Final report of the research group of

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Celsr genes in brain development and function

State of the art

Celsr (Cadherin, EGF-like, Laminin G-like, Seven pass, G-type Receptor) are developmentally regulated proteins with the ability to signal by homophilic and/or heterophilic interactions. Functional studies in the fruit fly have demonstrated a role for flamingo, the *Drosophila* Celsr, in the orientation of epidermal structures, such as wing hairs, abdominal bristles and the facets of the eye. These structures are organized in the plane of epithelium, orthogonal to the apical-basal polarity axis, by a process referred to as planar cell polarity (PCP). During the wing hair development, PCP is characterized by accumulation of Celsr/flamingo at cell boundaries. Celsr/flamingo triggers polarity by selectively recruiting frizzled to one side and van gogh to the opposite side of the cell.

When we started studying the mammalian Celsr genes in early 2000s, two members (*Celsr1* and *Celsr2*) were listed in databases, but little (if any) was known about their functions. We identified the third member (*Celsr3*), explored the expression patterns of the three genes and inactivated them in mice. Our analyses show that they are widely expressed in the nervous system where they play crucial roles in neural tube closure, neuronal migration, ependymal polarity, and axon guidance (reviewed in (Boutin et al., 2012; Tissir and Goffinet, 2010; Tissir and Goffinet, 2013)).

During the 3 years of this project, we have focussed mainly on the role of Celsr genes in ependymal polarity and ciliogenesis, and axon guidance.

Celsr1-3 in ependymal polarity and ciliogenesis

The wall of the lateral ventricles of the postnatal forebrain, hereafter referred to as lateral wall “LW”, is a region where neural stem cells coexist with ependymal cells. A striking feature of multiciliated ependymal cells is their high degree of polarization. At the cell level, all cilia need to beat in the same direction. Therefore, their basal feet (lateral extensions of BB that point to the direction of the effective stroke of cilia beat) rotate during development and adopt a homogeneous orientation (rotational polarity). Planar polarity is also observed at the tissue scale: all ependymal cells display a shift of their basal bodies (BB) to the anterior side of the cell (translational polarity). This specific organization of the lateral wall is essential for cerebrobrospinal fluid (CSF) circulation and its modification is thought to affect stem cell maintenance and adult neurogenesis.

In 2010, we showed that Celsr2 and Celsr3 impairs ciliogenesis and leads to defective CSF flow and lethal hydrocephalus. Mutant ependymal cilia never develop in normal numbers and display abnormalities in morphology, position, and planar organization. Ciliary basal feet are mis-oriented, and basal bodies were seen ectopically deep in the cytoplasm. The conventional method to analyze rotational polarity is to investigate the orientation of the basal foot by transmission electron microscopy. This method is time consuming. To speed up studies of LW in our mutants, we developed an alternative approach wherein we combined immunostaining and confocal microscopy. Gamma tubulin and phospho-beta-catenin localize at opposite sides of the BB and define a vector which nicely delineates rotational polarity of cilia. Using this method, we expanded on our initial finding and show that Celsr2 and Celsr3, together with

Fzd3 and Vangl2, control not only the orientation of motile cilia but also their spacing and heir lattice organization in individual cells.

To investigate the potential role of Celsrs in translational polarity, we performed immunostaining on LW whole-mounts. We used antibodies against ZO1 and gamma tubulin which label tight junctions and basal bodies respectively. We analyzed the position of BB patch relative to the center of the cell. In WT and PCP mutants, ependymal cells showed a displacement of cilia. However, while in WT animals, all ciliary tufts are systematically shifted toward the anterior pole, *Celsr1* mutant mice display abnormal translational polarity with cilia dispersed in any pole of ependymal cells. It has been suggested that the primary cilium of radial glial (RG) cells control the translational polarity of multicilia. We analyzed the presence of primary cilium in our mutants. Immunostaining against either gamma tubulin or acetylated tubulin demonstrated that virtually all RG cells bear a primary cilium at birth suggesting that the translational polarity defects observed in *Celsr1* are not due to lack of the primary cilium. We then carried-out a time course analysis and found that the primary cilium is progressively polarized to the anterior side of the cell in normal animals, anticipating ependymal cell translational polarity. In *Celsr1* mutant mice, the primary (mono) cilium migrates away of the center of the RG cells but not systematically toward the anterior side. The same phenotype is observed in mice mutant for *Fzd3*, and Vangl2. Interestingly RG polarity is not affected in *Celsr2* or *Celsr3* mutants. Our results show that *Celsr1*, *Fzd3* and *Vangl2* position the primary cilium in radial progenitors. In ependymal cells, whereas *Celsr2&3*, *Fzd3* and *Vangl2* work together to organize cilia tufts in a given cell; *Celsr1*, *Fzd3* and *Vangl2* coordinate polarity between cells. These signals are relayed by distinct cytoskeletal changes. These data reveal unreported functions of Celsr genes and PCP signaling and provide an integrated view as how polarity is set in radial progenitors and passed on to ependymal. In addition to the published articles listed below, the rest of the data has been compiled in a manuscript that is under revision in *PloS Biology*. The pdf file is attached (appendix 1)

Celsr3 in wiring of the nervous system

Since our initial report (Tissir et al., 2005), we and others have accumulated evidence that Celsr3 is a major player in directional growth of axons and wiring of the central nervous system(Feng et al., 2012; Fenstermaker et al., 2010; Lewis et al., 2011; Onishi et al., 2013; Price et al., 2006; Zhou et al., 2008a; Zhou et al., 2010; Zhou et al., 2008b; Zhou et al., 2009a; Zhou et al., 2007; Zhou et al., 2009b). However, whether Clesr3 acts in collaboration with, or in parallel to, other axon guidance systems such Eph/ephrins, Slit/Robo, Semaphorins/Plexins was not known; and whether it is involved in wiring of the peripheral and the enteric nervous systems was not investigated.

The enteric nervous system (ENS) constitutes a network of interconnected ganglia, which are arranged radially throughout the gut and integrate local and systemic signals to control gastrointestinal motility, secretion, and blood flow. In vertebrates, the majority of enteric neurons and glia are derived from neural crest cells which invade the foregut and, migrating rostro-caudally, colonize the entire length of the gastrointestinal tract. Considerable progress has identified a number of signaling pathways that control the migration of ENS progenitor cells and their differentiation into enteric neurons. However, the organizing principles of enteric connectivity and the mechanisms underlying the assembly of functional circuits from differentiated enteric neurons remain poorly understood.

In a collaborative study with the groups of Pieter Vanden Berghe (KUL, Leuven) and Vassilis Pachnis (MRC, London UK), we have combined in vivo and ex vivo physiological assays with gene inactivation and single-cell labeling to demonstrate that, in mice, Celsr3, together with Fzd3, controls gastrointestinal function by regulating the spatial organization of neuronal processes during gut organogenesis and the connectivity of ENS. In control guts, the vast majority of identifiable neuronal processes were directed anally parallel to the longitudinal axis. However, in both *Celsr3* and *Fzd3* mutants, a significantly larger fraction of neural projections were arranged circumferentially or directed orally. In addition to the altered trajectory, Celsr3- and Fzd3-deficient enteric neurons had shorter primary neurites, while a fraction of them acquired bipolar or multipolar morphology. Conditional inactivation of Celsr3 upon crossing Celsr3 floxed allele with Wnt-Cre transgenic mice demonstrated that Celsr3 is required cell autonomously in neural crest derivatives for normal development of ENC and for gastrointestinal function. Taken together, our studies identify Celsr3 as a critical regulator of ENS wiring in vivo and provide insight into the connection pathology that might underlie some gut motility disorders. This work was published in Journal of Clinical Investigation en 2013.

To probe the role of Celsr3 in the peripheral nervous system, we studied the consequences of its loss-of-function on limb innervation. We found that mice with conditional inactivation of Celsr3 in motor neurons often exhibit uni- or bilateral paralysis of the hindlimb. Muscles of the anterior compartment of the hindlimb, particularly the tibialis anterior, are very atrophic, pointing to a defect of peripheral motor innervation that was confirmed by electrophysiology. Further studies showed that *Celsr3* mutants have a selective deficit of innervation of extensor muscles innervated by the dorsal, peroneal nerve, whereas axons of tibial nerve that innervate ventral muscles are unaffected. Fzd3 mutants have an identical phenotype. EphA4 mutant mice as well as mice with inactivation of the GDNF receptor components Ret and GFRa1 have a similar phenotype, namely absence of dorsal peroneal nerve, with rerouting of axons ventrally, hinting at possible interactions between Celsr3/Fzd3 and those two important signals.

Detailed phenotype analysis showed that, in *Celsr3* mutant mice, axons of the peroneal nerve segregate from those of the tibial nerve, but fail to extend dorsally and stall near the branching point. Those axons are not rerouted ventrally; thus, the phenotype is not identical to that in *EphA4* and *GDNF* mutant animals. *Celsr3* mutant axons respond to the repulsive signal generated by ephrinsAs expressed in the ventral limb mesenchyme acting on EphA4 in motor neurons. They are also able to respond to the attractive signal of GDNF. By contrast, Celsr3 and Fzd3 mutant motor neurons, contrary to as the wildtype axons, are not attracted by EphA-Fc in the Dunn chamber assay. This clearly shows that Celsr3 and Fzd3-deficient axons are no longer able to respond to the reverse ephrin signaling triggered by EphA expressed in dorsal limb mesenchyme acting on ephrin A receptors in growth cones. Using *EphA4* mutant mice, we showed that Celsr3 interacts genetically with EphA4. We also demonstrated that Celsr3 associates physically with ephrinA2 and A5 in transfected HEK cells, and that Celsr3 co-immunoprecipitates with Fzd3 as predicted, as well as with Ret and GFRa1. Intriguingly, the peripheral axonal phenotype was not seen in mice with inactivation of the core planar polarity gene Vangl2, and no physical interaction between vangl2 and Celsr3 was detected, indicating that Celsr3 and Fzd3 regulate axon guidance in a Vangl2 independent manner. Our results provide strong evidence that Celsr3/Fzd3 interact with EphA:ephrinA reverse signaling to guide motor axons in the hindlimb.
This work is under revision in *Nature Neuroscience*. The pdf file of the manuscript is attached (appendix 2)

Peer-reviewed publications 2011-2013

2013:

- Onishi K, Shafer B, Lo C, **Tissir F**, **Goffinet AM**, Zou Y.
  Antagonistic functions of Dishevelleds regulate Frizzled3 endocytosis via filopodia tips in Wnt-mediated growth cone guidance.
  Journal of Neuroscience 33:19071-19085.
  IF : 6.91
- Tatin F, Taddei A, Weston A, Fuchs E, Devenport D, **Tissir F**, MakinenT.
  Planar cell polarity protein Celsr1 regulates endothelial adherens junctions and directed cell rearrangements during lymphatic valve morphogenesis.
  Developmental Cell 26: 31-44 (2013)
  IF: 14.03
- **Tissir F** & **Goffinet AM**.
  Shaping the nervous system: Role of the planar cell polarity genes.
  Nature Reviews Neuroscience 14: 525-535 (2013)
  IF: 30.45
- **Tissir F** & **Goffinet AM**.
  Atypical cadherins Celsr1-3 and planar cell polarity in vertebrates. The Molecular Biology of Cadherins, Edited by Franz van Roy; *Progress in Molecular Biology and Translational Science* 116: 193-214 (2013)
  (book chapter)
- Sasselli V, Boesmans W, Vanden Berghe P, **Tissir F**, **Goffinet AM**, Pachnis V.
  Planar cell polarity genes control the connectivity of enteric neurons.
  Journal of Clinical Investigation 123: 1763-1772 (2013)
  IF: 15.43
  Vardarajan B*****, Vergote D*****, **Tissir F***, Logue M, Yang J, Daude N, Ando K, Rogaeva E, Lee J, Cheng R, Brion JP, Ghani M, Shi B, Baldwin CT, Kar S, Mayeux R, Fraser P, Goffinet AM, George-Hyslop PS, Farrer LA, Westaway D.
  Role of p73 in Alzheimer disease: lack of association in mouse models or in human cohorts.
  Molecular Degeneration 8: 1-14 (2013)
  ***** : Equal contribution.
  IF: 4.01

2012:

- Boutin C, Goffinet AM, **Tissir F**.
  Celsr1-3 Cadherins in PCP and Brain Development.
  Current Topics in Developmental Biology 2012 ;101:161-183.
  IF: 6
- Feng J, Xu Y, Wang M, Ruan Y, So KF, **Tissir F**, Goffinet A, Zhou L.
  A role for atypical cadherin Celsr3 in hippocampal maturation and connectivity.
  Journal of Neuroscience 2012; 32(40):13729-13743
  IF: 7.11
- **Tissir F**, **Goffinet AM**.
  Cilia: conductors' batons of neuronal maturation.
  Nature Neuroscience 2012; 15(3):344-345.
  IF: 15.25
- Pietri S, Dimidschstein J, Tiberi L, Sotiropoulou PA, Bilheu A, **Goffinet A**, Achouri Y, **Tissir F**, Blanpain C, Jacquemin P, Vanderhaeghen P.
  Transcriptional Mechanisms of EphA7 Gene Expression in the Developing Cerebral Cortex. *Cerebral Cortex* 2012; 22(7):1678-89.
  IF: 6.28
- Cortijo C, Gouzi M, **Tissir F**, Grapin-Botton A. Planar Cell Polarity Controls Pancreatic Beta Cell Differentiation and Glucose Homeostasis.
  Cell Reports 2012; 2:1593-1606.
  IF: Unknown. *Cell Reports* is a new journal launched in 2012 by Cell press. The "predicted" IF is around 8 according to the number of articles and citations in 2012.

2011:

- **Tissir F**, **Goffinet AM**. p73 and p63: Estranged relatives?
  Cell Cycle 10:1351 (2011)
  IF: 5.35

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