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Configuring a robust nervous system with Fat cadherins

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Abstract

Atypical Fat cadherins represent a small but versatile group of signaling molecules that influence proliferation and tissue polarity. With huge extracellular domains and intracellular domains harboring many independent protein interaction sites, Fat cadherins are poised to translate local cell adhesion events into a variety of cell behaviors. The need for such global coordination is particularly prominent in the nervous system, where millions of morphologically diverse neurons are organized into functional networks. As we learn more about their biological functions and molecular properties, increasing evidence suggests that Fat cadherins mediate contact-induced changes that ultimately impose a structure to developing neuronal circuits.

Keywords

Fat cadherins; Fat-like cadherins; neural development; tissue polarity; planar polarity

1. Introduction

Cadherins constitute a superfamily of transmembrane proteins with varying numbers of calcium-binding cadherin domains that enable cell-cell interactions, in some cases resulting in the formation of stable junctions and in other cases activation of signaling pathways that impact the cytoskeleton, gene expression, and the cell cycle [1]. Initially described as cell adhesion molecules, cadherins vary widely in structure and fall into different subfamilies based on the presence of additional protein interaction domains extracellularly, the number of transmembrane domains, and the composition of their intracellular domains [2]. The classical cadherins, for instance, generally have five cadherin repeats and are characterized by the presence of catenin binding sites in the intracellular domain, consistent with their well-defined role in cell adhesion. All other molecules bearing cadherin domains are loosely defined as “protocadherins”, a group that comprises many additional subgroups based on their sequences and chromosome organization [3]. For example, the genes that encode the α -, β -, and γ -protocadherins are clustered in three distinct arrays in the genome, each of

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which contains several variable exons that are driven by their own promoters. Selection of a certain promoter and hence a certain exon seems to be achieved by differential promoter methylation and allows for the production of a variety of single transmembrane proteins with six cadherin domains that provides neuronal individuality [4]. The atypical Fat cadherins, on the other hand, stand out for their enormous size, up to 500–600 kDa, largely due to the presence of a huge extracellular domain containing 32–34 cadherin repeats, 1–2 laminin A–G and 4–5 EGF repeats [5,6]. Additionally, Fat cadherins have cytoplasmic domains that vary within the family and can recruit many different intracellular effectors, including transcription factors and actin regulators.

Cadherins play a particularly prominent role in the nervous system, which hosts a large population of diverse cell types that must be organized into coherent networks. Whereas classic cadherins and the clustered protocadherins direct neural circuit assembly through their effects on cell-type recognition and self-recognition [7,8], the role of Fat cadherins in the nervous system is less clear. In fact, Fat cadherins are present even in the simplest multicellular animals, predating the expansion of protocadherins in animals with more complex nervous systems [9]. Other cadherins have been proposed to be derivatives of the Fat cadherins, arising by steady loss of cadherin repeats and acquisition of new cytoplasmic domains, raising the possibility that Fat cadherins originally enabled basic cell-cell interactions that were adapted to new contexts in the nervous system, as witnessed by the evolution of subfamilies with more dedicated roles. With only 2–4 family members in any species and no evidence for the extensive diversity of the clustered protocadherins [7], Fat cadherins are not equipped to provide much in the way of specificity during cell-cell interactions. Instead, individual Fat cadherins stand out as versatile molecules that can influence cell adhesion, morphology, and proliferation via a range of extracellular interactions and intracellular effectors. Although it remains unclear whether there is one unifying function, emerging evidence suggests that Fat cadherins coordinate complex changes in cell morphology with the surrounding environment, thereby imposing a tissue-level order not only in epithelia such as the fly wing but also within developing circuits of the vertebrate nervous system.

1.1. Basic features of the Fat cadherins

What is best known about Fat function comes from studies in *Drosophila*, which have one *fat* (*ft*) gene and one *fat-like* gene (a.k.a *fat2* or *kugelei*). Analysis of these two huge atypical cadherins has defined two core functions: cell proliferation and tissue polarity [10]. Together with its Dachshous ligand, Ft appears to regulate proliferation and polarity through different intracellular pathways (Fig. 1A). Despite obvious structural and functional homologies to Ft, Fat-like acts through a different ligand and different effectors. Nonetheless, both molecules share the ability to mediate local cell-cell interactions and thereby coordinate cell behavior across tissues.

Ft was originally described as a tumor suppressor gene, as loss of function mutations led to increased cell proliferation and thus abnormal enlargement of larval imaginal discs [11], hence its name. In *Drosophila*, the Ft ligand is another unusually large cadherin, Dachshous (Ds) [12], which is also involved in cell proliferation [13]. Later findings positioned Ft and

Ds as upstream regulators of the Hippo pathway [14–19], which controls organ size by regulating transcription of pro-proliferation and cell death genes [20]. The Hippo pathway involves a series of phosphorylation events where Hippo (Hpo) and Salvador (Sav) form a kinase complex that phosphorylates and activates the kinase complex formed by Warts (Wts) and Mob-as-tumor-suppressor (Mats). One effector of this kinase cascade is the transcriptional co-activator Yorkie (Yki), whose Hippo-dependent phosphorylation prevents it from entering the nucleus and, therefore, acting as a transcriptional co-activator [20,21]. Ft-Ds has been proposed to serve as a receptor-ligand pair that regulates Hippo signaling, culminating in activation of the Hpo-Wts kinase cascade and inhibition of Yki by regulating components such as Expanded and Dachs [22].

In addition to its effects on proliferation, Ft also plays a key role in the polarization of cellular structures. Many cells acquire asymmetric morphologies that must be organized across an entire organ, a feature known as tissue polarity. Two classic examples are the *Drosophila* wing, where cells uniformly point trichomes distally, and the compound eye, where units of photoreceptors are oriented towards the equator. Genetic screens for defects in each of these tissues uncovered two molecular systems that control both the local organization of polarized cells within the epithelium (“planar cell polarity”) and the long-range organization of this polarity across the epithelium (“tissue polarity”) [23,24]. The orientation of neighboring cells depends on the “core” planar cell polarity (PCP) signaling pathway, which comprises Frizzled (Fz), Disheveled, Prickle, Van Gogh, Diego and Flamingo [23,25]. Ft participates in a second, less understood system that somehow coordinates more global features of polarity. As in cell proliferation, Ft works together with Ds to control tissue polarity, but in this case not strictly through the Hippo pathway. Although there is strong evidence that cells rely on systematic differences in Ft-Ds interactions to sense their position and hence orientation within an organ, exactly how changes in cell shape are brought about remains poorly understood.

In both the fly wing and eye, tissue polarity depends on gradients of Ft and Ds activity (Fig. 1B). In the wing, a Ft-Ds activity gradient results in the asymmetric distribution of the intracellular myosin Dachs and hence formation of distally pointing trichomes [26]. Additional positional information is provided by the Golgi kinase Four-jointed (Fj), which modulates the strength of the Ft/Ds interaction by phosphorylation of their extracellular domains [27]. Ft is uniformly expressed, but Ds and Fj are both expressed in gradients. Thus, heterotypic interactions with Ds result in graded activity of Ft, ultimately read out as asymmetric membrane localization of Dachs. Ft and Ds also influence each other’s localization, possibly transmitting asymmetric distribution within one cell to its neighbor.

Gradients of Ft and Ds activity also organize multicellular structures, such as the ommatidia of the eye. Each ommatidium consists of eight photoreceptors, seven of which are arranged into trapezoids that point away from the equator of the eye, with ommatidia in the dorsal half pointing up and those in the ventral half pointing down. The Ft/Ds system specifies the identity of the photoreceptor at the tip of the trapezoid and hence its orientation [28]. Thus, in both cases, gradients of Ft/Ds activity lead to tissue-wide changes in cell behavior. Importantly, Ft also has non-autonomous effects and can influence cells at a distance, though

it is unclear whether this is by serial propagation of local changes in protein distribution or instead through production of a long-range signaling molecule [29].

Ft appears to convey polarity information through a variety of downstream effectors that enable diverse cellular effects. For instance, distinct domains within the Ft cytoplasmic domain are required for proliferation or for planar polarity [30]. There is little consensus regarding the nature of the planar polarity effectors. In some contexts, different levels of Ft/Ds interactions influence protein distribution and perhaps establish an axis for the core PCP pathway. This is clearest in the wing, where the Ds/Ft/Fj module biases the asymmetric distribution of Fz in wing cells [31]. This may be through direct effects on the cytoskeleton, for example by orienting microtubules and hence the movement of vesicles carrying cargo such as Fz [32]. However, the relationship between these two systems remains controversial and may depend on context [29,33,34]. For example, in the eye, Ft/Ds interactions regulate the level of Fz signaling and hence the identity of the precursor closer to the equator of the eye [28], but these effects depend on regulation of gene expression by the transcriptional co-repressor Atrophin (Atro), which binds to the Fat intracellular domain (ICD) and is essential for tissue polarity [35]. Therefore, it is possible that Ft actually influences the global organization of the eye by defining its equator [36], perhaps via Atro-dependent regulation of an additional signaling molecule [37].

The Fat cytoplasmic domain also binds several proteins that modulate the levels, activation, and distribution of both Fat and its key effector, Dachs. For instance, the cytoplasmic protein Lowfat influences Ft localization, whereas the kinase Discs overgrown (Dco) activates Ft by phosphorylation of the intracellular domain. Activation of Ft leads to reduced accumulation of Dachs at the membrane, possibly via recruitment of the ubiquitin ligase Fbx17 [6,38,39]. Conversely, Ft activity is countered by associations with the palmitoyltransferase Approximated, which instead promotes localization of Dachs to the membrane, as does the cytoplasmic protein Dlish (also called Vamana) [38,40–42]. Hence, consistent with the presence of many protein interaction domains, Fat seems to act through multiple intracellular effectors that can act independently. However, synergistic interactions among the Hippo pathway and these various planar polarity effectors have been suggested to make optimal use of Ft's versatility as a signaling molecule [38].

The multi-functional nature of Fat cadherins is further emphasized by analysis of *fat-like*, which appears to share a role in polarity but through yet another mechanism. Like Ft, Fat-like has been implicated in the regulation of tissue polarity as well as morphogenesis of tubular structures [43–46]. No role for Fat-like has been described in proliferation. Fat-like influences polarity by modulating the cytoskeleton, perhaps by directing alignment of actin filaments [45] and microtubules in developing egg chambers [44,46]. This might occur via recruitment of the WAVE-regulatory complex (WRC) to the plasma membrane, as there are three WRC-interacting receptor sequences (WIRS) in the intracellular domain that are essential for WRC binding *in vitro*, for proper WRC localization *in vivo*, and for formation of polarized filopodia [47]. As a well-characterized regulator of the actin cytoskeleton, the WRC could serve as a direct link between Fat-like and local changes in cell structure.

This is not the whole story, as not all of Fat-like's functions require the intracellular domain [48]. At least in egg chambers, Fat-like does not mediate its polarity function via Ds [45]. In fact, no ligand has been defined. Nonetheless, there is clear evidence of non-autonomous functions. For instance, Fat-like activity in one cell stabilizes the asymmetric localization of the receptor tyrosine phosphatase Dlar in a neighboring cell, thereby ensuring directed collective cell migration [47,48]. Thus, some Fat-like functions may depend solely on non-autonomous signaling via the extracellular domain, while other functions may be mediated entirely by the intracellular domain. This seems to be the case for Ft, which is cleaved to release a cytosolic intracellular domain that can move into mitochondria and directly affect metabolism [49].

Despite their differences, Ft and Fat-like share several features that may underlie their common effects on polarity. First, both of these atypical Fat cadherins function in an asymmetric manner. In the case of Ft, asymmetric activation is achieved by graded expression of the Ds ligand and modification by Fj. Fat-like, on the other hand, is asymmetrically localized [48]. Second, both proteins exhibit autonomous and non-autonomous effects, emphasizing the importance both of the enormous extracellular domain as a potential conveyor of polarity information and of the variable intracellular domain as a mediator of this activity. Third, unlike the classical cadherins, there does not appear to be one core function for the Fat cadherin intracellular domain. Instead, Fat cadherins signal through multiple pathways to influence a range of effects, from cell proliferation to alignment of the cytoskeleton. Thus, cells may rely on different effectors to translate Fat-dependent positional information into an appropriate response.

1.2. The vertebrate Fat cadherins

Fat cadherins have expanded modestly in vertebrates, where four family members (Fat 1–4) share some basic functions with their invertebrate counterparts. Fat1, Fat2 and Fat3 are more closely related to *Drosophila* Fat-like [43], whereas vertebrate Fat4 is the Ft ortholog [5]. Indeed, Fat4 appears to be part of a conserved Ft/Ds system, while Fat1 and Fat3 share many properties with Fat-like (Fig. 1C). However, this is not a strict division of labor, as some features of Fat signaling are present in Fat-like homologs. Moreover, Fat and Fat-like proteins can interact with each other both genetically and biochemically, creating opportunities for further synergies amongst their diverse signaling outputs.

As predicted by strong sequence similarities with Ft, Fat4 regulates tissue polarity in several vertebrate organs, including the kidney and inner ear [50]. Consistent with its identity as the ortholog of *Drosophila* Ft, Fat4 binds to a *Dachsous* ortholog, *Dachsous1* (*Dchs1*) [51], and *Fat4* and *Dchs1* mutant mice exhibit many of the same phenotypes. There is a second *Dachsous* gene in mice, *Dchs2*, which functions in a partially redundant fashion with *Dchs1* [52]. In addition, members of the vertebrate *Atrophin* (*Atn*) family genetically interact with *Fat4* and influence its effects on vertebral arch fusion and renal tubular elongation [53], confirming *Atn* to be part of the Fat signaling pathway both in invertebrates and vertebrates. Thus, Fat4 seems analogous to Ft.

The other Fat family members share similarities with *Drosophila* Fat-like, but appear to have acquired novel properties as well. Most strikingly, there are multiple WIRS motifs in the

intracellular domains of both Fat1 and Fat3 [54], echoing the importance of the WRC for Fat-like function [47]. However, Fat1 and Fat3 also bind to members of the Ena/VASP family of actin regulators [55,56], acting through sites that are not present in *Drosophila* Fat-like or in the vertebrate Fat4. In addition, a number of other downstream binding partners have been reported for Fat1, such as β -catenin, Scribble, Homer1 and Homer3 [57–59]. Thus, Fat-like cadherins may play a general role in organizing the cell cytoskeleton, perhaps through direct interactions with actin regulators. How binding via the extracellular domain might contribute to these effects is unknown. Although ligands for Fat1 and Fat3 have not been identified, Fat2 binds homophilically [60]. It will be important to determine whether Fat1, 2, and 3 act independent of a Dachsous ligand like Fat-like does in flies, though genetic studies hint that Fat3 may signal with Dchs2 in some contexts [61].

Although it would be convenient to consider Fat4 separately from Fat1, 2, and 3, the situation appears to be more complicated. For example, Fat1 seems to share Fat4's use of Atrophin effectors [62]. Similarly, Fat3 interacts genetically with Atrophin2a (also called REREA) to control the polarized intercalation of chondrocytes in zebrafish, possibly leading to activation of a secondary long range signal much as Atro is proposed to act in flies [61]. Moreover, Fat1 can bind directly to Fat4 in *cis* [63], raising the possibility of cross-talk between pathways. Indeed, genetic interaction studies revealed that *fat4* synergizes with *fat1* and *fat3* in several tissues [53]. Fat1 and Fat4 also share the ability to regulate cell proliferation, perhaps via effects on the Wnt or Hippo signaling pathways [58,64], though a strong link between vertebrate Fats and the Hippo pathway has yet to be demonstrated [30]. Even more so than in flies, the picture for how atypical Fat cadherins work at the molecular level is far from complete, with a pressing need to identify relevant ligands and to piece together potential signaling pathways.

2. Fat cadherins in the nervous system

The vertebrate nervous system contains a stunning variety of neurons that form precisely ordered circuits that are located at stereotyped positions with respect to each other and to the body axis. The cadherin superfamily plays an important role at every step, from the formation of adherens junctions that ensure the integrity of the neuroepithelium to the emergence of cell-type specific synapses. Subfamilies such as the classical cadherins and clustered protocadherins are expanded in vertebrates and play a privileged role in the nervous system, likely providing molecular codes by which different types of neurons can recognize each other. The Fat cadherins, on the other hand, comprise a relatively small group with only a few alternative splice variants identified for *fat1* and *fat3* to date [65,66]. Rather, Fat cadherins stand out not for their diversity but for their flexibility as signaling molecules. Indeed, Fat cadherins influence many features of neural development, from morphogenesis of the neural tube to the assembly of complex circuits (Table 1). Efforts to understand how the Fat cadherins detect and transmit signals in each of these contexts are beginning to point to a general role in “polarity”, where Fat cadherins offer a cell adhesion system that transforms global positional information into local changes in cell shape.

Upon the identification of vertebrate *fat* genes, attention logically focused on possible roles in planar polarity in epithelia. One of the most obvious examples of vertebrate planar

polarity is the organization of hair cells in the cochlea, since each hair cell extends actin-rich stereocilia that are asymmetrically positioned on the apical surface, with all hair cells pointing in the same direction [23,67]. Planar polarity is also evident in the process of convergent extension, where migrating cells align with each other into streams that drive the narrowing and simultaneous lengthening of epithelial structures. When convergent extension fails, as occurs in mice lacking homologs of many “core” PCP genes, the cochlea is short and broad and the neural tube fails to close [68,69]. Likewise, Fat signaling appears to be required for proper morphogenesis of epithelial structures in the nervous system, but with important differences. Additionally, Fat cadherins influence many other features of neural circuit formation, from proliferation to migration to differentiation. Whether these diverse functions reflect a shared role in “polarity” remains to be determined.

2.1. Morphogenesis of epithelial structures in the nervous system

In both the cochlea and neural tube, the Ft/Ds system plays an important role that is related to yet distinct from that of the core PCP system. The cochlea is a structure of the inner ear that contains the sensory organ for hearing, the organ of Corti. The organ of Corti is composed of hair cells – mechanosensory cells responsible for detecting sounds – that are arranged in three rows of outer hair cells (OHCs) and one row of inner hair cells (IHCs) (Fig. 2A). *fat4* mutants develop shorter cochleae, with misoriented hair cells and an extra fourth row of OHCs [50,53], phenotypes that are consistent with a role in polarity. However, these phenotypes are subtle compared to what occurs when the core PCP system is disrupted, suggesting different cellular mechanisms. This fits with the fact that the Ft/Ds system is not equivalent to the core PCP system in flies. Consistent with *in vitro* binding assays [51], *Dchs1* likely acts as the relevant ligand, since *dchs1* mutants show similar cochlear defects, as do *dchs1; fat4* double mutants [70].

The Fat-like cadherins Fat1 and Fat3 also appear to influence cochlear patterning, though in less familiar ways. *fat1* mutant mice do not show classic signs of planar polarity defects [53]. However, loss of one copy of *fat1* enhances the effects of *fat4* mutations on both the length of the cochlea and the appearance of an extra row of OHCs [53]. Conversely, the *fat4* phenotype is suppressed in *fat3; fat4* double mutant mice. These opposing effects of Fat3 and Fat4 may reflect differential recruitment of Atn effectors, since *Atn1* and *fat3* mutants both show loss of the 3rd row of OHCs, whereas loss of one allele of *Atn2* enhances the appearance of a 4th OHC row in *fat4* mutants [53]. These genetic observations fit with a model where Fat1 and Fat4 act through a common Atn2 pathway, whereas Fat3 antagonizes Fat4 by an Atn1-mediated mechanism. Whether such parallel Fat pathways exist remains to be proven.

In contrast to the stereotyped neural tube defects observed upon loss of the core PCP system, disruption of Fat cadherin function affects neural tube closure in a different way. The developing neural tube closes at three independent sites [71]. Closure at site 1 depends on well-characterized PCP events that ensure convergent extension and hence elongation of the caudal neural tube. When these events fail, the hindbrain and spinal cord remain open, as occurs for example in *Vangl2* mutant mice [72]. None of the Fat cadherins have been implicated in this type of neural tube closure. Instead, Fat1 and Fat4 appear to cooperate to

ensure closure at site 2, further rostrally in the brain. In *fat1* mutant embryos, the neural tube remains open in cranial regions, leading to exencephaly, but only in 20–40% of the animals and in a strain dependent manner [53,63]. Some of this variation may reflect a redundant role for Fat4, as deletion of one allele of *fat4* enhanced the penetrance of exencephaly in *fat1* mutant mice. Similarly, *fjx1*, the sole *fj* gene in vertebrates, synergizes with *fat1* to regulate neural tube closure [53], as would be expected if Fjx1 modifies Fat1 and Fat4 in parallel. Downstream signaling might also be preserved, as mouse mutants for *Atn2* also exhibit an anterior open neural tube [73], consistent with Atro's role in the Fat pathway in *Drosophila* [35] and vertebrates [53]. However, it remains unclear whether the observed phenotypes involve the same types of planar polarity mechanisms associated with the core PCP system or even whether different Fat cadherins contribute to neural tube morphogenesis in the same way.

2.2. Development of neural circuits

Although polarity is most obvious in epithelial structures such as the cochlea and neural tube, many other types of cells exhibit asymmetric morphologies that are coordinated with each other within a tissue. This is particularly apparent in the organization of neural circuits, where axons and dendrites ensure the polarized flow of information through the nervous system. Accumulating evidence suggests that Fat cadherins, particularly Fat-like cadherins, may influence “polarity” in these non-canonical situations.

Amacrine cells (ACs) are polarized interneurons that modulate the propagation of visual information across the retina (Fig. 2B). In vertebrates, light is detected by photoreceptors in the outer retina and visual information is transmitted through bipolar cells toward retinal ganglion cells (RGCs) in the inner retina and on to the brain. ACs form synapses with RGCs and bipolar cells (BCs) in the inner plexiform layer (IPL) and thus extend dendrites from one side of the cell soma exclusively into this layer. After an early multipolar stage, developing AC precursors assume a bipolar morphology as they migrate through the tissue. Upon contact in the IPL, ACs become unipolar by stabilizing the process that is in the IPL and retracting the other. In mature retinas, *fat3* mutant ACs develop an abnormal bipolar morphology with two primary dendrites, one pointing into the IPL and an ectopic one pointing into the inner nuclear layer (INL) or the ganglion cell layer (GCL) in the case of displaced ACs [74]. As a result, two ectopic plexiform layers form, disrupting the overall laminar organization of the retina.

Upon closer examination of Fat3 and its effectors, there are hints that Fat3 regulates AC morphology by transducing an asymmetrically localized signal to the cytoskeleton. One of the hallmarks of polarity proteins is their ability to distinguish one side of a cell from the other, generally through localized distribution or activation [23]. Similarly, Fat3 protein is localized to processes in the IPL, where AC primary dendrites arborize [74,75]. One way that polarized localization may be achieved is through kinesin-mediated transport of Fat3 isoforms generated by alternative splicing, as has been demonstrated in cultured hippocampal neurons [66]. The fact that the primary consequence of Fat3 loss occurs opposite to where the protein is localized favors a model where Fat3 senses and transduces a local signal at the IPL that induces an overall change of the cytoskeleton, possibly via

interactions with Ena/VASP family actin regulators [55]. Although there are strong indications that Fat3 affects the cytoskeleton in some way, it is not clear that Ena/VASP proteins are the sole or primary effectors *in vivo*. Indeed, it is possible that Fat3 acts through adhesive mechanisms, for example, firmly attaching Fat3-bearing processes to each other in the developing IPL, with only indirect effects on the actin cytoskeleton. This would fit with recent observations that *Drosophila* Fat-like does not always require its intracellular domains to have an effect on polarity [48]. Although a ligand for Fat3 has not been defined, *fjx1* interacts genetically with *fat3* in the retina, indicating that at least some features of the Ft/Ds system are shared [74].

Laminar organization is a common feature throughout the nervous system, raising the possibility that Fat cadherins act more generally to organize neurons such that their axons and dendrites point in the right direction. Like the retina, the cerebellum is a stratified structure, with cerebellar neurons residing in cellular layers and extending projections to form synapses in stereotyped positions. In the rat adult brain, Fat2 is expressed exclusively in the cerebellum [76] and localizes to the parallel fibers of cerebellar granule cells [60]. Fat2 mediates homophilic interactions in trans [60], suggesting that it might facilitate cell-cell interactions important for the parallel fiber development [60]. However, to date, no specific functions for Fat2 have been described, leaving it unclear whether this family member has any role in polarization. Given its limited expression, especially relative to the other Fat cadherins, Fat2 may have unique functions that remain to be characterized.

2.2.1 Neuronal migration—Polarized neural circuits such as those in the retina emerge gradually, raising the possibility that the primary role of Fat cadherins is to impose order within the dynamic environment of the developing neuroepithelium. One of the earliest signs of polarity in developing laminar structures is the directed migration of neuronal precursors. For example, in the developing cortex, neuronal precursors migrate from the ventricular zone towards the cortical plate, leaving the trailing process to develop as the axon and the leading process as the apical dendrite, events that depend on a conserved apical- basal polarization pathway [77]. Similarly, Fat cadherins may influence the formation of polarized circuits by orienting the cytoskeleton within migrating neuronal precursors, with all four family members contributing to cell migration [55,56,58,74,78].

After neurons are born, they migrate to their final position where they elaborate axons and dendrites that reach out to contact synaptic partners. For example, in the developing retina, AC precursors migrate from the neuroblast layer to either the inner nuclear layer (INL) or further to the ganglion cell layer (GCL). A subpopulation of ACs, the GABAergic ACs, are normally restricted to the INL. In *fat3* mutants, excess GABAergic ACs accumulate in the GCL [74]. Time-lapse imaging studies revealed that *fat3* also affects the migration of ACs that remain in the INL. *fat3* mutant ACs vacillate on their journey to the INL, without affecting motility *per se* [55]. Thus, Fat3 may act to promote directed migration of all ACs towards the INL and, concomitantly, to prevent migration of GABAergic ACs into the inner layers of the retina. Likewise, Fat3 was put forward as a candidate to regulate the choice of a tangential route by migrating interneurons as they travel from the subpallium to the cortical plate [79], though a specific role for Fat3 in cortical development has not been described. Fat3's apparent effects on migration are reminiscent of Fat-like's effects on migration of

follicle cells in the fly ovary [47,48], raising the intriguing possibility that Fat3 similarly controls collective migration of ACs, for example by mediating interactions between leading and trailing processes of migrating AC precursors.

A role in directed migration does not appear to be restricted to the Fat-like branch of the family. Fat4 and Dchs1 are also required for tangential lateral migration of facial brachiomotor (FBM) neurons in the developing hindbrain [80]. Mice lacking both *fat4* and *dchs1* show delayed migration of FBM neurons to the same extent as the single mutants of each gene, suggesting that they function in the same pathway, most likely as a ligand-receptor pair. FBM neurons migrate through the neuroepithelium, where Fat4 and Dchs1 are expressed in opposing gradients, with neurons migrating towards regions with higher Fat4 expression. How signaling operates to guide FBM neurons is unclear, as Fat4 and Dchs1 are required both cell autonomously and non-cell autonomously, suggesting that intrinsic mechanisms in FBM operate as well as interactions with the environment [80]. Similarly, in *C. elegans*, the Fat-like cadherin CDH-4 regulates anterior-posterior migration of the Q neuroblasts in a cell-non-autonomous fashion [81,82].

As in the cochlea, different members of the Fat cadherin family cooperate to control neuronal migration. For example, acute downregulation of *fat1* disrupts radial migration in the developing cortex and causes differentiated neurons to accumulate abnormally below the cortical plate (Fig. 2C). This effect is enhanced by *fat4* knockdown [63], suggesting that Fat1 and Fat4 work together to control migration of neurons to the cortical plate. In support of this idea, downregulation of *fat4* or *dchs1* leads to an accumulation of neurons in areas closer to the ventricles [83]. In this system, the Fat4/Dchs1 system may be linked to the Hippo pathway. Normally, activation of the Hippo pathway leads to phosphorylation of Yap (the Yki ortholog) and its consequent degradation and inability to activate gene expression. Downregulation of either *fat4* or *dchs1* results in activation of Yap-mediated transcription. Moreover, concomitant downregulation of *yap* and *fat4* or *dchs1* rescues the migration defects observed after loss of Fat4/Dchs1 function in the cortex [83]. How Fat4 control of Hippo activity intersects with Fat1-dependent mechanisms in developing cortical progenitors is not known.

2.2.2 Neuronal proliferation—As in flies, vertebrate Fat cadherins also influence proliferation in the vertebrate nervous system, though the precise mechanism is not known. Fat1 inhibits proliferation of cortical neuronal progenitors, which are called radial precursors. In exencephalic *fat1* mutant embryos, radial precursors overproliferate, leading to an expansion of the ventricular zone [63] (Fig. 2C). Importantly, acute downregulation of *fat1* also leads to excess proliferation of radial precursors in the absence of neural tube defects, suggesting that Fat1 directly regulates proliferation. Reduced expression of Dchs1 and Fat4 also leads to an increase in neuronal precursors in the neocortex [83]. In addition, the spinal cord is wider in *fat4* single mutants, which do not show cranial neural tube defects [50,53]. Downregulation of *fat4* in the chick neural tube induces overproliferation of a subset of progenitor cells via a mechanism that involves the Hippo pathway [64], providing a possible explanation for the wider spinal cord observed in *fat4* mutant mice.

Although the Hippo pathway could mediate direct effects on cell proliferation, there is also evidence that Fat cadherins can affect the morphology of neuronal progenitors, affecting proliferation secondarily. Radial precursors in the cortex exhibit an apico-basal polarity, where the apical side lines the lateral ventricles and the basal side reaches up to the pial surface. Neighboring progenitor cells are connected by adherens junctions (AJs). Just apical to the AJs, the plasma membranes of neighboring radial precursors contact each other in a region that has been called the “subapical membrane apposition” [51]. Fat4 interacts with Dchs1 specifically in the subapical membrane apposition in the mouse cortex. Moreover, the intracellular domain of Fat4 binds to the Mupp1/Pals1 complex that is also localized here, and knock-down of either Fat4 or Pals1 disrupts the emergence of this region [51]. Thus, Fat4 and Dchs1 could influence the basic organization of progenitors within the ventricular zone and hence alter patterns of proliferation without directly controlling the cell cycle. Alternatively, Fat cadherins may induce proliferation via one set of effectors and drive the changes in cell morphology underlying migration and differentiation through different effectors. By working in *cis*, collections of family members could simultaneously create junctions, orient cells and stimulate appropriate changes in gene expression within the neuroepithelium. This would offer a powerful mechanism for translating a local Fat-dependent adhesion among progenitors into a tissue-wide order in the mature brain.

2.3 Neuronal diseases

Considering the varied functions of different Fat family members in so many stages of nervous system development, it is not surprising that these atypical cadherins are involved in neuronal diseases. Several members of the cadherin family have been associated with intellectual disability, autism, bipolar disease, epilepsy or schizophrenia [84]. However, only a handful of studies have directly linked lack of Fat function to human neurological disorders. In humans, the Van Maldergem syndrome is an autosomal-recessive disorder characterized by intellectual disability, among other symptoms. In affected patients, cortical neurons are mislocalized away from the gray matter and closer to the ventricles, indicating defective neuronal migration. *dacsh1* and *fat4* were identified as the two genes that were mutated and caused deleterious effects in all the analyzed individuals [83]. Therefore, malfunction of Dchs1/Fat4 signaling and hence abnormal cortical neuron migration could be the cause of intellectual disability in Van Maldergem syndrome patients. The *fat1* gene was identified as a susceptibility gene for bipolar disorder [85]. Furthermore, in mice, classic drugs used to treat this disorder – Lithium and Valproate – concomitantly induced downregulation of *fat1* and upregulation of *Enah* and *Catnb*, genes that code for the Fat1-associated proteins Ena/VASP and β -catenin [85]. These results suggested that Fat1 and its partners belong to a signaling pathway involved in susceptibility to bipolar disorder. However, not all genetic studies agree about the relationship between *fat1* and bipolar disorder [86,87]. Given the known role of Fat cadherins in the formation of properly oriented dendrites, one possibility is that loss of Fat1 leads to subtle changes in the organization of neural circuits and hence bipolar disorder.

3 Concluding remarks

As ancient members of the Cadherin superfamily, Fat cadherins represent a fascinating group of molecules that appear to play a basic role in the coordination of cell behaviors across a tissue. In the nervous system, Fat cadherins may enable developing progenitors to sense their position and then make appropriate decisions, such as whether to divide, which way to migrate, and where to make a dendrite. In the future, it will be important to determine whether all of these outcomes actually reflect a common core role as a “polarity” sensing system, as well as how diverse effects are brought about in each cellular context. In contrast to other types of cadherins, the specificity of Fat cadherin activity, at least in vertebrates, may be determined not by which ligand or receptor is present, but more by which combination of ligands, receptors, and effectors are present, with many opportunities for cross-talk.

To see whether there is any truth in these ideas, we need a much more complete picture of how Fat cadherins actually function at the molecular level. Although Fat4 appears to work through Dchs1, no ligands for Fat1 or Fat3 have been defined. Homophilic and heterophilic interactions offer one possibility, especially given evidence that Fat2 can bind to itself [60]. The role for Dchs2 also remains unclear, although it may function redundantly with Dchs1 in some situations and together with Fat3 in others [52,61]. Without a ligand in hand, it is hard to envision how the Fat-like branch of cadherins detect position or whether they serve more of an adhesive role in some tissues. It would seem likely that the remarkable size of the extracellular domain must serve a purpose in some situations. For example, two other unusually large cadherins, Cdh23 and Pcdh15, act through a structural mechanism to form tip links in inner ear hair cells [88]. Even more perplexing is the diversity within and across intracellular domains, which may even be cleaved to produce cytoplasmic fragments with effects of their own. Since Fat cadherins have both autonomous and non- autonomous effects, it will be important to examine whether some activities are devoted to the extracellular domain and others to the intracellular domain. For example, the Fat1 intracellular domain is also imported to mitochondria and plays an important role in respiration [89]. The Fat1 intracellular domain is also able to translocate to the nucleus, though its function here is undefined [58,90]. Might Fat intracellular domains also be imported into neuronal mitochondria or neuronal nuclei to affect metabolism or transcription, respectively? Moreover, Fat cadherins continue to be expressed in the mature nervous system, raising the possibility of additional functions, such as synaptic maintenance or perhaps ongoing asymmetric trafficking of material needed at the synapse. With the identification of more binding partners, both intracellular and extracellular, we need to determine the rules of engagement, both for any individual Fat cadherin but also across different family members, which may cooperate in some contexts. Although the enormous size of the Fat cadherins will make it challenging to answer many of these questions, by persevering, we may gain new insights into the mechanisms that establish the basic organization of neural circuits.

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the Ft/Ds system is activated along a gradient, extending along the proximal (Px)-distal (Dt) axis of the wing or from the poles (Pl) to the equator (Eq) of the eye. Ft is uniformly expressed, with Ds and Fj in opposing gradients. The resulting gradient of Ft activity results in asymmetric localization of Dachs (D) and hence polarized alignment of cells along this gradient. (C) In vertebrates, the Ft ortholog Fat4 binds to the Ds ortholog Dchs1. Intracellularly, Fat4 binds to the MUPP1-Pals1 complex, and possibly to Atrophin1 and Atrophin 2L (Atn1/2L). Fat2 mediates homophilic interactions, whereas the ligands for Fat1 and Fat3 remain to be elucidated. Fat1 binds to a variety of intracellular molecules, including cytoskeletal regulators such as Ena/VASP, Homer1 and Homer3, and probably WRC, as well as the Atn transcriptional regulators. Fat3 binds to WRC and Ena/VASP. How any of these interactions actually influence polarity remains unclear.

Figure 2. Fat cadherins influence multiple features of neuronal development

(A) In wild-type (WT) animals, the cochlea contains one row of inner hair cells (IHC) and three rows of outer hair cells (OHC1-3), each with a single kinocilium (green) asymmetrically positioned at the edge of the apical surface, adjacent to a polarized bundle of actin-rich stereocilia (red). In $fat4$ mutants, HCs are mildly misaligned and an extra fourth row of OHCs appears in some cochlear turns. $fat4$ synergizes with $fat1$, whereas loss of $fat3$ suppresses the $fat4$ phenotype. (B) During development of the WT retina, amacrine cell precursors (green) migrate through the neuroblast layer (NBL) to the inner nuclear layer (INL) and the ganglion cell layer (GCL), gradually acquiring a unipolar morphology with one primary dendrite extending into the inner plexiform layer (IPL). $fat3$ mutant amacrine cell precursors vacillate as they travel toward the IPL and then often retain neurites outside of the IPL, exhibiting a bipolar shape in the mature retina. OLM: outer limiting membrane,

ONL: outer nuclear layer, OPL: outer plexiform layer. (C) In the WT neocortex, neuroepithelial cells (purple) proliferate in the ventricular zone (VZ) and give rise to neurons (yellow) that migrate along the radial glia to the cortical plate (CP), where they differentiate. Within the ventricular zone, the progenitors form Fat4/Dchs1-positive “subapical membrane appositions” (SAMA) just apical to the adherens junctions (AJ). Depletion of either *fat1* or *fat4* induces overproliferation of neuronal precursors and accumulation of differentiated neurons below the CP, accompanied by disruptions in the SAMA. SVZ: subventricular zone, IZ: intermediate zone, MZ: marginal zone.

Table 1

Fat functions in the nervous system

	Tissue	Gene	Function	Genetic/Biochemical Interactions	References
Epithelial morphogenesis	Cochlea (Mouse)	<i>fat1</i>	Cochlear length and patterning	Synergizes with <i>fat4</i> genetically	[53]
		<i>fat3</i>	Cochlear length and patterning	Antagonizes <i>fat4</i> genetically; May be <i>Atn1</i> -dependent.	[53]
		<i>fat4</i>	Cochlear length and patterning	Dchs1-dependent; Interacts genetically with <i>Atn2</i>	[50,53,70]
	Neural tube (Mouse)	<i>fat1</i>	Cranial neural tube closure	Interacts with <i>fat4</i> and <i>fatx</i> genetically; <i>Atn2</i> mutants show similar defects	[53,63,73]
Neuronal circuit formation	Retina (Mouse)	<i>fat3</i>	Regulates lamination by controlling retraction of ACs dendrites	Interaction with <i>fatx</i> genetically; Links to actin cytoskeleton (<i>Ena/VASP?</i>)	[55,74]
			Regulates directed migration of ACs toward INL; Restricts GABAergic ACs to the INL		[55,74]
	Hindbrain (Mouse)	<i>fat4</i>	Required for tangential lateral migration of FBM neurons	Dchs1-dependent	[80]
	Cortex (Mouse)	<i>fat1</i>	Regulates migration to cortical plate	Exacerbated by <i>fat4</i> knockdown	[63]
			Inhibits proliferation of cortical neuron progenitors	Forms <i>cis</i> heterodimers with <i>Fat4</i>	[63]
		<i>fat4</i>	Regulates migration to cortical plate	Dchs1-dependent; Exacerbated by <i>fat1</i> knockdown; Possible connection to Hippo pathway	[83]
			Inhibits proliferation of cortical precursors	Dchs1-dependent	[83]
	Neural tube (Chick)	<i>fat4</i>	Inhibits proliferation of progenitor cells	Hippo pathway is involved	[64]
	Cerebellum (Rat)	<i>fat2</i>	Unknown	Mediates homophilic interactions	[60]