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Minireview

Three Ways to Make Two Sides: Genetic Models of Asymmetric Nervous System Development

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Many anatomical and functional features of nervous systems are asymmetric about the left-right axis. These asymmetries can exhibit either random or invariant laterality at the population level. Recent studies in fish and worms provide insight into the developmental mechanisms used to create both types of asymmetry. These studies reveal diverse and molecularly complex strategies for developing asymmetric nervous systems.

Although animals are largely bilaterally symmetric, most exhibit differences between the left and right sides of the body. Among the most fascinating of these asymmetries are those that affect the function of the nervous system. Many aspects of the way we receive sensory information, process it in the central nervous system, and generate motor behaviors exhibit strong left-right bias, although the underlying anatomical bases for these functional asymmetries are largely unknown. Over the past 10 years there has been intense interest among developmental biologists in the molecular cascades and tissue interactions that determine the left-right axis and, to a lesser extent, the morphogenetic processes that create asymmetric organs. The field initially focused on the asymmetry of internal organs, but there is increasing attention to left-right asymmetry in nervous systems. Progress has been made largely as a result of genetic studies in model organisms. Among the questions that these studies address are how organs become bilaterally asymmetric at morphological and molecular levels, whether the pathways that control the left-right arrangement of internal organs also influence neuronal asymmetries, and how neuroanatomical asymmetries ultimately affect the function of the nervous system.

Anatomical and functional asymmetries can be classified into two categories—those that exhibit randomized laterality (sometimes called "antisymmetries") and those that exhibit consistent laterality in most animals in a population (called "directional asymmetries"). Both kinds of asymmetries—randomized and directional—have been observed in nervous systems at neuroanatomical and behavior levels. Handedness in humans provides an example of directional functional asymmetry, with ~90% of people exhibiting right-handedness (reviewed in Sun and Walsh, 2006). In contrast, the laterality of handedness in mice, dogs, and cats is random. Each animal has a dominant paw, but half of the animals in a population are "right-pawed" and the other half are "left-pawed." This review focuses on progress made in dissecting the molecular pathways controlling both directional and randomized neuronal asymmetries in model organisms. Studies in these models have uncovered unexpected diversity and complexity in the regulatory pathways that create neuroanatomical asymmetries and help to explain how functional asymmetries arise.

Genetic Dissection of a Randomly Lateralized Asymmetry in *C. elegans* Neurons

A pair of olfactory neurons in *C. elegans* called AWCL and AWCR (on the left and right, respectively) exemplifies an asymmetry with randomized laterality (Troemel et al., 1999) (Figure 1). Morphologically, the AWC neurons are mirror images of each other, but the G protein-coupled olfactory receptor *str-2* is expressed in only one of the two AWC neurons. Half of the animals in a population express *str-2* in AWCL, and half express it in AWCR. This molecular asymmetry correlates with a functional asymmetry: The *str-2*-expressing neuron and the non-*str-2*-expressing neuron are each specialized to sense a distinct set of odorants (Wes and Bargmann, 2001).

AWC neurons are unrelated by lineage and are born on opposite sides of the animal. To achieve random *str-2* asymmetry, the left and right neurons must therefore signal in a reciprocal manner to make a coordinated decision ensuring that each adopts a distinct sensory cell fate. Although the type of cooperative signaling that occurs in the AWC neuron pair bears strong conceptual similarity to lateral signaling mediated by the Notch pathway, Notch signaling itself is not involved in the generation of AWC asymmetry. Nonetheless, the lateral signaling paradigm provides a useful conceptual framework for understanding how signaling between equivalent cells can result in an asymmetric outcome. As in Notch-mediated lateral signaling, the two AWC cells initially must be capable of signaling in both directions, until stochastically the strength of signaling from one side gains an "advantage" over the other. Positive and/or negative feedback loops then amplify this signaling asymmetry until the two cells reach distinct stable states and adopt molecularly and functionally different fates. Although the AWC neurons are on opposite sides of the animal, they can communicate directly, since they send axons across the midline that contact

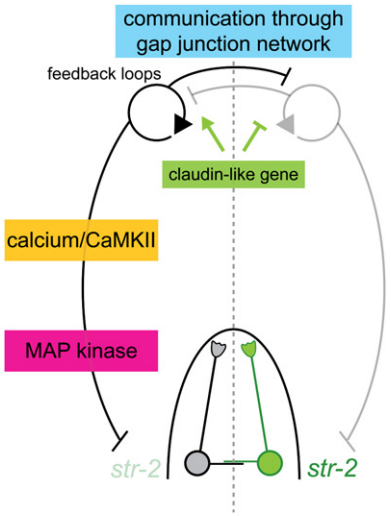

Figure 1. A Model for the Regulation of Randomized Asymmetry in *C. elegans* AWC Olfactory Neurons

each other. Mutations in axon guidance genes that disrupt these contacts disrupt AWC asymmetry, implying that communication occurs at least in part in the axons (Troemel et al., 1999).

Mutagenesis screens with a GFP reporter for *str-2* expression led to the identification of several neuronal symmetry (*nsy*) mutants that express *str-2* symmetrically, either in both or in neither AWC neuron (Troemel et al., 1999; Vanhoven et al., 2006; Chuang et al., 2007). Molecular identification of the mutated genes responsible for the *nsy* phenotypes has uncovered components of a novel genetic pathway controlling AWC asymmetry. Epistasis experiments and analysis of mosaic animals with some mutant and some wild-type cells have clarified when and where in the pathway each gene acts. Several of the *nsy* genes are required cell-autonomously to execute the fate of the AWC neuron that does not express *str-2*, but do not participate in communication between the two cells (Sagasti et al., 2001; Tanaka-Hino et al., 2002). This "execution" portion of the pathway begins with calcium entry through voltage-gated calcium channels that activates the calcium/calmodulin-dependent protein kinase CaMKII, which in turn activates a MAP kinase cascade. Activation of MAP kinase signaling represses *str-2* expression through as yet unidentified transcription factors. A putative scaffolding protein that associates with CaMKII is also required in this cell fate execution pathway (Chuang and Bargmann, 2005). A relative difference in the strength of calcium signaling between the two cells determines asymmetry. Calcium signaling is relatively low in the cell that expresses *str-2* and high in the cell that does not, leading to differences in the strength of MAP kinase signaling, ultimately causing each neuron to adopt distinct fates.

What determines the relative difference in calcium signaling between the two AWC cells? By analogy to the Notch-mediated lateral signaling model, there must be genes that participate in reciprocal communication between the two cells and feedback loops to amplify stochastic differences between them. Two *nsy* genes that appear to participate in this portion of the pathway have been identified so far (Vanhoven et al., 2006; Chuang et al., 2007). One of these genes, *nsy-4*, encodes a claudin-like protein that may modulate ion channel function or cell adhesion, and the other, *nsy-5*, encodes an innexin protein that forms gap junction channels. While it is not clear exactly how these genes function, genetic analysis suggests that they act in parallel pathways to impinge upon AWC calcium signaling.

Characterization of *nsy-5* gene function uncovered two aspects of unanticipated complexity in AWC signaling (Chuang et al., 2007). First, mosaic analysis and expression experiments revealed that in addition to its function in the AWC neurons themselves, the *nsy-5* gene can function in several other bilaterally symmetric sensory neurons to influence AWC cell fate. This suggests that a network of cells connected by gap junctions mediates signaling between the left and right sides of the nervous system. Second, mosaic analysis suggested that despite the randomness of the outcome of AWC communication, the *nsy-4* and *nsy-5* genes both possess directional biases in their function (*nsy-5* appears to be more effective in AWCR and *nsy-4* more effective in AWCL). These biases must presumably cancel each other out during AWC signaling to achieve an unbiased outcome. Thus, although the classical lateral signaling pathway has provided a useful paradigm for thinking about AWC asymmetry, AWC signaling appears to involve not only an entirely different set of genes, but perhaps also a substantially different logic. It will be interesting to determine whether this signaling pathway is used in other instances to partition neuronal cell fates. One asymmetry that could follow a similar signaling logic occurs between lobster claws (Govind, 1992). In lobsters, stochastic differences in the activity of each claw during a specific developmental stage causes the left and right to adopt distinct, but randomly lateralized, claw morphologies. More broadly, making use of neuronal circuitry and genes controlling excitability could be a generally useful strategy to diversify cell fates during neuronal development.

Genetic Dissection of a Directional Asymmetry in *C. elegans* Neurons

Like the AWC neuron pair, the ASE neurons are a bilateral pair of sensory neurons located in the head of *C. elegans* that express sensory signaling genes asymmetrically (Figure 2). In contrast to AWC asymmetry, however, asymmetry between the ASE neurons is directional. This pair of gustatory neurons expresses several guanalyl cyclase (gcy) genes, transcription factors, and neuropeptides in an invariantly lateralized asymmetric fashion (Yu et al., 1997; Johnston et al., 2005). For example, in every worm, *gcy-7* is expressed on the left and *gcy-5* is expressed on the right. Like AWC, each ASE neuron is

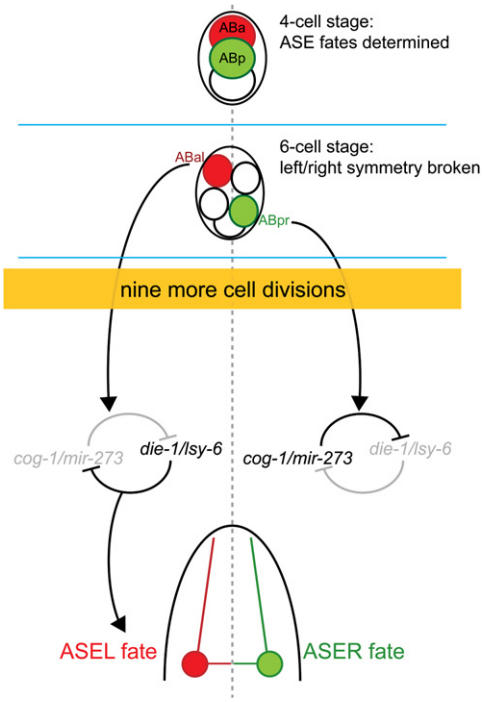

Figure 2. A Model for the Regulation of Directional Asymmetry in *C. elegans* ASE Gustatory Neurons

specialized to sense a characteristic set of chemicals (Pierce-Shimomura et al., 2001).

Despite the fact that the two ASE neurons derive from different lineages, they are born in initially similar states, with both cells expressing ASEL and ASER genes (Johnston et al., 2005). Signaling pathways resolve this initial equivalency into distinct gene expression profiles for each cell soon after their birth. Importantly, unlike the AWC asymmetry decision, interactions between the left and right ASE neurons are not required for the signaling that causes them to adopt different fates. Surprisingly, genetic analysis so far suggests that there is little or no overlap with the pathway that determines AWC asymmetry (Chang et al., 2003).

Just as with the AWC pathway, a forward genetics approach was used to uncover genes regulating ASE asymmetry, and epistasis and mosaic analysis were used to order those genes into a pathway. At the core of the ASE fate pathway is a genetic circuit made up of microRNAs (miRNAs) and transcription factors that reciprocally repress each other to achieve one of two mutually exclusive stable outcomes (Johnston and Hobert, 2003; Chang et al., 2004; Johnston et al., 2005). In ASEL, the DIE-1 transcription factor promotes expression of the miRNA *lsy-6*, which downregulates expression of the transcription factor gene *cog-1*, in turn preventing it from activating the miRNA *mir-273*. In ASER, the situation is reversed: COG-1 activates transcription of the *mir-273* miRNA, which represses *die-1* and *lsy-6* expression. The fate of each ASE neuron is ultimately executed by transcription factors acting downstream of the feedback loop, including the Lim homeobox gene *lim-6* and the zinc-finger gene *fozi-1*, which are partially responsible for executing the ASEL cell fate under the control of DIE-1 (Johnston et al., 2006). The bilaterally expressed zinc-finger transcription factor LSY-2 is required permissively for expression of the *lsy-6* gene in ASEL (Johnston and Hobert, 2005). In *lsy-2* mutants, the *cog-1/mir-273* module "wins" over the *die-1/lsy-6* module, causing both cells to take on the ASER cell fate.

The miRNA/transcription factor feedback circuit explains molecularly how the two ASE cells stably adopt distinct fates, but it does not explain how asymmetry is established in the first place. What determines which half of the feedback loop gets activated in each cell? In principle, the information leading to ASE asymmetry may be established in two ways. In one scenario, another left-right asymmetric structure could send a signal to one of the two ASE cells relatively late in development to differentiate it from its contralateral equivalent. Alternatively, asymmetries early in the lineage could provide different cell-intrinsic information to the two ASE neurons that biases their fates. With a series of elegant experiments involving cell ablations and genetic manipulations that alter early embryonic lineages, Poole and Hobert (2006) demonstrated that the left-right positions of the ASE neurons do not influence their fates, implying that reception of a nonautonomous left-right asymmetric signal is not required for ASE laterality. However, the particular embryonic lineage from which an ASE neuron descends always correlates with its fate, suggesting that ASE asymmetry is determined very early in development. ASE fate determinants are already asymmetrically segregated along the A/P axis at the four-cell stage, before left-right symmetry is broken. Reversing left-right asymmetry reverses asymmetry of ASE, but only because it reverses the relative positions of the lineages that give rise to the two ASE neurons. Remarkably, the ASE cells retain, through ten cell divisions, a "memory" of a factor that was asymmetrically expressed at the four-cell stage. This mechanism for left-right determination is probably only possible in organisms with highly stereotyped developmental lineages like *C. elegans*. Understanding the nature of the ASE fate-determining asymmetric mark, how it is retained through the lineage, and how it impinges on the ASE signaling circuitry will be exciting questions for future study.

A Directional Asymmetry in the Zebrafish Brain

The epithalamus is a region in the dorsal diencephalon that is anatomically asymmetric in many vertebrate species (reviewed in Concha and Wilson, 2001). In zebrafish, the main constituents of the epithalamus are a sensory/neuroendocrine complex, consisting of the pineal and the parapineal, and the paired habenular nuclei. The pineal gland is a stalk-shaped organ located at the midline, and the parapineal is an asymmetric structure located invariably to the left of the midline. The habenula functions as a relay center connecting telencephalic inputs with

a structure in the ventral mesencephalon known as the interpeduncular nucleus (IPN). The left habenula also receives inputs from the parapineal. Each habenular nucleus consists of several subnuclei that are defined by gene expression and axon projection pattern (Aizawa et al., 2005; Gamse et al., 2005). A subnucleus that is located laterally in both habenula sends projections primarily to the dorsal IPN, and a medially located subnucleus sends projections to the ventral IPN. Like the pineal complex, the habenula exhibits marked bilateral asymmetry. In the left habenula, the lateral subnucleus is substantially larger than the medial subnucleus, so the left habenula sends more inputs to the dorsal IPN. Conversely, on the right, the medial subnucleus is bigger than the lateral subnucleus, so the right habenula sends more inputs to the ventral IPN. Thus, the medio-lateral segregation of information in the habenula, and the characteristic left-right differences in its organization, is maintained in downstream neurons, albeit in a dorso-ventral orientation. Anatomical left-right asymmetry in the habenula is accompanied by molecular asymmetries. For example, three members of the *potassium channel tetramerization domain containing* (KCTD) gene family are expressed asymmetrically—the *leftover* gene is expressed primarily in the left habenula, and *righton* and *dexter* are expressed primarily in the right habenula (Gamse et al., 2005). Recent work addresses both how the epithalamus adopts consistent laterality and how its constituents become morphologically asymmetric (Figure 3).

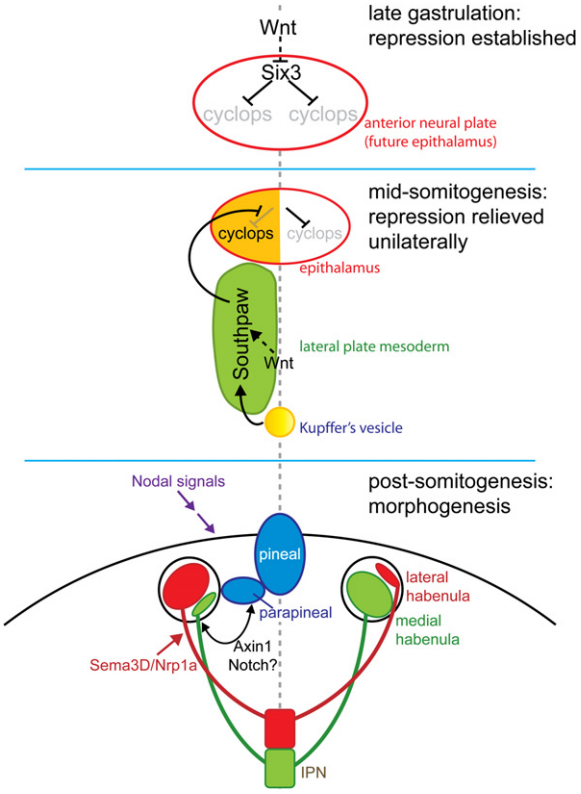

Figure 3. A Model for the Regulation of Directional Asymmetry in the Zebrafish Epithalamus
Relative sizes of structures and distances between them are not to scale.

Imposing Laterality on the Zebrafish Brain

Work over the past 10 years has defined a pathway for establishing bilateral asymmetry in the viscera of vertebrates (reviewed in Raya and Belmonte, 2006). According to the predominant model, after the dorsal-ventral and anterior-posterior axes have been defined, bilateral symmetry is broken at the node in mice, or a related structure called Kupffer's vesicle in zebrafish, in a process requiring the function of motile cilia whose beating creates an asymmetric "flow." Nodal flow is thought to localize morphogens asymmetrically around the node. This asymmetry at the node is transferred to the lateral plate mesoderm (LPM), where *nodal*, a gene encoding a member of the transforming growth factor-β superfamily, is expressed exclusively on the left. *nodal* expression is confined to the left LPM by barriers at the midline. Left-sided *nodal* expression in turn leads to asymmetric expression of the transcription factor *pitx2*, which controls the laterality of asymmetric organs. Although details of the pathways that determine the left-right axis are not identical in every animal, early asymmetry in the node, broad left-sided expression of Nodal ligands, and the role of the midline as a barrier are conserved features of the pathway in all vertebrates.

Studies of epithalamic development in zebrafish have made it possible to assess the relationship between visceral and brain asymmetry. Genetic analysis has revealed that epithalamic laterality is controlled by Nodal signaling, the same pathway that determines laterality of internal organs (Concha et al., 2000; Liang et al., 2000). Several components of the Nodal pathway, including the nodal ligand Cyclops, the transcription factor Pitx2, and the Nodal antagonist Lefty1, are expressed exclusively on the left side of the epithalamus. In embryos in which Nodal signaling is reduced or bilateral, parapineal and habenular asymmetry is randomized. Nodal signaling is therefore not required for asymmetry itself, but rather for imposing a directional laterality upon it.

Another zebrafish Nodal gene, *southpaw*, is required for determining the laterality of both the viscera and the epithalamus, but is expressed only in the LPM and not the brain (Long et al., 2003). Laterality of the viscera and brain, which are normally concordant, are uncoupled in embryos lacking *southpaw* function, identifying Southpaw as a major component of the signal that coordinates laterality. This result also suggests that signals specifying left-sidedness spread from the LPM to the ectoderm, rather than arising there independently.

Two new studies published in this issue of *Neuron* address how laterality signals are transferred from the mesoderm to the brain (Carl et al., 2007; Inbal et al., 2007). Carl et al. (2007) demonstrate a role for the Wnt pathway in regulating Nodal expression in the epithalamus. Activating Wnt signaling with a mutation in the *axin1* gene, which

encodes a negative regulator of the Wnt pathway, or briefly treating embryos with LiCl during late gastrulation led to bilateral Nodal pathway gene expression in the epithalamus. Manipulation of the Wnt pathway at these early stages specifically affected Nodal pathway gene expression in the brain, but not the LPM, leading to randomization of epithalamic asymmetry but normal visceral asymmetry. Altering Wnt pathway activity at later (mid-somitogenesis) stages also led to bilateral Nodal pathway gene expression, but in this case, both the viscera and the brain were affected. The authors propose a model in which Wnt influences asymmetry decisions twice. First, during late gastrulation, Wnt signals can repress a repressor of Nodal gene expression specifically in the anterior neural plate. Axin1 activity at these stages represses Wnt signaling to allow, in turn, the repression of Nodal signaling. Later, during mid-somitogenesis, Southpaw signaling overcomes this repression on the left side of the epithalamus. Manipulating Wnt signaling at this later stage can perturb Southpaw signaling, thus affecting laterality of both the viscera and the brain.

Another study in this issue of *Neuron* finds that reducing the function of Six family transcription factors in the anterior neural plate causes a similar phenotype to Wnt pathway activation—in Six3-depleted embryos, Nodal genes are expressed bilaterally specifically in the brain, and, as a result, epithalamic asymmetry is randomized (Inbal et al., 2007). Conversely, excess Six3 function results in bilateral repression of Nodal activity in the epithalamus. Since Six3 and Wnt have been shown to negatively regulate each other, Six3 may act downstream of Wnt signaling during early somitogenesis to repress Nodal gene expression bilaterally in the epithalamus (Lagutin et al., 2003). It is important to emphasize that, in these models, Wnt signals and Six3 function symmetrically. Epistasis analysis demonstrated that the asymmetric signal that lateralizes the brain comes from Southpaw in the left LPM, which relieves the Six3-dependent repression of Nodal activity specifically on the left side of the epithalamus.

Morphogenesis of Asymmetry in the Zebrafish Epithalamus

Nodal signaling imposes directional laterality on epithalamic asymmetry but is not required for asymmetry itself (Concha et al., 2000). Several recent studies have begun to reveal how, morphogenetically, the parapineal and habenula become asymmetric. These studies uncover a rich variety of tissue interactions and signaling events that ensure coordination and precision of epithalamic circuitry.

There are at least two possible strategies for creating asymmetric organs: A structure could be born on one side or the other, making use of signals that have already been confined to one side of the embryo, or communication across the midline could be used to make a coordinated decision leading to asymmetry. Cell tracing studies suggest that the latter strategy is used by the parapineal (Concha et al., 2003). These studies showed that parapineal precursors arise on both sides of the midline, but then migrate to one side where they form the parapineal. In wild-type animals, the influence of the left-sided Nodal pathway biases migration of parapineal precursors so that it invariably occurs from right to left. In the absence of Nodal signaling, although migration occurs normally, the direction of migration is randomized. Removing Nodal signaling, therefore, uncovers a state analogous to the asymmetry of AWC neurons in *C. elegans*—cells on the left and right coordinately determine asymmetry. Wnt signaling may influence this migration because in *axin1* mutants parapineal cell migration is often delayed (Carl et al., 2007).

Although epithalamic laterality is randomized in Nodal mutants, parapineal asymmetry is always coordinated with habenular asymmetry, suggesting that at least one of these structures influences the other. Indeed, habenular and parapineal precursors signal reciprocally to ensure concordance of asymmetry. Ablation of the parapineal reduces morphological and molecular asymmetry in the habenulae, while ablation of habenular precursors influences the direction of parapineal migration (Concha et al., 2003; Gamse et al., 2003). Intriguingly, Carl et al. (2007) show that concordance of these two structures is uncoupled in *axin1* mutants. In these mutants, parapineal asymmetry is often unaffected, but both habenulae display right-sided features. This observation suggests that Wnt signaling (or another pathway in which Axin1 plays a role) contributes to the coordination of the two structures.

Progress has recently been made in understanding how asymmetry in the relative sizes of habenular subnuclei develops. Differences in subnuclear size in the left and right can be attributed to bilateral differences in the timing of neurogenesis (Aizawa et al., 2007). During a specific developmental time window, most neurons (on both sides) are specified to reside in the lateral subnuclei, and later in development, most neurons are specified to reside in the medial subnuclei. The difference between the left and right is in the timing of the generation of neurons—more neurons are born on the left at the earlier stage, thus creating a relatively larger lateral nucleus, and on the right more are born later, creating a relatively larger medial subnucleus. Aizawa et al. (2007) altered the timing of neurogenesis by altering Notch signaling and thus manipulated the relative sizes of the two subnuclei. Notch signaling itself may therefore be a good candidate for participating in a signal that leads to left-right asymmetry. Given the influence of the parapineal on habenular asymmetry, it is possible that the signal causing the left-right difference in habenular neurogenesis is sent from the parapineal.

The final level at which epithalamic asymmetry is manifested is the targeting of habenular efferents to the IPN. One gene that plays a role in determining connectivity of habenular neurons is *neuropilin1a* (*nrp1a*), which encodes a coreceptor for secreted members of the semaphorin family of axon guidance molecules (Kuan et al., 2007). *nrp1a* is expressed in a portion of the left habenula, but is not prominently expressed on the right. Laterality of

nrp1a expression is under control of the Nodal pathway and influenced by the parapineal. Depletion of Nrp1a or of the secreted semaphorin, Sema3D, reduced dorsal innervation of the IPN without affecting habenular asymmetry. Thus, semaphorin signaling is required downstream of pathways that establish epithalamic asymmetry to execute a left-specific pattern of connectivity.

Behavioral Consequences of Asymmetry

Functional lateralization and asymmetry of animal behavior is common, but the developmental bases for these behavioral asymmetries are not well understood. The genetic models of asymmetry provide a means for exploring the behavioral consequences of perturbing neuroanatomical asymmetry. Despite the very different molecular underpinnings of AWC and ASE asymmetry, in both neuron pairs, asymmetry serves a similar purpose (Pierce-Shimomura et al., 2001; Wes and Bargmann, 2001). In both cases, each neuron of the bilateral pair is specialized to sense a distinct set of chemicals. Segregating receptors into different neurons allows the worm to distinguish between more chemicals. For example, a mutant in which both ASE neurons express left- and right-sided markers simultaneously is able to sense the normal set of chemicals, but when a saturating level of one chemical is present, they cannot sense any of the others (Pierce-Shimomura et al., 2001). Thus, dividing sensory labor between the left and right sides of the very compact *C. elegans* nervous system allows the animal to sense more chemicals at once and potentially expands its behavioral repertoire.

Although the specific function of epithalamic asymmetry has not been identified, zebrafish with reversed laterality of both internal organs and the epithalamus exhibit reversed laterality of several asymmetric behaviors (Barth et al., 2005). For example, fish fry display characteristic eye preferences when they look at themselves in a mirror. These eye preferences are reversed in fish with anatomically reversed laterality. Notably, other left-right asymmetric behaviors are unaffected in these reversed animals, and new behaviors that are not observed in wild-type fish appear. Imposing laterality on brain asymmetries thus ensures that all lateralized behaviors are coordinated, regardless of whether they are under the same molecular control, and prevents new potentially maladaptive behaviors from arising in a population.

More Ways to Make Two Sides?

Studies in the three models discussed here reveal surprising diversity in the strategies used by nervous systems to develop asymmetry, implying that they arose by diverse evolutionary mechanisms. It has been proposed that randomized organ asymmetries evolved first and that uniform directional laterality was imposed later to ensure organ concordance (for example, see Palmer, 1996; Capdevila et al., 2000). This is a plausible explanation for the development of asymmetries in the epithalamus, where removal of laterality signals reveals an underlying asymmetric state. However, in *C. elegans* the fact that ASE does not require extrinsic left-right signals to achieve directional asymmetry, coupled with the unexpectedly discovery of left-right biased gene function in AWC, suggests that perhaps the opposite evolutionary scenario could have occurred in *C. elegans*. In this scenario, a pre-existing intrinsic bias in AWC signaling, similar to that which occurs in ASE, evolved first, and a mechanism to ensure randomness of AWC signaling was overlaid on top of that. If this was the case, the random sidedness of AWC function may have had some advantage at the population level.

It is possible that further studies in these systems and in new models may reveal some conserved principles, but they are also likely to reveal more diversity. Although the zebrafish studies correlate the laterality of several behaviors with the laterality of anatomical asymmetries, fish exhibit other asymmetric behaviors that do not correlate with epithalamic laterality (Barth et al., 2005). Similarly, in humans functional asymmetries such as lateralization of handedness and hemispheric language dominance do not correlate with laterality of internal organs, suggesting that an entirely novel genetic pathway may determine the neuroanatomical and molecular asymmetries that underlie these functional asymmetries (reviewed in Sun and Walsh, 2006).

Insight into the basis for functional asymmetries could potentially be gained by screening directly for mutants with defects in robustly lateralized behaviors in zebrafish. Characterizing such mutants could help identify other molecular and neuroanatomical asymmetries in the brain that are directly responsible for specific behaviors. Alternatively, developing more models of neuroanatomical asymmetries could identify new pathways. A good candidate for such a model is the "asymmetric body" of *Drosophila*, a structure that is located only on the right side of the brain of most flies (Pascual et al., 2004). This *Drosophila* model of brain asymmetry remains so far unexploited, but the powerful genetic and molecular tools available in *Drosophila* make it likely that it could contribute to our understanding of the development of brain asymmetry and laterality.

A more direct approach to understanding human functional asymmetries would be to use mammals themselves for studying asymmetric brain development. A set of genes that is differentially expressed in the two hemispheres of the human brain has recently been identified (Sun et al., 2005). Using these genes as starting points for mouse and human genetic studies could provide insight into how several fundamental human behaviors are biased to the left or right.

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