. 2F). Mutant females also have defective oocyte maturation (Fig. 2G, H). CELSR1 is not detected at the oocyte surface but is highly expressed at junction between granulosa cells, suggesting that the oocyte maturation defect is non cell autonomous.

### 3.4. CELSR1 and neural tube closure

The implication of PCP in convergent extension and neural tube closure was initially demonstrated in *Xenopus* and zebrafish [31–35]. In mice, inactivation of *VANGL2*, *DVL1&2*, *CELSR1, FZD3* or *FZD3&6* leads to craniorachischisis, the most severe neural tube closure defect [20,22,24,25,36–40]. In humans, craniorachischisis is associated with mutations in *VANGL2* and *CELSR1* [41–43], and myelomeningocele with mutations in *VANGL1*, *VANGL2*, *FZD6*, *PRICKLE1* and *CELSR1* [41–46]. Neural tube closure defects are attributed to defective cell intercalation at the midline, leading to broadening of the floor plate that prevents the dorsal fusion of neural folds. In the bending neural plate, CELSR1 is concentrated in adherens junctions oriented toward the midline and this may explain its role during neural tube closure [47]. In line with this, mutations associated with human craniorachischisis impair the trafficking of CELSR1, reducing its membrane localization *in vitro* [43]. Interestingly, craniorachischisis is also found in humans and mice with mutations in gene SEC24b, which encodes a component of the COPII complex implicated in trafficking of the PCP gene VANGL2 [48–50].

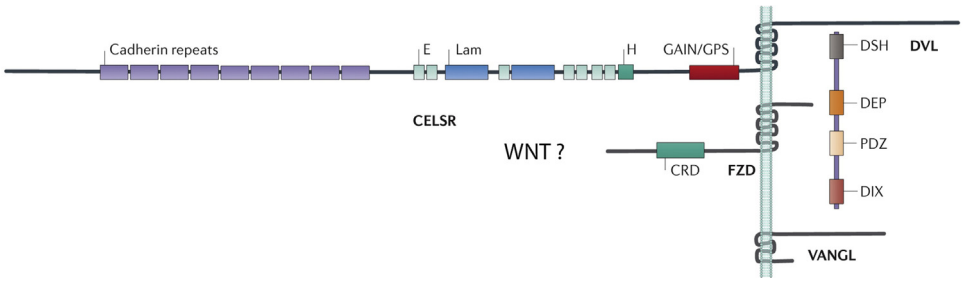


**Fig. 1.** Schematic representation of CELSR proteins and partners of PCP signaling.
Extracellular domains of CELSR1-3 includes nine cadherin repeats, seven epidermal growth factor (EGF)-like domains (E), two laminin G repeats (Lam), one hormone receptor (HRM, H) motif, and a G-protein-coupled receptor proteolytic site (GAIN/GPS). CELSR1, 2 and 3 cadherins are anchored to the plasma membrane by seven transmembrane domains; their cytoplasmic tails vary in size and are poorly conserved among the three CELSRs. Partners of PCP signaling are Frizzled, especially FZD3 and FZD6, Van Gogh-like (VANGL) and Disheveled (DVL). CRD: Frizzled cysteine-rich domain, to which WNT factors bind. In DVL, the main protein interaction motifs are DSH, DEP, PDZ and DIX. Adapted from [7].

### 3.5. *CELSR1, -2 and -3 in cilia development and organization*

Based on their structure and motility, cilia are classified into motile, primary and nodal [51]. Motile cilia garnish the apical surface of epithelial cells lining airways, reproductive tracts and cerebral ventricles. Ciliary microtubules anchored to a basal body in the apical cortex generate directional cilia beats and fluid flow. This is crucial for clearing mucus and debris in airways and for transit of sperm and eggs in genital tracts [52,53]. In the postanatal mouse brain, multicilia develop at the surface of neuroepithelial cells around cerebral ventricles when they differentiate into ependymal cells. The basal body of each cilium has a lateral "basal foot" that points in the effective beat direction and is used as a hallmark of cilia polarity [54–56]. To generate efficient flow, cilia and basal feet are aligned within each individual cell, as well as in all cells in the epithelial sheet [57,58]. *CELSR1*, *2* and *3* are expressed in the developing ependyma. Mutation of *CELSR2* and *CELSR3* impairs ependymal ciliogenesis at the individual cell level, resulting in defective beats and fluid flow, with lethal hydrocephalus [59]. Inactivation of CELSR1 does not impact the formation of cilia but perturbs the coordinated alignment of cilia tufts along the ventricle axis, which results in mild hydrocephalus [60]. The membrane localization of VANGL2 and FZD3 is disrupted in CELSR2 and CELSR3 mutant ependymal cells, providing evidence that CELSR proteins regulate ciliogenesis via PCP signaling. In accord with this, downregulation of core PCP genes affects the orientation of multicilia in *Xenopus* [61,62], and ependymal cells from *VANGL2* mutant mice fail to align their motile cilia in response to hydrodynamic forces *in vitro* [63]. The role of CELSR in human ciliopathies was recently emphasized with the demonstration that mutated CELSR2 is implicated in Joubert syndrome [64–66].

### 3.6. *CELSR1, -2 and -3 in neuronal migration*

The migration of facial branchiomotor neurons (FBMN) in the developing rhombencephalon combines tangential and radial migration [67–69]. Neurons are generated in rhombomere 4 (r4) at E10.5 and immediately extend their axons towards facial muscles. From E11.5, cell bodies migrate caudally from r4, traverse r5 and move laterally and dorsally in r6 [70] where they move to a subpial location and form the facial nucleus [67]. In zebrafish, mutations of *CELSR2* [71] perturb FBMN migration, and phenotypes are aggravated upon morpholino knock-down of *CELSR1a* and *1b*. PCP genes *VANGL2*, *FZD3a*, *PRICKLE1a*, and *PRICKLE1b* are also required for caudal migration of FBMN [35,72–75].

In *CELSR1* mutant mice, most FBMN are able to leave r4, but a subset migrate rostrally rather than caudally, and then move laterally prematurely in r5 and r6. In the hindbrain, *CELSR1* is expressed in NPC and in the floor plate, but not in postmitotic FBMN. CELSR1 protein is detected in apical junctions between floor and roof plate cells, and between NPC. In addition CELSR1 is present in the basal domain, at the end-feet abutting the pial basement membrane [4,76]. Abnormal migration is FBMN non cell autonomous: conditional inactivation of *CELSR1* in progenitors, but not in FBMN, mimics the phenotype [76,77]. In *CELSR2* mutant mice, there is no rostral migration, but caudal migration is diminished and an elongated ectopic facial nucleus forms in r4 and r5 instead of r6. Contrary to CELSR1, CELSR2 is required cell autonomously in FBMN. Inactivation of *CELSR3* does not affect FBMN migration, but exacerbates the *CELSR2* mutant phenotype and increases FBMN apoptosis in a cell autonomous manner. The phenotype of *CELSR2; CELSR3* double mutants is similar to that of *FZD3* mutants, and other PCP related genes such as *VANGL2* and *WNT5a* are also implicated in FBMN migration [78,79].

### 3.7. *CELSR2 and CELSR3 and brain wiring*

The formation of neural networks is orchestrated by genetic programs in interaction with the environment. During and after migration, neurons extend axons that are guided by intrinsic programs, guidepost cells and attractive and repulsive cues, and then ramify dendritic fields. CELSR proteins are implicated in axon guidance and dendrite development, from *C. elegans* and *Drosophila* to mammals [80–82], reviewed in [7].

In flies, dendrites from homologous sensory neurons in opposite hemisegments avoid each other, and this is defective in *fmi/stan* mutants [81], who also have defective dendritic extension of mushroom body neurons [83]. In mammals, RNAi-induced downregulation of *CELSR2* in brain slices reduces the length of dendrites in cortical pyramids, and their complexity in Purkinje cells, whereas silencing *CELSR3* results in dendrite overextension [84,85]. Those opposite effects of CELSR2 and CELSR3 *in vitro* need to be confirmed by studies of mutant brains.

Evidence for a pivotal role of CELSR in axonal development is even stronger. In flies, inactivation of *fmi/stan* generates abnormalities in longitudinal axonal tracts [8]. In the visual system, Fmi/Stan mediates axon–axon and axon-target interactions required for guidance of photoreceptor axons, and this is independent of Fz and Vangl [86–89]. WNT and PCP genes are required for axonal targeting and branching of mushroom body neurons [90,91] and, intriguingly, the amyloid precursor protein is involved in this process [92]. In *C. elegans*, *FMI-1* controls pioneer-dependent axon guidance in the ventral nerve cord [93], and *FMI-1* and *WNT* pathway components control the growth of anteroposterior axons of VD GABAergic neurons [94,95].

In mice, *CELSR3* mutation results in pathfinding defects in the anterior commissure (Fig. 3A), internal capsule (Fig. 3B), medial lemniscus and corticospinal tract [96]. CELSR3 is required for the

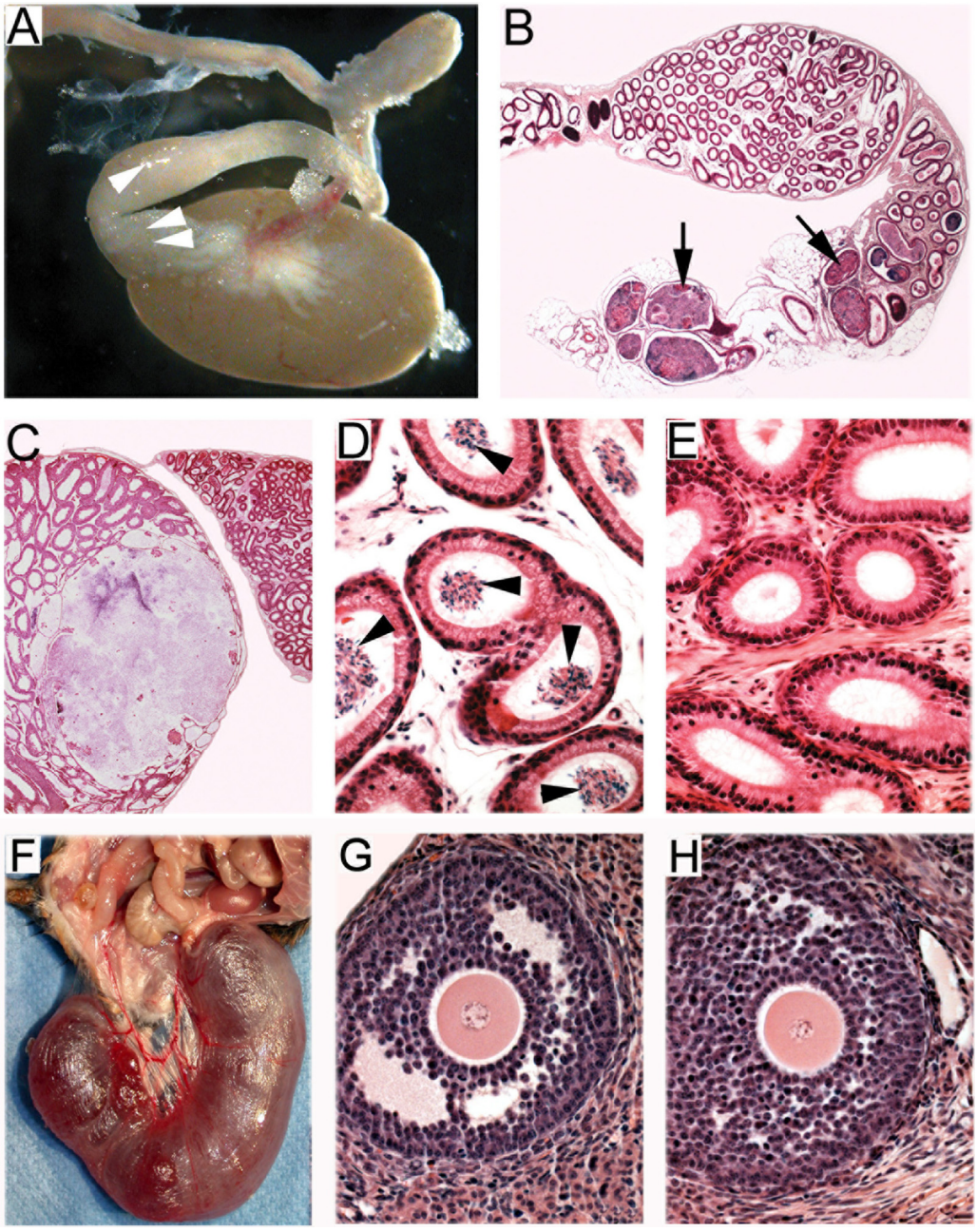


**Fig. 2.** Role of CELSR1 in reproductive system.
A–E. Testes from *CELSR1* adult mutant males (A, B, C, E) show whitish material in the rete testis (A, arrowheads), which corresponds to sperm debris (B,C, arrows). Contrary to the epididymis of normal mice (D, arrowheads), that of *CELSR1* mutants contains no sperm (E).
F–H. In *CELSR1* mutant females (F, H), large cysts often develop in the uterus asymmetrically (F), and there is abnormal organization of the granulosa layer in the ovary follicles, resulting in poor definition of antral cavity (compare control in G with mutant in H).

anterior-posterior organisation of monoaminergic axons in the brainstem [97] and for the rostral turning of commissural axons after midline crossing in the spinal cord [98,99]. The defects are not limited to central axons, as peripheral axons are also affected [17,100]. Axonal phenotypes observed in *CELSR3* and in *CELSR2*; *CELSR3* double mutant mice are copied in *FZD3* [101–105] but not in *VANGL2* mutants, although this remains somewhat debated (see for example [106]). CELSR3 and FZD3 do not affect axonal growth *per se*, but rather guidance [96,107]. Conditional inactivation using tissue specific Cre lines showed that, in most cases such as corticospinal tract, anterior commissure and peripheral motor axons, CELSR3 and FZD3 are required cell autonomously in the neurons of origin [100,102,103,108]. An exception is the development of reciprocal thalamocortical connections, for which CELSR3 and FZD3 are required in "guidepost cells" acting as intermediate compartments along the pathway [108], but not in corticothalamic or thalamocortical neurons [104]. This non cell autonomy is explained by the role of CELSR3 and FZD3 in patterning a scaffold of early pioneer axons that extend from prethalamus to ventral telencephalon and telencephalic preplate. This CELSR3 and FZD3-dependent scaffold develops prior to the emergence of cortical and dorsal thalamic axons and serves as a bridge to guide them to their reciprocal targets [109].

In addition to their role in the neuroepithelium, PCP genes such as PRICKLE1, PRICKLE2 and VANGL2 have been implicated in synaptic function [110,111]. There is evidence that CELSR3 and VANGL2

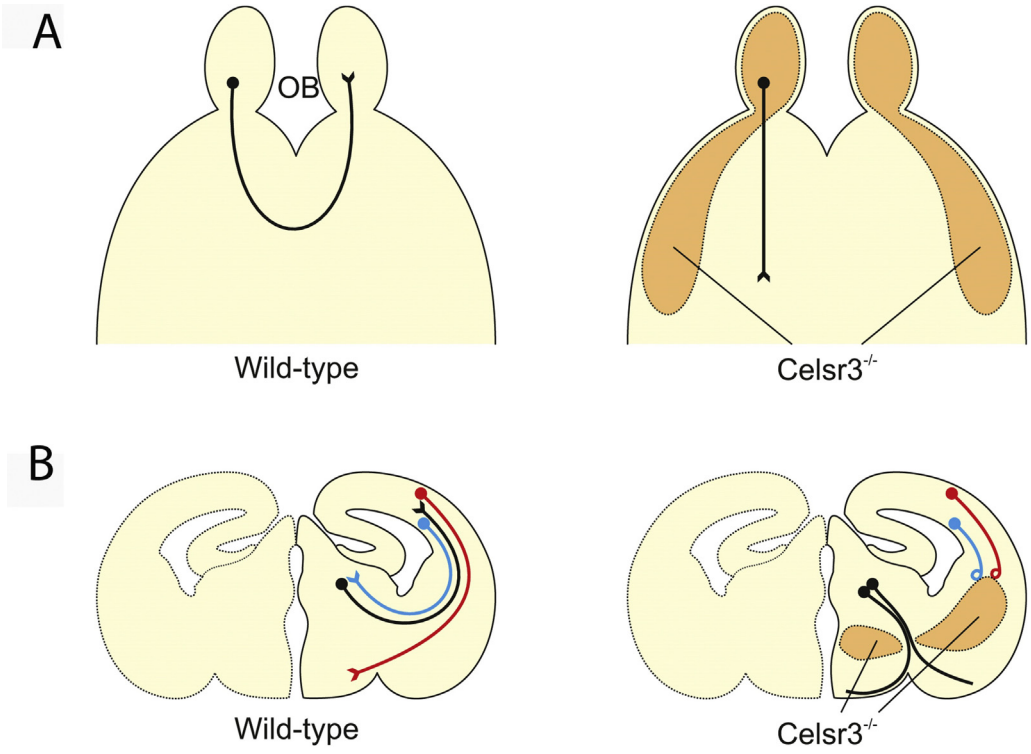


**Fig. 3.** Role of CELSR3 in axon guidance.
A. Commissural fibers from the olfactory bulbs (OB) cross the midline in the anterior commissure in wild-type control (left) but not in mice in which CELSR3 is inactivated in cortical areas (right, dark area).
B. The main components of the internal capsule in wild-type mice are thalamocortical fibers (black), corticothalamic fibers (blue) and corticosubcortical axons (red). All three are defective when CELSR3 is inactivated in forebrain guidepost cells (dark areas). Adapted from [7].

play opposite roles in glutamatergic synapse formation [112], and connectivity is affected upon CELSR3 inactivation in hippocampus [113]. Those observations suggest a role of CELSR proteins in synaptic function, which remains to be defined in future studies.

## 4. Speculations about mechanisms

The actions of CELSR in epithelial PCP should be understood in the context of PCP signaling which is reviewed extensively elsewhere [6]. Here, we will rather focus on other mechanisms.

### 4.1. Structure-activity data

Like other cadherins, CELSR presumably mediate adhesion. In fly S2 cells, Fmi induces cell aggregation [8], and mammalian CELSR2 has adhesive properties that map to the cadherin repeats [85]. Most dendritic defects of *fmi* mutants are rescued by full length Fmi and by mutant proteins lacking most ectodomain. By contrast, C-terminally deleted Fmi rescues self avoidance but not dendritic overgrowth [114]. In worms, flies and mice, the function of Fmi/CELSR in axon guidance requires interactions of the C-terminus with unknown partners [93,108,115]. *In vitro*, CELSR2 and CELSR3 have opposing roles on dendrites, with CELSR2 promoting, and CELSR3 restricting growth [84,85]. Studies of constructs in which ectodomains are swapped, show that, whereas ectodomains mediate homophilic interactions, the transmembrane domains and C-terminus determine the dendrite growth. In zebrafish, injection of a C-terminally truncated CELSR in wild type embryos generates epiboly defects [116]. The mutant protein is sequestered in the Golgi, where it behaves as a dominant negative and impacts trafficking of the wild-type protein. Injection of the cytoplasmic tail of CELSR2 fused to a membrane localization signal generates convergent extension defects without affecting epiboly, and perturbs the Frizzled-induced membrane localization of Disheveled and PCP signaling. In mammals, knock-in of C-terminal CELSR1 fused to a membrane localization signal also perturbs the trafficking of DVL2 and VANGL2 [117]. As the CELSR1-3 cytoplasmic tails display no similarity, differences in the C-terminus may account for functional differences. In *Drosophila*, Fmi, Fz and Dsh depend on each other for their membrane localization [118]. Fmi is expressed on both membranes and promotes homophilic binding. It further interacts physically with Fz and selectively recruits Fz and Vang to opposing cell boundaries [119]. In mice, the membrane localization of FZD3 and VANGL2 in ependymal cells, of FZD6 and VANGL2 in skin epithelial cells, and of DVL2 and VANGL2 in visceral endoderm cells all depend on CELSR [27,59,117], suggesting that a mechanism analogous to that in flies operates.

### 4.2. Role of WNT ligands

Studies of conditional mutant phenotypes provide overwhelming evidence that CELSR3 and FZD3 interact in the same cells, and this is confirmed by co-immunoprecipitation of these proteins [100,120]. This raises the question of the ligand(s) of the CELSR/FZD complex, among which WNT factors – also implicated in PCP – are obvious candidates [121]. Spinal commissural axons turn rostrally after crossing the midline, and this is defective in *FZD3* and in *CELSR3* mutant mice. WNT4 and WNT7b form an anterior high – posterior low gradient in the floor plate. WNT4 is able to attract post-crossing commissural axons, and inactivation of WNTs by sFRP impairs rostral turning, whereas WNT4 expressing cells placed ectopically are able to re-route axons towards them [105]. In explants, WNT5a attracts serotoninergic but repels dopaminergic axons, whereas WNT7b attracts dopaminergic axons, and

*FZD3* mutant dopaminergic axons cannot respond to WNT5a and WNT7b [97]. Another argument is the implication of WNT5a in FBMN migration mentioned above [79]. Lastly, in the late embryonic cortex, WNT7a and WNT7b act on migrating neurons through CELSR3/FZD3, to increase their expression of JAG1 and thereby regulate NOTCH signaling in apical neural progenitors, as discussed further below [122]. Although the importance of WNTs is evident, several questions remain. For instance, no ligand receptor-binding interaction was clearly identified, let alone quantified, and WNT mutant mice do not harbor phenotypes evocative of those in CELSR mutants. Studies are complicated by the existence of nineteen WNTs and ten FZD receptors (in mammals), and by the fact that some WNTs bind to other receptors such as the tyrosine kinases ROR1,2 [123] or RYK ("receptor related to tyrosine kinase") [124].

### 4.3. Interactions of CELSR3/FZD3 with other guidance receptor complexes

Growth cones encounter and integrate multiple guidance cues with which CELSR3/FZD3 could interact, as indicated by studies of hindlimb innervation [100,103]. In the developing hindlimb, the sciatic nerve divides into dorsal/peroneal and ventral/tibial nerves, in response to at least two signals: i) EPHRIN-As in ventral mesenchyme repel dorsal peroneal axons by binding to their EPH-A receptors; ii) EPH-A receptors and GDNF in dorsal mesenchyme bind to EPHRIN-A2/A5 and GDNF receptors (RET and GFRA1) and synergistically attract dorsal peroneal growth cones [125]. *CELSR3* and *FZD3* mutant axons of the dorsal peroneal nerve stall after the sciatic nerve division and fail to extend distally in the dorsal hindlimb. Although they remain able to respond to attractive GDNF or repellent EPHRIN-As, mutant axons cannot respond to attractive EPHRIN-A reverse signaling (EPH-As in the limb acting on EPHRIN-As in the axon). Moreover, in transfected cells, CELSR3 and FZD3 co-immunoprecipitate with EPHRIN-A2 and -A5, and RET. EPHRIN-As are GPI-anchored proteins located in membrane microdomains and could help assemble signaling platforms containing CELSR3/FZD3, RET/GFRA1 and perhaps other partners, enabling growth cones to integrate multiple steering signals.

Another interesting interaction occurs between CELSR3/FZD3 and NOTCH signaling. CELSR3 and FZD3-deficient cerebral cortices are characterized by increased neuronal and decreased glial density, indicating defective timing of neurogenesis and gliogenesis in radial NPC. Intriguingly, that phenotype results from absence of CELSR3 or FZD3 in immature neurons, not in NPC. Expression of NOTCH ligand JAG1 is decreased in CELSR3 and FZD3-deficient neurons compared to control. This fails to activate NOTCH properly in NPC, resulting in increased neurogenesis at the expense of gliogenesis. JAG1 mRNA is upregulated upon treatment by WNT7, the most abundant WNT factor in the embryonic cortex, but this is blunted in CELSR3 and FZD3 mutant neurons. There is thus a feedback loop whereby CELSR3 and FZD3 are required in immature neurons to bind WNT7 and upregulate JAG1, and thereby activate NOTCH signaling in NPC to tune their fate and the timing of the gliogenic switch [122]. This is reminiscent of the situation in the fly retina, where a decreasing equatorial-polar gradient of WNT signals via Fz in photoreceptor R3, triggering Delta expression, which activates Notch signaling in adjacent R4 cells [126,127].

### 4.4. Potential partners identified through mutant phenotype similarity

LINX (a.k.a. ISLR2) is a transmembrane tyrosine kinase that regulates the extension and guidance of corticofugal, thalamocortical, sensory and spinal motor axons [128,129]. LINX interacts with other receptor tyrosine kinases such as TRKA, RET, and TRK–associated protein p75NTR. Peripheral nerve projection defects in *LINX* mutants are reminiscent of those in *NGF*, *TRKA* and *RET* mutants. *LINX* mutants also share multiple phenotypic traits with *CELSR3* and FZD*3* deficient mice. Like CELSR3 and FZD3, LINX is required in guidepost cells in the basal forebrain for internal capsule formation, in neocortex for the development of corticospinal tracts, and in motor neurons for hindlimb innervation. These data suggest that LINX may interact with CELSR3/FZD3.

Another gene whose mutant phenotype resembles that in *CELSR3/FZD3* is *PHR1* (a.k.a. *Mycbp2*), an E3 ubiquitin ligase expressed in the nervous system. Like *CELSR3* and *FZD3* mutants, *PHR1* knockout mice die at birth and exhibit prominent axonal defects: corticofugal and thalamocortical projections are lost, the anterior commissure is absent, the corpus callosum and peripheral nerves are reduced [130,131]. PHR1 inhibits the dual leucine kinase MAP3K12 (a.k.a. DLK) and forms a complex with the F-box protein FBXO45 [132,133]. Intriguingly, *MAP3K12* [134] and *FBXO45* [132] mutant mice also share phenotypes with *CELSR3/FZD3* mutants, such as absence of anterior commissure and abnormal internal capsule.

### 4.5. Downstream effectors of CELSR3/FZD3 signaling. Role of DVL?

Disheveled (DVL) is a key cytoplasmic adapter of FZD receptors and PCP signaling. All three *DVL* (*DVL1- 3*) are expressed in the mouse nervous system [135]. Single *DVL* mutants exhibit mild phenotypes, *DVL1* and *DVL2* double mutants show neural tube closure defects, and *DVL1-3* triple mutants cannot undergo gastrulation, indicating that DVL1-3 have redundant functions. FZD3 can recruit and activate DVL, which further signals to JNK, RHOA and RAC to regulate the cytoskeleton [136]. DVL1-3 are therefore potential downstream effectors of CELSR3/FZD3 signaling, in axon guidance like in PCP. The lack of conditional alleles hampers genetic studies *in vivo*, but there is evidence for a role of DVL in axon development. Overexpression of DVL in cortical or commissural neurons enhances axon outgrowth. In dissociated commissural neurons, FZD3 is able to recruit DVL from intracellular vesicles to the membrane [106].

Phosphorylation of DVL by the ABELSON tyrosine kinase is required for its activity in PCP [137,138], and a feedback in the FRIZZLED/DVL signal may result from the ability of DVL to increase the hyperphosphorylation of FZD3 and prevent its internalization, thereby inhibiting PCP signaling [106]. Formins such as DAAM1 and DAAM2 dock to DVL [139] and activate RHO signaling [140]. As they regulate actin bundle elongation and microtubule dynamics, formins are candidates to relay a signal to the cytoskeleton. Thus far, however, there is no direct *in vivo* evidence implicating formins in CELSR/FZD signaling, perhaps owing to genetic redundancy among the fifteen members of the formin family [141].

## 5. Conclusions

Atypical cadherins CELSR1-3 are important molecules. They interact closely and most probably form molecular assemblies with FZD3 and FZD6. Those assemblies may interact with other signaling systems such as EPH-EPHRINs or NOTCH. Although direct experimental evidence is somewhat lacking, CELSR/FZD receptors are probably activated by WNT ligands. Several questions remain about the intracellular partners and mechanisms that mediate their role in target cells and growth cones. Future work to address those questions will help unravel the role of those important proteins in development, regeneration and disease.

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