CHAPTER 9

LATROPHILIN SIGNALLING IN TISSUE POLARITY AND MORPHOGENESIS

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Abstract: Understanding the mechanisms that coordinate the polarity of cells and tissues during embryogenesis and morphogenesis is a fundamental problem in developmental biology. We have recently demonstrated that the putative neurotoxin receptor *lat-1* defines a mechanism required for the alignment of cell division planes in the early embryo of the nematode *C. elegans*. Our analysis suggests that *lat-1* is required for the propagation rather than the initial establishment of polarity signals. Similar to the role of the flamingo/CELSR protein family in the control of planar cell polarity, these results implicate an evolutionary conserved subfamily of adhesion-GPCRs in the control of tissue polarity and morphogenesis.

INTRODUCTION

A fundamental requirement in all multicellular organisms is a robust program to achieve the correct spatial arrangement of cells. Cell fate decisions, the orientation of mitotic divisions, the migration of individual cells and morphogenetic movements of cell groups have to be tightly coordinated. While our understanding of the molecular mechanisms controlling asymmetric cell fate decisions and mitotic spindle orientation in certain types of cell-cell interaction is advanced (reviewed in refs. 1,2), it is less well understood how signals are propagated in larger groups of cells to align cell polarity and division plane orientation and how tissue polarity is coordinated with morphogenetic movements. The analysis of planar cell polarity (PCP) in epithelial sheets and the study of convergence and extension (C and E) movements during gastrulation in vertebrates have implicated signalling by the Wnt/PCP, Fat/Dachsous/Four-jointed (Fat/Ds/Fj) and

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anterior-posterior (a-p) tissue polarity pathways in the co-ordination of cell division plane orientation (reviewed by ref. 3, see Formstone, this volume).

Cells also have to find and maintain their correct positions in relation to surrounding cells. Since the pioneering experiments of Townes and Holtfreter[4] the molecular basis for the directed movement and selective adhesion of embryonic cells has been an area of intense interest. While substantial progress has been made in elucidating the formation and maintenance of boundaries between compartments and tissues, the segregation and sorting of mixed cell populations is much less well understood.[5] Widely accepted hypotheses are the thermodynamic model, mainly based on differential adhesion mediated by cadherin-based mechanisms[6,7] and the activity of cell guidance systems transmitting attractive or repulsive cues to migrating cells.[8] However, the currently known mechanisms do not yet fully explain the developmental processes shaping embryos and organs.

The Role of Adhesion-GPCRs in Development

An interesting class of candidate molecules for the control of cell-cell interactions are the adhesion-GPCRs,[9,10] which combine extracellular domain features of adhesion molecules with transmembrane regions characteristic for G protein coupled receptors. Vertebrate genomes encode 30 or more adhesion-GPCRs with at least 8 different extracellular domain architectures[11] (see Schioth et al, this volume), making it the second largest group of seven-pass transmembrane (7TM) receptors. Adhesion-GPCRs are implicated in immune functions[12,13] and in rare inherited developmental disorders[14] but there is little information about the physiological function of most members of the protein family. A key role in development has been defined for the cadherin-like flamingo/starry night (FMI) and its vertebrate homologs (CELSR), which have essential and conserved functions in the PCP pathway and in neuronal development[15-23] (see Formstone, this volume).

Comparative genomics of the highly divergent adhesion-GPCR family shows that next to FMI only the domain architecture of latrophilins (LPHN; synonyms CL/CIRL/Lph/Lectomedin; see Ushkaryov, in this volume) is strictly conserved across phyla (see below).[11] The lectin-like latrophilins were originally described as cellular receptors for latrotoxin (α-LTX), the main neurotoxin of the Black Widow spider *Latrodectus mactans*.[24,25] They have been implicated as modulators of neurotransmitter release[26-28] (Silva et al, this volume) and are thought to act as components of the fusion machinery that regulates discharge of the pool of biogenic amine vesicles (i.e., norepinephrin, GABA, glutamate) in several neuron types and vesicles carrying insulin in pancreatic β-cells. However, the physiological function of this highly conserved receptor is not well defined and its endogenous ligands are unknown. Recent work from our laboratory has identified an unexpected role for latrophilins as essential regulators of tissue polarity in embryonic development.[29]

Adhesion-GPCRs in *C. elegans*

A major challenge in the genetic analysis of orphan adhesion-GPCRs is the complexity of the gene family. The large number of different domain architectures raises issues about general conservation of function versus species-specific diversification. In addition, the presence of up to 3-5 paralogs for some receptor subfamilies in vertebrates indicates possible functional overlap and compensation between paralogs. To investigate the

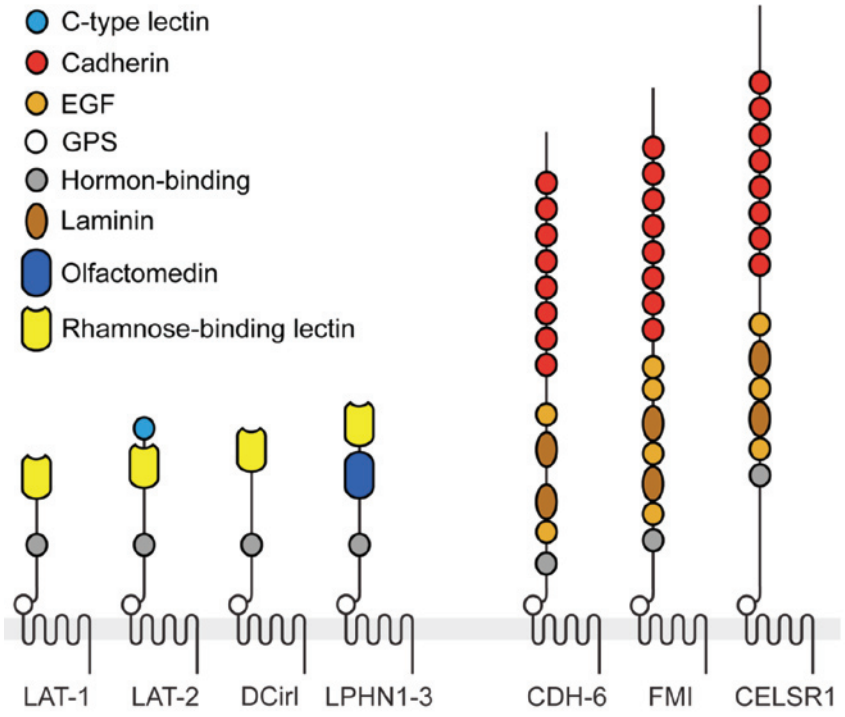

Figure 1. Adhesion-GPCR classes conserved between vertebrates and invertebrates. The domain architecture of adhesion-GPCRs is conserved from nematodes to mammals and characterized by an extracellular GPS motif in close proximity to the outer face of the 7TM region. The RBL domain is the hallmark for receptors of the latrophilin subfamily (LPHN), whereas the Flamingo (FMI) group is determined by the presence of cadherin, EGF and laminin domain repeats. Reprinted from Langenhan et al, Dev Cell 2009; 17(4):494-504,[29] ©2009 with permisson from Elsevier.

physiological function of adhesion-GPCRs in a less complex system we turned to the nematode *C. elegans*.

The *C. elegans* genome contains two LPHN genes, *lat-1* and *lat-2*[28,30] and a single FMI homolog (*cdh-6*)[31,32] (Fig. 3). Similarly, FMI and LPHN (dCIRL) are the only conserved adhesion-GPCR architectures in Drosophila (Fig. 1). Other *C. elegans* or *Drosophila* genes showing similarity to adhesion-GPCRs are highly divergent with little sequence homology to adhesion-GPCRs in vertebrates,[11,33] while FMI and LPHN are conserved in other nematode and insect species. This suggests that FMI and LPHN represent the core functions of adhesion-GPCRs that are highly conserved in the evolution of bilateral animals.

The small number of adhesion-GPCRs implies a low level of functional redundancy in the worm and offers the possibility to separate and dissect the role of individual genes and to assign the physiological function to each member of the receptor class. Based on loss-of-function mutants, molecular requirements of different receptor domains can be tested by transgenic complementation. Quantitative assays provide a means to distinguish the different signalling properties of receptor mutants under physiological

conditions even without knowing the identity of the endogenous ligand(s). Further, the interaction of adhesion-GPCR signalling with other molecular pathways can be tested by epistasis experiments.

Introduction into *C. elegans* Embryonic Anatomy and Development

C. elegans has an essentially invariant embryonic cell lineage,[34] which unfolds by a sequence of asymmetric cell divisions and intercellular induction events.[35,36] Starting from the zygote (P0), the three body axes of the embryo are established within the first three cleavage divisions (Fig. 1). The first cleavage event generates the anterior AB and posterior P1 blastomers thereby assigning the primordial antero-posterior (a-p) axis to the early embryo. In the next round of cell divisions, AB is divided into an anterior (ABa) and posterior daughter (ABp), whereas P1 gives rise to the ventral EMS blastomere (Endoderm/MeSoderm) and the posterior P2 cell, thus defining the dorso-ventral (d-v) body axis. During the following third cleavage, ABa/p divide perpendicular to the a-p and d-v axes into ABal and ABpl on the left side of the embryo and ABar and ABpr on the right hand side. This establishes the left-right (l-r) axis and the slightly more anterior position of ABal/pl compared to ABar/pr defines a handed bilateral asymmetry (Fig. 2).

In subsequent asymmetric blastomere divisions, the P1-derived blastomere EMS divides into E and MS. P2 gives rise to C and P3 and the latter divides into D and P4. At the end of these first divisions all three body axes are laid down and 6 founder blastomeres have been generated, which eventually give rise to clonally expanding tissues that form the embryo: AB, MS, E, C, D, P4. Germ-line potential is always retained in the posterior blastomere Px. With the exception of E, which gives rise to all gut cells, i.e., endoderm, the founder blastomeres only loosely correspond to the classical germ layers. The AB, MS and C lineages can give rise to cell types with ectodermal and mesodermal characteristics (Fig. 3).

Contrary to a common misconception the invariant embryonic cell lineage of *C. elegans* is not a form of "mosaic" development determined exclusively by the segregation of preformed cell-autonomous determinants. Rather, it is established by a sequence of controlled asymmetric cell divisions and intercellular induction events very similar to the ones seen in the embryonic development of "higher" animals. Due to the small number of cells and their precisely reproduced locations and interactions in the nematode embryo, cell fates and cell division planes are coordinated so tightly that the lineage and fate of each cell appears to be invariant. The regulative features of *C. elegans* development have been identified by the analysis of mutations in signalling pathways and by the ablation of blastomeres with laser microbeams.[34,36-39]

The Wnt/β-catenin asymmetry pathway has been shown to be essential for cell fate decisions (reviewed by ref. 40) while a noncanonical Wnt/Frizzled (Wnt/Fz) pathway is required for the orientation of mitotic spindles (reviewed by ref. 41). The mechanisms controlling cell polarity in the first, second and third round of embryonic cell divisions are understood in considerable detail.[41,42] A posterior polarising centre is located in the descendants of the founder blastomere P1[43] and can orient the division planes of immediately adjacent cells.[44] The polarisation of EMS by P2 at the four-cell stage is thought to require an instructive Wnt/Fz signal and a permissive activity of *scr-1*/SRC oncogene and the receptor tyrosine kinase *mes-1*.[45-50] While the P2-EMS interaction at the 4-cell stage has served as an excellent paradigm to study the molecular mechanisms of a polarising induction, it is not well understood how the polarising information is propagated and coordinated as the complexity of the embryo increases rapidly from the

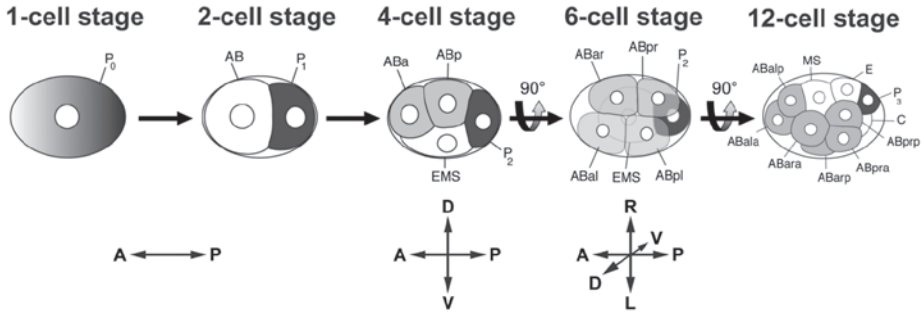

Figure 2. Establishment of principal body axes and founder blastomers through asymmetric divisions in the early *C. elegans* embryo. Within the first division rounds the three body axes are generated and after the fifth round (not depicted) all six founder lineages have been established. Germline precursors are labelled dark grey, AB lineage light grey, all other (P-derived) blastomeres white.

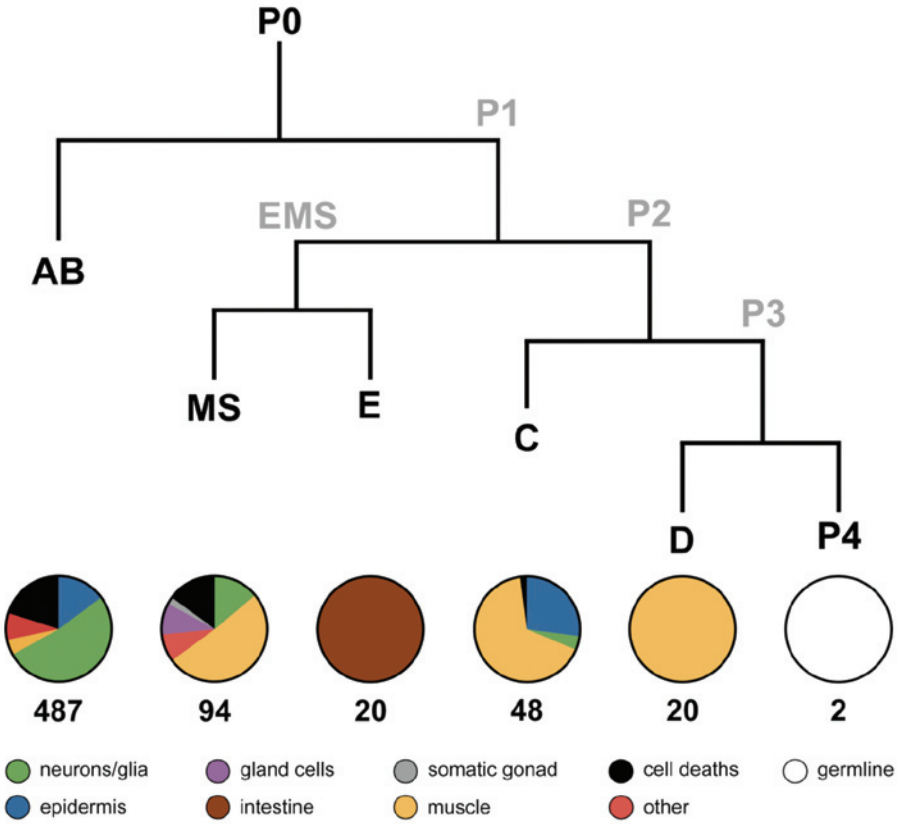

Figure 3. Embryonic lineage of *C. elegans*. Six founder blastomers give rise to all tissue types of the developed animal. Numbers below pie charts indicate total number of cells generated within this lineage during embryogenesis.[34] Transient blastomere names in grey.

second (4 cells) to the 10th division cycle (~1000 cells). A *wnt*-dependent relay mechanism has been proposed,[51] but it is a matter of debate how this mechanism relates to existing models for PCP or a-p tissue polarity signalling.[52,53] A clear functional equivalent of PCP in *Drosophila* has not yet been described in *C. elegans*.

LATROPHILINS AND TISSUE POLARITY

Maternal and Zygotic *lat-1* Expression Is Required for *C. elegans* Development

Homozygous offspring of nematodes heterozyous for a mutant *lat-1* allele develop with normal morphology to the first larval stage and display disturbed pharyngeal motor behaviour reminiscent of synaptic dysfunction.[28,30] A more detailed examination revealed that the offspring of *lat-1* homozygotes show additional severe defects in embryonic and larval development, leading to drastically reduced adult brood sizes for *lat-1* mutants.[29] The early defects in homozygous mutant embryos can be suppressed by the presence of maternal LAT-1 protein, while the phenotype is observed in heterozygous embryos created by mating of homozygous hermaphrodites with normal males, which lack maternal but not zygotic LAT-1. This indicates that maternal gene product is required and sufficient to support normal early development. The dependency on maternal *lat-1* gene product coincides with high levels of *lat-1* mRNA in the maternal germline and in all blastomeres during the first cleavage rounds of the zygote.[29]

The examination of embryos lacking maternal and zygotic gene product revealed a defect in the division plane orientation of 8-cell *lat-1* embryos that is distinct from polarity or patterning mutations described in the literature. In normal development, the division plane of ABal, the most anterior blastomere, is oriented in the anterior-posterior direction typical for most embryonic cell divisions. The mitotic spindle is skewed towards the putative a-p axis of the embryo, allowing only the posterior daughter ABalp to contact the posterior neighbour MS, while ABala assumes the most anterior position within the egg shell and does not touch MS (Fig. 4a,f). In *lat-1* mutants, the ABal axis is positioned perpendicular to the embryonic a-p axis, suggesting that anterior-posterior tissue polarity is defective (Fig. 4b,g).[29]

Although cell fate changes can be detected in several embryonic sublineages, the division of ABal appears to remain asymmetric, suggesting that *lat-1* is required for a-p cell polarity, but not for cell fate determination in ABal.

lat-1 Is Required for Tissue Polarity in Anterior Blastomeres

A posterior signalling centre formed by descendants of the P1 blastomere polarizes the a-p axis of the *C. elegans* embryo.[43,44] Blastomere recombination experiments have suggested that Wnt-dependent signalling activity of P2 can provide an instructive cue to orient the EMS spindle[45] and that polarizing signals can be propagated to align cleavage planes in a larger group of cells by a Wnt-dependent relay mechanism.[51]

The current literature suggests that in normal development the division plane orientations of the blastomeres ABpl and ABpr are determined by Wnt/Fz-dependent signalling from E, while a different Wnt/Fz signal emanating from C orients the ABar spindle into its characteristic orientation perpendicular to ABpl/r.[41,49] However, the

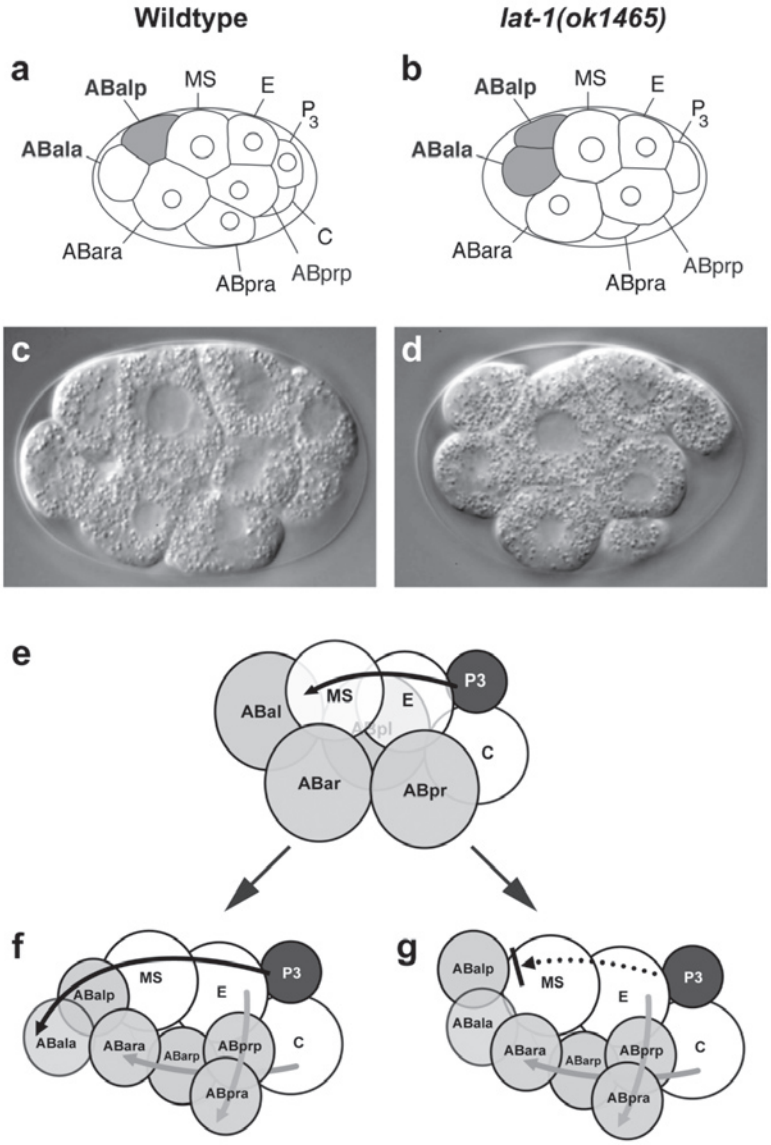

Figure 4. Division plane defect of *lat-1* mutants. a,c, during the transition from 8→12-cell stage the ABal blastomere divides in a plane (c) allowing only the posterior daughter ABalp to contact MS (grey cell), whereas ABala is separated from MS. b,d, in embryos deficient of *lat-1* the ABal division plane is skewed such that both daughters contact MS (grey cells). e, at the 4-8-cell stage a polarizing signal originating from P2/3 aligns the embryo along the the a-p axis (black arrow) f, when the embryo transits to the 12-cell stage ABal is furthest away from P3 and requires a polarizing signal to align the ABal daughters in an a-p direction. This putative signal is propagated via E and MS (black arrow). ABar, ABpr and ABpl are oriented by signals from E and C (grey arrows). g, in *lat-1* mutants, ABal daughters divide perpendicularly to the a-p axis indicating loss of the P3-polarizing signal. All other AB-derived blastomeres are in direct contact with primary or secondary polarizing cells (P3, E, C, MS) and thus still align appropriately. Reprinted from Langenhan et al, Dev Cell 2009; 17(4):494-504,[29] ©2009 with permission from Elsevier.

anterior ABal blastomere is only in contact with MS and AB descendants rather than E or C. It has been shown that the E, C and MS blastomeres acquire the capacity to transduce polarizing signals of different strength and quality, but that only E and C derived signals are equivalent to P2 signals.[53] Until recently no specific molecular requirements for the division plane orientation of ABal had been described, but a mechanism propagating the polarising signal from the primary source P2/3 via E to MS has been assumed.[51]

It could now be shown that *lat-1* is essential to align the mitotic spindles and division planes of the E-MS-ABal cell group to a common a-p axis (Fig. 4).[29] The blastomere ABal occupies the most anterior position in the 8-cell stage and fails to align in embryos lacking maternal and zygotic *lat-1* protein. Consistent with the model that a putative polarizing signal emanating from P2/3 would have to be transmitted through E and MS to reach ABal, the alignment of the MS spindle is also affected in *lat-1* mutants. The timing of spindle rotations suggests that successful alignment of E can "rescue" the alignment defect of MS, but not ABal, which has already undergone mitosis at this time. In contrast, *lat-1* is not required for the division plane orientation of EMS, E, or C, which are in direct contact to P2/3 and thus receive a polarizing signal directly. In turn, E and C retain most or all of their ability to orient ABar and ABpl/pr.[49,52]

While lack of *lat-1* function has little or no effect on the division planes of blastomeres that are in direct contact with the primary or secondary signalling cells P2/3, E and C, the spindle alignment of the next generation of ABalx or ABarp descendants is frequently delayed or failing. These results can be explained by a simple model in which *lat-1* is required to efficiently propagate spindle alignment cues from a posterior source towards the anterior through the growing cellular array.[29] In this model, ABal is a weak spot as its orientation relies on MS which is a "tertiary cell" not in direct contact to the primary source P2[53] and which itself shows delays and errors of a-p orientation in *lat-1* mutants. Later ABa descendants have more diverse cell contacts that could provide compensating signals and underlie stronger spatial constraints, leading to a lower penetrance of the overt spindle alignment phenotype.

Interaction of Latrophilin Signalling and the Wnt/Frizzled Spindle Orientation Pathway

The genetic analysis of wnt/frizzled-dependent signalling in the early *C. elegans* embryo suggests that multiple parallel Wnt signals transmit the polarizing information.[46,48] *lat-1* might be specifically required to propagate one of these parallel signals, or an as-yet unknown Wnt-independent signal. Alternatively, *lat-1* function might be required for the efficient propagation of all parallel signals, e.g., for an essential response of the cells in the path of the signal(s). Alternative models for *lat-1* function are also plausible, but more complex. *lat-1* might be required for an anterior-to-posterior alignment activity overlapping and opposing the posterior-to-anterior signal, similar to the model recently presented for vulval precursor cell organisation.[54] The predictions made by the alternative models have not been tested in detail yet.

The analysis of differentiation markers and embryonic cell lineages shows that *lat-1* is not required for endoderm induction and does not appear to have a strong direct effect on cell fate in asymmetric cell divisions.[29] This indicates that *lat-1* is not an essential component of the transcription-dependent Wnt/β-catenin asymmetry pathway. In *lat-1*

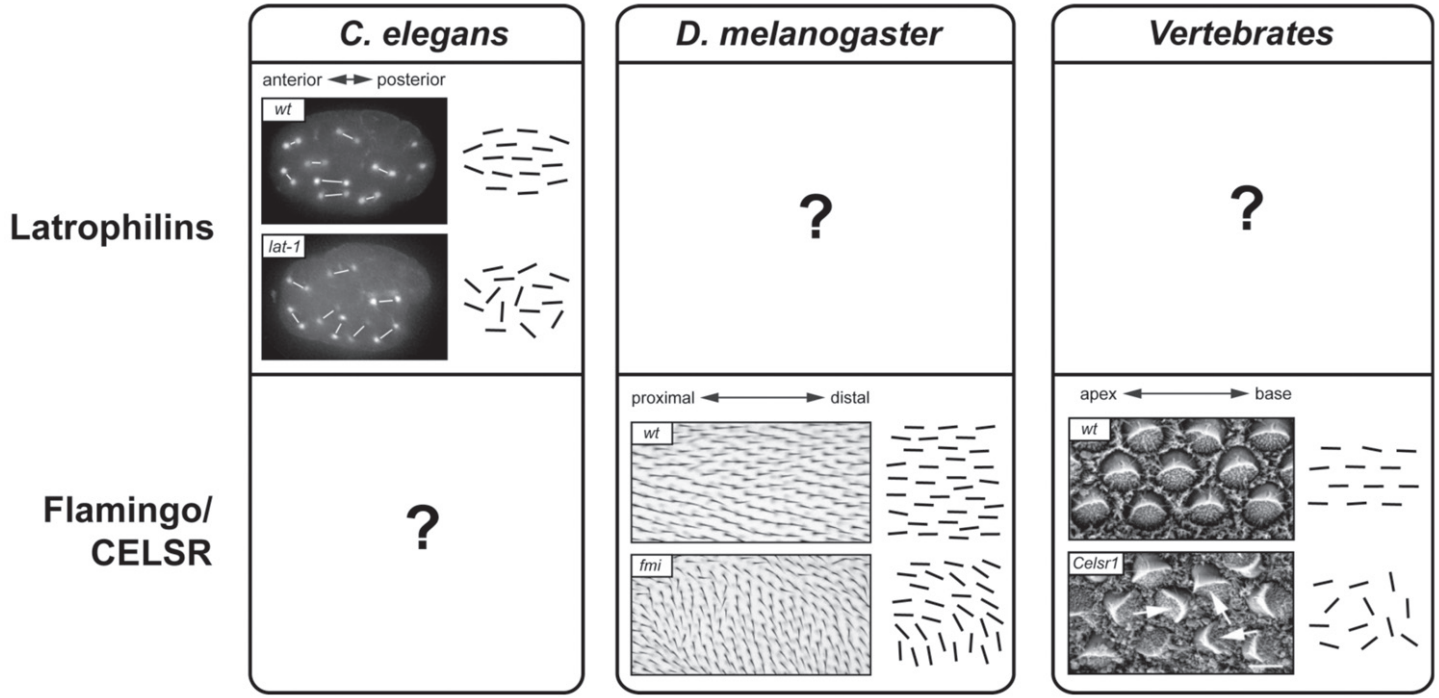

Figure 5. Adhesion-GPCRs and the control of tissue polarity. The FMI class of adhesion-GPCRs has been associated with defects in planar cell polarity: mutations in the fmi gene of Drosophila disturb the organwide polarity of wing bristles (middle panel), dendritic trees and ommatidial rotation (reproduced from Usui T, Cell 1999; 98(5):585-595,[15] ©1999 with permission from Elsevier); the vertebrate FMI homolog CELSR1 is involved in the establishment of inner ear sensory epithelium polarity (right panel: reproduced from Curtin et al, Curr Biol 2003; 13(13):1129-1133,[21] ©2003 with permission from Elsevier). Recent evidence shows that LPHN receptors are required for the correct establishment of tissue polarity in the developing C. elegans embryo (left panel: reproduced from Langenhan et al, Dev Cell 2009; 17(4):494-504,[29] ©2009 with permission from Elsevier). It is still unclear whether LPHNs act in similar phenomena in higher organisms and FMI has a polarizing role in *C. elegans*.

mutants, the ABal division still generates asymmetric cell fates in most cases and the normal ABala cell fate is surprisingly robust against altered cell position and ectopic cell contact to MS.

The Molecular Mechanism of Latrophilin Signalling

Adhesion-GPCRs are heterodimers composed of an extracellular "adhesion" subunit and a GPCR-like domain with seven transmembrane helices. The heterodimers are derived from monomeric precursor proteins by cleavage at the GPS domain[24,55] (see chapter by Lin, this volume). The lectin-like RBL domain, the defining feature of LPHNs[56] is absolutely required for all functions of *lat-1*.[29] In contrast to results recently described for FMI,[57] constructs lacking the RBL domain but retaining the hormone-binding domain (HRM), GPS and 7TM domains have not shown partial activity. This is consistent with an essential role of the RBL domain in ligand binding and implies that the 7TM domain transduces an "outside-in" signal that is dependent on an extracellular interaction. Recent biochemical data argue strongly against a carbohydrate ligand for the lectin-like RBL domain and do not support homodimer formation mediated by the RBL domain.[29,56]

CONCLUSION

The control of mitotic spindle orientation in the *C. elegans* embryo has been investigated intensively and the roles of PAR proteins and heterotrimeric G-proteins in establishing zygotic polarity[35] and of Wnt/Fz and SRC-1/MES-1 pathways in P2/EMS signalling at the four-cell stage[41] have been identified. However, it is still poorly understood how spindle orientation and cell fate asymmetry are coordinated from the 8-cell stage onwards and clear equivalents of PCP or a-p tissue polarity pathways have not yet been defined in *C. elegans* embryogenesis.[49,52] Latrophilins are structurally very similar to FMI proteins, a related subfamily of highly conserved adhesion-GPCRs that are essential for PCP signalling in *Drosophila* and a-p tissue polarity in vertebrates[3,18] (see chapter by Formstone, this volume).

Unexpectedly, the study of *C. elegans* embryogenesis has revealed that the putative neurotoxin receptor *lat-1* defines a mechanism required for the alignment of cell division planes.[29] Similar to the role of FMI in PCP, this implicates an evolutionary conserved subfamily of adhesion-GPCRs in the control of tissue polarity and morphogenesis (Fig. 5). It also suggests that the expansion of adhesion-GPCRs in vertebrates might contribute to the larger variety of organ and tissue architectures in these species.[14,21,23,58] Further studies will be required to define the up- and downstream components of adhesion-GPCR signalling.

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