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Introduction

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Introduction to provocative questions in left–right asymmetry

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Left–right asymmetry is a phenomenon that has a broad appeal—to anatomists, developmental biologists and evolutionary biologists—because it is a morphological feature of organisms that spans scales of size and levels of organization, from unicellular protists, to vertebrate organs, to social behaviour. Here, we highlight a number of important aspects of asymmetry that encompass several areas of biology—cell-level, physiological, genetic, anatomical and evolutionary components—and that are based on research conducted in diverse model systems, ranging from single cells to invertebrates to human developmental disorders. Together, the contributions in this issue reveal a heretofore-unsuspected variety in asymmetry mechanisms, including ancient chirality elements that could underlie a much more universal basis to asymmetry development, and provide much fodder for thought with far reaching implications in biomedical, developmental, evolutionary and synthetic biology. The new emerging theme of binary cell-fate choice, promoted by asymmetric cell division of a deterministic cell, has focused on investigating asymmetry mechanisms functioning at the single cell level. These include cytoskeleton and DNA chain asymmetry—mechanisms that are amplified and coordinated with those employed for the determination of the anterior–posterior and dorsal–ventral axes of the embryo.

This article is part of the themed issue 'Provocative questions in left–right asymmetry'.

1. Introduction

From the nano to the macro, the man-made to the natural and the simple to the complex, the world we inhabit is rich in pattern. Although there is extensive variance in the intricacy and magnitude of the patterns that surround us, they fundamentally stem from only two core motifs: symmetry and asymmetry. Prevalent in our art, architecture, dance, music, fashion and other design—symmetry evokes order, desire, harmony and perfection. Indeed, we place such high value on symmetry that its presence in facial features can be used to define physical beauty. Like symmetry, asymmetry is also pervasive in our aesthetics, where it lends a provocative contrast, representing the dynamic, the unexpected, the emerging and the innovative. Together, symmetry and asymmetry comprise the proverbial Yin and Yang, the black and white, the metaphorical good and sinister. Both are essential for completeness, but too much in either direction disrupts a critical balance—symmetry unchecked by asymmetry transmutes order, harmony and beauty into static, sterile and monotonous. Asymmetry unchecked by symmetry becomes aberrant, unrestrained and chaotic.

Biology holds no exception to this duet. At first glance, symmetry may appear to be the predominant pattern in nature; indeed, animal cognitive systems have evolved sensitive symmetry detectors that function during mating choice and predator detection. However, organisms ranging from protists to plants to

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vertebrates possess a fascinating collection of asymmetries within their body structures. Such morphological asymmetries exist with respect to the left–right (L/R) axis, spanning scales of size and levels of anatomical organization. The ability of biological forms to reliably generate consistent asymmetry in a universe that does not distinguish left from right (except at the quantum level) [1,2] is a remarkable phenomenon that attracts significant interest from many disciplines. As the first laterality genes were discovered only about 20 years ago, the field of L/R asymmetry is in a vibrant state of advancement, with emerging insights into deep unresolved questions about the cell biology and evolutionary history of chirality, propelled by identification of a steady stream of molecular-level work performed in a range of model systems.

In higher organisms, the establishment of body shape requires precise development and coordination of anterior–posterior (A/P), dorsal–ventral (D/V) and L/R body axes during embryogenesis. D/V and A/P axes are specified by external forces that operate on the oocyte, such as the point of sperm entry, maternal animal–vegetal gradients and the effect of gravity. Of the three body axes, the most open questions concern the mechanisms of specifying and patterning the L/R axis. Models proposed to explain laterality mechanisms remain controversial, but it is clear that anomalies of L/R axis determination can lead to a spectrum of congenital disorders that affect both arrangement and morphology of the heart and visceral organs. L/R visceral organ asymmetry anomalies are broadly classified as two disorders, *situs ambiguus* and *situs inversus*. Also termed heterotaxy, *situs ambiguus* is a disorder of discordance in which some organs develop normal L/R asymmetry and others do not. This class of laterality disorders includes isomerism, in which some normally asymmetric organs instead develop symmetrically (e.g. left or right atrial or pulmonary isomerism) with health consequences, including prenatal death. *Situs ambiguus* is associated with significant foetal and perinatal lethality in humans and other vertebrates, largely owing to the development of complex congenital heart defects. Comparatively less mortality is associated with *situs inversus*, a disorder in which all organs are concordantly (mirror-image) reversed with respect to the other two body axes. Nevertheless, as ciliary defects are often associated with *situs inversus*, respiratory, kidney and reproductive function are frequently compromised in affected individuals. Moreover, developmental anomalies of unknown cause with ectodermal derived tissues representing brain hemispheric L/R asymmetry are associated with debilitating schizophrenia and bipolar psychoses disorders found in approximately 2.0% individuals worldwide and for that of the very rare mirror-hand movements disorder found in humans.

Because of the importance of L/R axis determination for vertebrates' health and the many questions surrounding the mechanisms involved, it was an obvious choice for the Royal Society to publish a monograph on this subject. The editors' intention in accepting this assignment was to highlight recent research achievements, current controversies and remaining gaps in our knowledge in the field, with the goal to help guide future research. Experts in this field were asked in their contributions to summarize the progress made thus far, either as review articles or original research articles. We believe this monograph will be useful for researchers in this specific field, as a tool for teaching developmental biology in graduate-level courses and for teaching the biology of origin of congenital disorders to medical students. The field of L/R asymmetry contains a number of basic puzzles, including:

— How do embryos orient the L/R axis reliably with respect to the other two axes and the midplane?
— When does this occur—how early do embryos first tell their L from their R?
— How is this information propagated to cell fields, resulting in regions with L and R identity?
— What is the relationship between chirality (seen in snails, behaviour of cells in culture) and directional asymmetry (e.g. vertebrate body plan)?
— How is the lateralization of other aspects of the body—behavioural, immunological, parietal hair whorls' clockwise versus anti-clockwise orientation, etc., related to pathways that set organ positioning?
— How conserved are the answers to the above questions for organisms across phyla, and what are the evolutionary relationships between the different ways these problems are solved across the tree of life?
— After finding the biological basis of human congenital laterality disorders, how can that knowledge be used for biomedical applications?

2. Synopsis of laterality chapters

As defining the mechanisms driving L/R asymmetric phenotypes is the main subject of this theme issue, it begins with a compact yet comprehensive review by Palmer [3], who discusses the ways in which asymmetries arise in otherwise bilaterally symmetrical organisms. This review beautifully highlights very different phenotypes of laterality development in diverse organisms, tackling the questions of how symmetry is first broken during embryogenesis, whether it is determined by genes, environment and chance, whether direction of asymmetry is maintained during regeneration of a tissue and whether asymmetry is produced at the organ level or globally at the organismal level. By reviewing asymmetry across multiple species and their morphogenetic features, such as gastropod coiling, flatfish eye sidedness, directional crossbill crossing, claw asymmetry in lobsters, crabs and shrimp, katydid sound-producing structures and various plant asymmetries—the message is reinforced of the diversity that exists across organisms in the process of asymmetry, with different mechanisms likely having evolved multiple times for the benefit of each species. One particularly illuminating example of this is presented by the genetics of coiling direction controlled by two of the three alleles of a single locus of gastropods. They are the *right*, *left* and *stochastic* alleles, where the *stochastic* allele is recessive to both the dominant *right* or *left* alleles of a specific species. One key question this raises for future research is whether there is a commonality of biological mechanisms used for evolution of novel phenotypic variation of L/R asymmetry? The study of Schilthuizen *et al.* [4] adds to the understanding of evolution of asymmetry in the context of another coiling macroscopic feature: the asymmetric genitalia in Coleoptera. Asymmetry in male genitalia is a pervasive aspect of much of the animal kingdom, raising interesting questions about the physical and behavioural dynamics that drive this outcome in animals with highly diverse body plans and lifestyles.

Regarding the molecular origin of asymmetry, one popular model suggests that ciliary movement during neurulation initiates asymmetry in those organisms that have cilia prior to the appearance of laterality. For some vertebrates, ciliary

movement during neurulation and the subsequent calcium signal is a crucial event [5]. An excellent review of this model with details on the role of motile cilia in leftward fluid flow in humans and animal models is provided by Dasgupta and Amack [6]. They explain that while there is consensus that a transient leftward fluid flow can direct left-side asymmetric activation of the conserved Nodal pathway, namely in mouse and zebrafish, there is evidence indicating that not all vertebrates use this mechanism. Moreover, as also discussed by Dasgupta and Amack [6], and elsewhere [7], this model has faced a number of problems, including the question of what mechanism(s) are used to sense the fluid flow—e.g. mechanosensitive cilia, morphogen gradients or vesicular particles. More recent studies have shown that primary cilia are not in fact calcium-responsive mechanosensors [8], and that asymmetries in cleavage-stage frog embryos have not only consistently asymmetric metabolic profiles [9], but also protein components with functional roles in asymmetry development [10]. Consistent with the existence of L/R asymmetry at such an early stage of development, Dasgupta and Amack [6] used mosaic cell labelling and time-lapse imaging in the zebrafish embryo to demonstrate that ciliated cells within Kupffer's vesicle—a structure that is functionally analogous to the embryonic node and alternatively termed the 'L/R organizer'—are non-stochastically arranged, suggesting that they may be propagating L/R positional information that exists prior to L/R Organizer morphogenesis and hence, generation of directional fluid flow.

If asymmetry precedes the L/R organizer, then what is the symmetry-breaking event and when does it occur? A common view of development suggests that cells in the embryo initially are undifferentiated, and that a morphogen gradient is generated to specify gene regulatory networks on cells located at different locations in the embryo [11]. This model was adapted by Wolpert [12] for explaining general development, including L/R axis determination [13], and has constituted the major paradigm for guiding developmental biology research conducted for decades. The variety of data described in this issue reveals that asymmetry mechanisms are far broader. In fact, in a 2009 interview, Wolpert discarded the morphogen model by stating: 'diffusible gradients are out' [14, p. 659]. In our view, the mechanisms of L/R axis remain open, with two emerging (and non-mutually exclusive) alternatives to the morphogen model: both place the element of initial L/R asymmetry inside the cell, as opposed to extracellular fluid flow, and notably at a much earlier time point (early cleavage).

As expertly presented by Klar [15], one intriguing model that is fundamentally different from the commonly invoked morphogen model is based on DNA strand-specific segregation, driven by the mechanism of somatic strand-specific imprinting and selective chromatid segregation (SSIS). This mechanism invokes the processes of monochromatid gene expression, and that is based on the asymmetry of DNA chains of developmental control gene(s), followed by selective segregation of thus epigenetically differentiated sister chromatids in the mitosis of a deterministic cell to produce developmentally unequal sister cells. This paper summarizes previous application of this mechanism to explain the genetically caused variation of development of visceral organs' laterality in vertebrates, the piebald (i.e. two different colours; black spots on one wing and red spots on the lateral wing) mutant phenotype of the *Bruchus* beetle and anomalies of brain hemispheric asymmetry owing to chromosome 11 rearrangements that are associated with psychoses and for mirror-hand movement disorders owing to *RAD51* haploinsufficiency in humans. Klar [15] has here extended the application of this developmental mechanism to explain the genetics of congenital limb developmental anomaly in humans. This anomaly is called the split hand/foot malformation, where the index and middle fingers fail to develop with variable penetrance even for different limbs of the same person. It is known that deletions and conventional mutations of *DLX5* (the *Drosophila distal-less dll* homologue) in chromosome 7 cause the anomaly with incomplete penetrance owing to haploinsufficiency, but paradoxically, the vast majority of cases result from chromosomal aberrations where the *DLX5* locus itself is not mutated, and moreover, where the aberration breakpoints are often located millions of bases away from the *DLX5* locus. Klar [15] concludes that chromosome 7 translocations and inversions associated with the disorder (whose genetic aetiology has remained unexplained thus far) have satisfied developmental biology predictions of the SSIS mechanism. It is argued that the double-helix structure of DNA itself forms the physical basis of turning the developmental control gene *ON* in one chromatid and *OFF* in sister chromatid during replication of the progenitor cell's gene, and this binary-coded gene expression switching mechanism may be the underlying mechanism of development in general. By evolving factors that can vary the selective strand/chromatid segregation process functioning at the centromere of the relevant chromosome, both regulated asymmetric versus symmetric cell divisions can be produced in different cell types by the same intracellular SSIS mechanism, providing a possible molecular explanation for the Ying and Yang developmental outcome described above.

Another alternative model of symmetry breaking is grounded in the role of the cytoskeleton, with a mechanism that has strong implications for evolution. It should be appreciated that intracellular features of cellular polarity and cytoskeleton comprising cellular chirality (to be presented below) must play a critical role for the SSIS mechanism to operate. Nodal ciliary flow is absent in pig and chick, as well as *Drosophila*, snails and *Caenorhabditis elegans*; thus, if cilia are crucial in some situations, then the origin of asymmetry must be highly divergent across phyla. By contrast, intracellular cytoskeletal elements are highly conserved; recent data revealed a microtubule protein that was functionally implicated in asymmetry of plants, *C. elegans*, *Xenopus* and human cells [10]. One possibility is that asymmetry is an ancient property that predates the origins of multicellularity; even bacteria [16], unicellular ciliates [17] and slime moulds [18] exhibit asymmetry. Satir's paper [19] deftly overviews the many actin filaments and microtubule processes in cells that exhibit chiral properties, and illustrates how helical properties of cytoskeletal elements instruct cell behaviour and subsequent morphogenesis.

Consistent with this model, exciting recent data have revealed that individual cells have many directionally oriented asymmetric behaviours that can be explained by chiral properties that have an impact on large-scale structures to which these cells contribute [20–23]. The ability of single cells (even *in vitro*) to determine their L/R axis in the absence of ciliated organs [24–26] suggests that in at least some cases, asymmetry is leveraged intracellularly and may be present throughout the body. The link between single-cell asymmetry and whole-body asymmetry is one of the most exciting future aspects of this field. Inaki *et al.* [27] have reviewed studies with model systems addressing the role of cellular chirality

in L/R axis determination and asymmetric organ development. They review various cultured cell lines of vertebrates that show intrinsic chirality affecting cellular behaviours, and suggest that chirality derives from cellular structures such as basal bodies and/or cytoskeleton. Specific examples include blastomere chirality, which dictates L/R body asymmetry at or before the eight-cell stage in *C. elegans* embryos and dextral versus sinistral shell coiling in snails, and myosin31DF as a key player in controlling directionality of gut looping in *Drosophila*. Wan *et al.* [28] also critically review cell chirality, focusing on *in vitro* cell systems involving microprinting and degradable hydrogels as well as mathematical models of biophysical mechanisms that have been used to probe multicellular chirality and its propagation during collective cell migration. In addition, they discuss a recently developed three-dimensional spheroid rotation assay that can be used to determine chirality of less adhesive cells. As chirality appears to be an intrinsic property of all cells, and is readily demonstrable with experimental micropatterning systems, one intriguing application of this phenomenon is use of cell chirality as an endpoint in screening for nanomaterial toxicity and putative teratogens.

An important avenue for future work is the development of computational models that reveal how large-scale body plans arise from the activities of single cells. In the L/R asymmetry field, this amounts to multi-scale biophysical models that quantitatively explain the integration of chiral cell behaviours towards whole body axes. Recent work has begun this task [20,23,29–31], and the contribution by Martinez *et al.* [32] extends the effort to patterns of plant leaf asymmetry. Analysis of several mutants with spiral phyllotaxis found the mirrored patterns of L/R leaflet displacement as predicted by the model, as were the results of analysis of leaf pair shapes in plants with distichous phyllotaxis. The model was based on the dynamic of fluxes in auxin concentrations, which is interesting, because auxin is a signalling molecule very similar to serotonin—a neurotransmitter with important L/R patterning roles [33].

For L/R axis patterning, clearly, laterality-specifying genes and their precise regulation are required. But how does a cell regulate laterality gene expression according to whether it lies on the left versus right side of the embryo? Transcriptional processes cannot distinguish spatial directions by themselves, and any gene regulatory circuit has to be painted onto a shape that is in part dictated by physical forces. In the L/R axis, regardless of which of the initiation models turns out to be correct, a microscopic, physical process (structure of cytoskeletal or DNA elements) is amplified into signals across whole cell fields. This is an important example of biological information processing, as chiral physical elements provide direction (e.g. leftward or clockwise), whereas L/R-asymmetric gene expression requires position (left side relative to midline). During the early phases of asymmetry, the embryo converts microscopic directional cues into macroscopic positional cues, which feed into differential cascades of gene expression on the L and R sides. Burdine and Grimes [34] exploit the strength of the zebrafish model system, performing a large number of elegant transplantation and ablation studies in mutant lines that show how events in the zebrafish L/R organizer result in the reliable unilateral expression of key L/R patterning genes. These events involve tissue interactions between the notochord, lateral plate mesoderm and the Kupffer's vesicle, revealing how the transcriptional cascades operate in the context of a complex embryo that is simultaneously patterning numerous other aspects. An essential component is the notion of midline; the midline is essential for defining asymmetry in the first place, but how an early embryo bisects itself in a consistent manner is a key unanswered question. Asymmetric cell division occurring very early on to bisect the embryo, followed by each laterality-specified daughter cell's progenies executing the specific left- versus right-sided developmental programme, should be considered as one of the possibilities.

In addition to an essential role in maintaining L/R asymmetry, midline interactions also simultaneously operate to preserve symmetry in bilaterally paired organs that arise from lateral tissues. Robichaux *et al.* [35] explore this in the context of mammary gland development in mice lacking a functional allele for an A/P patterning gene, *retinoid X receptor alpha* (RXRα). In this study, the authors demonstrate that the genetically engineered haploinsufficiency in the RXRα locus causes a substantial fraction of mammary glands (in approx. 50% of all animals analysed) to demonstrate distinct patterning asymmetry when left breast tissues are compared with their right counterparts, as evidenced by quantifying such anatomic parameters as ductal network area, density of terminal end buds and branch points, and relative mass of gland tissue. No variances in L/R morphometrics were present in wild-type mice carrying both functional RXRα alleles. Even more notably, in RXRα$^{+/-}$ animals, the effect was restricted to thoracic mammary glands but not detected in inguinal mammary glands, an observation that underscores previously made assumptions of differential tissue response to retinoid-mediated signalling along the A/P axis. As parenchymal L/R asymmetry in breast tissue is an early and sensitive predictor of breast cancer risk, this study suggests that heightened risk for neoplasia may actually be rooted in L/R asymmetric tissue disorganization that results from relatively subtle embryonic axial patterning defects.

Two laterality genes with highly conserved expression and function in midline and lateral patterning events include *Nodal* and *Pitx2*. Martin-Duran *et al.* [36] cover evolution of L/R asymmetry of spiralians, which includes the most celebrated phenotype of the direction of coiling of snail shells, which they associate with embryonic chirality. In this study, they describe in detail the pattern of gene expression of a *Nodal* orthologue in a brachiopod species and the pattern of *PitX* orthologues expression in an annelid and pripulid species. Their description helps us appreciate the evidence depicting patterning of L/R asymmetries in early embryonic cell lineages of the Spiralia group. Studies of these taxa indicate that embryonic chirality is a widespread feature of development, and this investigation highlights the features of asymmetric *Nodal* and *Pitx2* orthologue expression in embryonic cells even in species that develop without obvious adult L/R asymmetries. From their description, it is appreciated that the Nodal signalling pathway functioned even in the ancestors of all bilaterally symmetric animals. Consistent with other studies presented in this issue, Martin-Duran *et al.* [36] demonstrate that *Nodal* and *Pitx* genes are not expressed in the earliest cleavage stages of the embryo, indicating that a separate upstream genetic mechanism defines the L/R axis in spiralian embryos. Indeed, a recent study has demonstrated that the cytoskeleton formin gene (*ldia2*) dictates the handedness of shell coiling through its differential expression at the two-cell stage of the embryo [37]. Thus, as in other species, such a very early embryonic symmetry-breaking event must influence expression of genes such as *Nodal* for L/R axis

specification to occur later on. The next challenge is to define how such very early decisions control later L/R axis programmes later on during embryogenesis.

Also focused on *Nodal*, Signore *et al.* [38] take a comparative approach to cover the role of this pathway in the development of nervous system asymmetries in fish and ascidian species. These authors make an important distinction between Nodal as a laterality inducer and Nodal as a modulator of pre-existing identity, and describe experimental conditions in which the precise role of Nodal can be investigated. The main lesson spelled out is that bilaterally paired and midline-unpaired structures respond differently to perturbations of *Nodal* expression. Therefore, effects on paired structures should be interpreted separately from effects on unpaired strictures. These lessons can be applied to other systems for defining the role of Nodal signalling, such as for mammalian visceral and brain hemispheric laterality specification.

Subsequently, physics enters the picture again, as asymmetric protein products ultimately ensure distinct tension, stretching and bending forces that implement asymmetric morphogenesis. L/R patterning is thus not only an example of multiscale integration, but also of the continuous interplay of physics and genetics, aspects that are ubiquitous in living systems and yet still poorly understood. Another way in which L/R asymmetry sheds light on biology beyond embryonic laterality is in the implications for regenerative repair. McDowell *et al.* [39] report that the L/R transcriptional cascade, which is known to be highly conserved, has the capability of self-repair: early treatments that efficiently randomize early asymmetric genes such as the left-sided *nodal* often give rise to a smaller percentage of embryos with abnormal sidedness of downstream genes such as *Lefty* or *Pitx2*, and an even smaller percentage of animals with abnormal organ laterality. This reveals that although molecular functional data in specific assays identify genes necessary and sufficient for induction of the downstream genes (e.g. the *Nodal* → *Lefty* → *Pitx2* cascade), the story *in vivo* is more complex, owing to the presence of parallel repair pathways that can apparently detect and reverse molecular abnormalities, so that subsequent steps have normal laterality.

Despite advances made in identifying many of the cellular and molecular processes that control L/R axis determination, the genetic aetiology of L/R asymmetry disorders remains largely unknown. Whereas the majority of human laterality defects are believed to be sporadic, there is also an appreciable incidence of heritable transmission, including X-linked, autosomal dominant and autosomal recessive modes. As discussed by Cowan *et al.* [40], familial studies have led to identification of mutations in *Nodal* and other L/R patterning genes (e.g. *ZIC3*); however, surprisingly, these mutations underlie only a minority of sporadic heterotaxy cases. To identity additional causes, Cowan *et al.* [40] used array-based genotyping methods to discover that nearly 20% of patients with heterotaxy and heterotaxy-associated congenital heart defects have copy number variants (CNVs) in their genome. As analysis included only affected individuals but not their parents, it is unclear whether CNVs result from de novo mutations or if they are familially inherited. Regardless, a number of genes with cilia-related functions, including *TTC21B*, *CEP290*, *TTBK2* and *CFPAP126*, were implicated for L/R anomalies. Duplications or deletion of several other loci have been identified. One interesting case is that of *phosphofructokinase-1* (*PFKP*), a gene whose heterotaxy pathogenesis was confirmed by morpholino-based experiments in *Xenopus laevis*. This enzymatic activity is required for glycolysis, raising the question of how could it be involved in L/R asymmetry development? The authors speculate that there is a non-glycolytic role of *PFKP* in L/R patterning because its isoforms are known to bind the α-subunit of H^+-V-ATPase, a membrane-bound proton pump implicated in laterality determination in *Xenopus* and chick. Given the success achieved in this study, future continued study of additional human subjects is anticipated to be highly rewarding.

Genetic analysis of heterotaxy is also being conducted by Alcorn *et al.* [41], who use *C. elegans* in their search for disease loci. The commonly used laboratory strain of this nematode exhibits a stereotypical gut–gonad L/R asymmetry that has been shown to be established during early embryogenesis, i.e. during the division of the two anterior granddaughter cells of four-cell stage embryo. In examining nearly 100 evolutionarily diverged isolates of this species, Alcorn *et al.* [41] discovered that there is naturally existing variation in L/R organ asymmetry, and moreover, that some species exhibit heterotaxy in up to 11–12% of the animals. By conducting genetic crosses of lines showing normal L/R *situs* with lines showing heterotaxy, these investigators found that heterotaxy is associated with three genomic regions. Moreover, they report that temperature elevation prior to gastrulation can cause heterotaxy, consistent with an early embryonic symmetry-breaking event existing in this research model organism.

In addition to gut–gonad asymmetry, nervous system asymmetry can be readily investigated in *C. elegans*. Laterality of the nervous system is observed across many species, including brain hemispheric laterality development in humans that is driven by poorly defined mechanisms. Focusing on the pair of amphid wing 'C' (AWC) olfactory neurons in *C. elegans*, Alqadah *et al.* [42] review recent progress made in defining the mechanisms that regulate their functional and molecular L/R asymmetric development. The AWC neuron pair differentiates asymmetrically into AWC^{ON} and AWC^{OFF} subtypes, which sense different odours, and curiously these two neurons are randomly (stochastically) distributed with respect to the L/R axis. The AWC asymmetry is established by exposure of cells to different levels of calcium signalling during embryogenesis, and following this specification neuronal identities are maintained throughout the life of the animal. Because of its genetic tractability and the simplicity of its nervous system's laterality, *C. elegans* has emerged as a powerful model organism to define molecular mechanisms of neuronal asymmetry and the laterality of gut and gonad organs. Very interesting mutations that affect the neuronal asymmetry development are highlighted in the review. The authors note that, as found in studies of AWC asymmetry in the nematode, calcium signalling is implicated in L/R organ patterning in vertebrates.

3. Concluding remarks

L/R asymmetry is one of the most exciting problems under investigation in modern biology. Far from being solved, recent progress only highlights the remarkable opportunities highlighted by this aspect of pattern formation. A study of L/R asymmetry, in its deep aspects, will reward not only the novice student of biology, but also the seasoned expert in other fields. Spanning scales of organization, from cell to whole organism, and disciplines, from evolution to cell biology to clinical medicine, it provides fertile ground for research for generations of workers to come. Biological asymmetry's

fundamental feature is the continuous, tight integration of physical and genetic mechanisms; understanding this is a fundamental challenge, while learning to exploit it will enable powerful new roadmaps for biomedicine.

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