The evolution of left–right asymmetry in chordates

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Summary

The internal organs of all vertebrates are asymmetrically organised across the left–right axis. The development of this asymmetry is controlled by a molecular pathway that includes the signalling molecule Nodal and the transcription factor Pitx2, proteins encoded by genes that are predominantly expressed on the left side of all vertebrate embryos studied to date. Vertebrates share Phylum Chordata with two other groups of animals, amphioxus and the urochordates (including ascidians). Both these taxa develop left–right asymmetries, and recent studies have begun to address the degree of conservation of *nodal* and *Pitx2* in this process. *Pitx2* is a member of the Pitx homeobox gene family, and in both amphioxus and ascidians *Pitx* gene expression is predominantly left sided. These studies suggest that left–right asymmetry in all chordates is regulated by a conserved developmental pathway, and that this pathway evolved before the separation of the lineages leading to living chordates. *BioEssays* 24: 1004–1011, 2002. © 2002 Wiley Periodicals, Inc.

Left–right asymmetry in vertebrates; conservation and divergence

Most animals, with the exception of early diverging lineages such as the sponges, comb jellies and cnidarians, are members of the Bilateria. A key, primitive feature of the Bilateria is, as the name suggests, bilateral symmetry; their bodies are organised with clear anteroposterior (AP) and dorsoventral (DV) axes and with left and right sides reflected around the midline. However most, if not all, individuals do deviate to some degree from true bilateral symmetry. This is known as asymmetry, or left–right asymmetry, and is typically classified into three different types.(1) Firstly, minor differences between the left and right sides of an individual can result from subtle differences in environmental perturbation of developmental processes either side of the midline. These are not heritable, and are referred to as fluctuating asymmetries. Secondly, antisymmetry describes heritable morphological differences between left and right in which sidedness is randomly determined such that, on examining a population, half will show one asymmetric phenotype and half the mirror image of this. Finally, directional asymmetry describes asymmetric morphology in which sidedness is fixed for a population or, more usually, for a species or higher taxon. Directional asymmetry implies a consistent, heritable difference in the regulation of developmental processes on either side of the midline.

Directional asymmetries are widely distributed in bilaterian animal taxa. Sometimes they are obvious, for example most gastropod molluscs have a fixed direction of shell coiling, and many tube-dwelling polychaete worms have a fixed tube spiral direction. More commonly, however, directional asymmetries are subtle and have only been detected following careful examination, for example the wing and gut morphology of *Drosophila melanogaster* and the mouthpart asymmetry of thrips.(2–5) An informative approach to understanding the evolution of asymmetry has been to map asymmetries onto phylogenies.(1) Such studies allow the timing and direction of evolutionary change to be inferred and have suggested that, for individual structures, directional asymmetry has evolved numerous times from an ancestral antisymmetric state. In some invertebrate groups, reversals of directional symmetry are also common; for example, while most gastropods show dextral shell coiling, species with sinistral coiling are also widely distributed in gastropod phylogeny, indicating the repeated evolution of sinistrality from dextrality. Symmetry reversal is here apparently dependent on reversing the direction of spiral cleavage and, in the pond snail *Lymnaea peregra*, symmetry reversal in different strains can be traced to a single recessive locus, suggesting a genetic mechanism for the evolution of symmetry reversal.(6)

Most vertebrates are externally bilaterally symmetrical. However, as James Bond discovered on confronting arch-villain Dr No in Ian Fleming's novel, the internal organs such as the heart and gut are asymmetrically distributed. Dr No had situs inversus, a condition in which the asymmetry of internal organs is reversed, and had correspondingly survived an assassin's bullet aimed at his left chest where his heart should have been.(7) Comparative anatomy and embryology show all vertebrates share this same basic orientation of directional asymmetry, in that the first major morphological asymmetry is

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a directional kink in the median, symmetrical heart tube. Naturally occurring reversed-symmetry populations have not been identified; animals like flatfish do show reversals of their obvious postmetamorphic external asymmetry, but it is unclear whether this is related to internal visceral and heart asymmetry.(8) Similarly mutations like Dr No's that lead to full inversion of symmetry are rare. These observations suggest that vertebrate internal asymmetry arose and became fixed early in vertebrate evolution, and furthermore suggest that reversed symmetry phenotypes are subject to purifying selection.

The molecular mechanisms controlling the development of asymmetry in vertebrates have been dissected in some detail. They have also been recently and comprehensively reviewed,(9,10) and hence we will not discuss all these findings here but rather will summarise some key points (Fig. 1). Conceptually, an egg or embryo with specified AP and DV axes has equivalent left and right sides, and thus we must invoke an initial symmetry-breaking event to differentiate between these two. Some studies suggest distinct mechanisms operate to break symmetry in different vertebrate taxa. For example, experiments in *Xenopus* embryos suggest a role for the TGFβ superfamily protein Vg1,(11) while some experiments suggest that, in mouse development, symmetry is broken comparatively late in development by cilial activity at the node.(12) Interestingly, nodal cilia appear to be conserved amongst the jawed vertebrates, although their function is as yet unclear.(13) Furthermore, experiments by Levin and coworkers suggest that patterned gap junction communication in the early *Xenopus* and chick embryos is important for establishing asymmetry,(14,15) perhaps resulting in the directional transport of regulatory molecules driven by the differential activity of ion channels.(10,16) This is an additional pathway that must be reconciled with the observations discussed above. These studies may suggest some divergence of the upstream asymmetry pathway between different vertebrates, perhaps an example of the evolutionary divergence of a developmental pathway by the addition or substitution of different components on different lineages.(17) It is also, however, quite possible that the apparent differences are artefacts, generated by the different techniques and approaches used in different model systems; this remains to be determined.

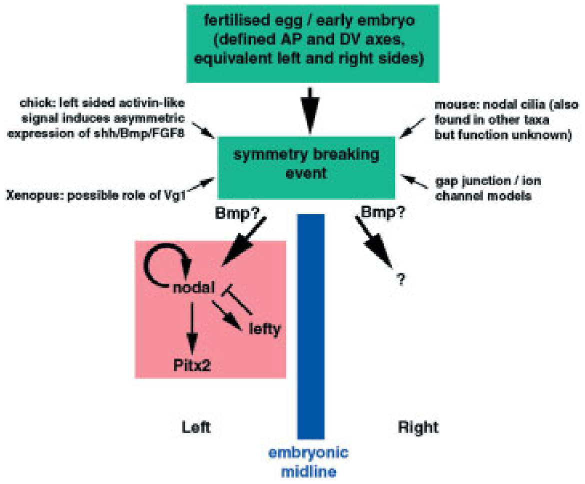

Figure 1. Simplified model of the regulation of left–right asymmetry in jawed vertebrates. An initial symmetry breaking event is assumed to break the symmetry of early embryos with equivalent left and right sides. Different groups of vertebrates appear to have different mechanisms for initiating or interpreting this event, although it is by no means clear if these reflect genuine differences between taxa, experimental artefacts or different facets of the same process, or even if the early embryo is really bilaterally symmetric. Downstream of this event, experiments suggest Bmps play a role in several taxa, although how they do this and whether it reflects a conserved pathway component is unclear.(41–44,53,54) On the left side of all jawed vertebrates, a conserved pathway including *nodal*, *lefty* and *Pitx2* operates, with Nodal inducing itself (in an autoregulatory loop), *lefty* and *Pitx2*, while Lefty represses *nodal* in a negative feedback loop. A similar conserved pathway has not been identified on the right, though the best candidate is the zinc finger transcription factor gene *snail*, which is asymmetric in chick and mouse.(55)

Downstream of the symmetry-breaking event, numerous genes have been identified for which expression patterns or functional studies suggest a role in regulating asymmetry. Some appear specific to different groups of vertebrates; however, in all vertebrates studied, three genes have been found to be pivotal in the regulatory cascade that differentiates left from right: *nodal*, *lefty* and *Pitx2* (Fig. 1). *nodal* and *lefty* encode TGFβ superfamily members, while *Pitx2* encodes a paired-superclass homeobox transcription factor. All three are 'left' genes, in that they are preferentially expressed on the left side of vertebrate embryos. Together these three genes form a conserved signalling 'cassette' whose role in regulating left–right asymmetry at least predates the radiation of bony vertebrates.

Thus, all bony vertebrates have conserved asymmetric morphology together with a conserved molecular pathway regulating its development. While comparable molecular studies have not yet been undertaken in more basal vertebrates (cartilaginous fish and jawless fish such as lampreys; a report of a *Pitx* gene expressed in the lamprey(18) is inconclusive), we can infer from the similarity of early cardiac development in these taxa that the conserved molecular pathway is also present, suggesting it is a character of all living vertebrates. *Pitx* genes have also been identified in two Protostomes, *Caenorhabditis elegans* and *D. melanogaster*, however, neither shows asymmetric expression or an asymmetric phenotype when mutated.(19,20) Furthermore, orthologs of the vertebrate *nodal* genes do not seem to be present in the *C. elegans* or *D. melanogaster* genomes. This suggests that the role of *Pitx2* and *nodal* in vertebrate asymmetry is not shared with these protostome lineages. A key question, then, becomes when did this mechanism for regulating asymmetry evolve? Recent comparative studies of taxa of increasingly distant relations to the vertebrates are beginning to answer this question.

Asymmetry in other chordates: amphioxus and tunicates

Vertebrates are members of Phylum Chordata, a position that they share with two other taxa, the cephalochordates (often called amphioxus) and the urochordates (also know as tunicates). These three taxa are united by a number of distinctive characters, including the notochord, dorsal neural tube, endostyle and postanal tail. Within the chordates, most molecular and morphological analyses place amphioxus as the sister group to the vertebrates (Fig. 2).

Amphioxus is a broadcast spawner, with planktonic embryos that hatch at the early neurula stage and develop rapidly to form a feeding larva. The larva remains in the plankton for some weeks before metamorphosing and taking up the benthic lifestyle of the adult. The first described asymmetry in amphioxus development is the spiral swimming of hatched neurulae. Neurulae swim using cilia rather than muscles, and can repeatedly reverse the direction of spiralling, suggesting this asymmetry is behavioural rather than constrained by underlying asymmetric morphology.(21) The first observable morphological asymmetry occurs a little later in development when (in neurulae with 4 to 5 somites) the boundaries of the anterior somites shift out of register (Fig. 3A). This asymmetry is reflected by an asymmetric shift in the AP extent of gene expression patterns and nerve exits points in the neural tube.(22–24) As amphioxus embryos finish neurulation, they begin to undergo a series of morphological movements that generate a radically asymmetric larval head. Anterior to the somites and lateral to the notochord, paired endodermal head cavities known as Hatschek's Diverticula are formed. The left cavity then migrates posteriorly to form the pre-oral pit, a structure that following metamorphosis will become Hatschek's pit, the probable homolog of the pituitary (Fig. 3B). The right cavity expands concomitantly to fill the vacated space in the head. Subsequently the mouth opens on the left and the gill slits, which initiate on the ventral midline, spread up the right side (Fig. 3C,D).

Amphioxus larvae retain this radical head asymmetry until metamorphosis, when the mouth moves to a symmetric midline position (Fig. 3E). The existing right-sided gill slits now migrate to the left side, and new gill slits open on the right. Combined, these processes re-establish gross pharyngeal symmetry. However clear morphological asymmetries remain in the adult, including the positioning of Hatschek's pit and of a blind gut diverticulum (Fig. 3F,G).

The majority of investigations into urochordate development have focused on ascidians, a paraphyletic assemblage of taxa with sessile adults. Unlike the gross morphological asymmetry seen in amphioxus development, ascidian embryos and larvae show little sign of asymmetry. A dorsal view of the head of an ascidian tadpole larva shows asymmetric positioning of the two sensory pigment spots, the ocellus and otolith, and of the adjoining regions of the brain (Fig. 3H). Ascidian directional asymmetry becomes most apparent at metamorphosis (Fig. 3I). Specifically, the adult ascidian gut is asymmetrically folded in the body cavity. Interestingly larvaceans, a group of pelagic tunicates that achieve sexual maturity as a feeding tadpole, also have asymmetrically folded guts (Fig. 3J,K). Larvaceans are likely to be the most basal

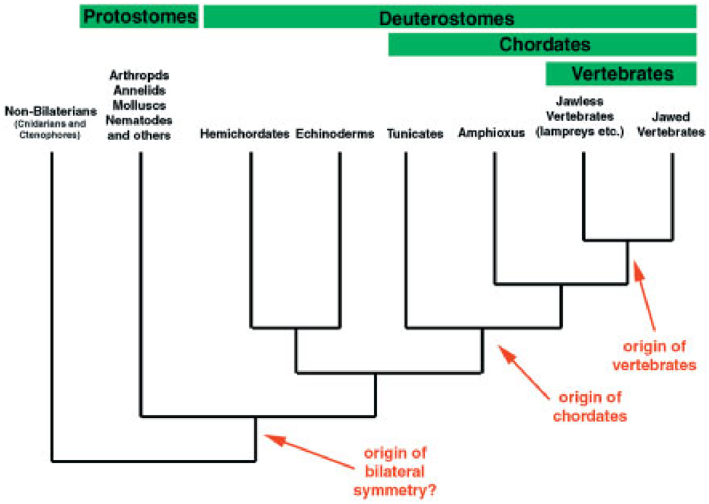

Figure 2. A simplified phylogeny of the animals, focused on the Deuterostome taxa. Bilaterians are subdivisible into two major clades, the Protostomes and the Deuterostomes. We can infer the origins of bilateral symmetry predate the separation of these lineages, an hypothesis supported by the likely positioning of the bilaterally symmetrical acoel flatworms (not shown) basal to Protostomes and Deuterostomes.(56) Basal to the Bilateria are the diploblastic Phyla including the Cnidaria (sea anemones, jellyfish and allies) and Ctenophores (comb jellies). These are often described as radially symmetric, though might be better thought of as biradial. Within the Deuterostomes, the hemichordates and echinoderms are sister Phyla. Vertebrates share Phylum Chordata with two other lineages, the amphioxus and the tunicates.

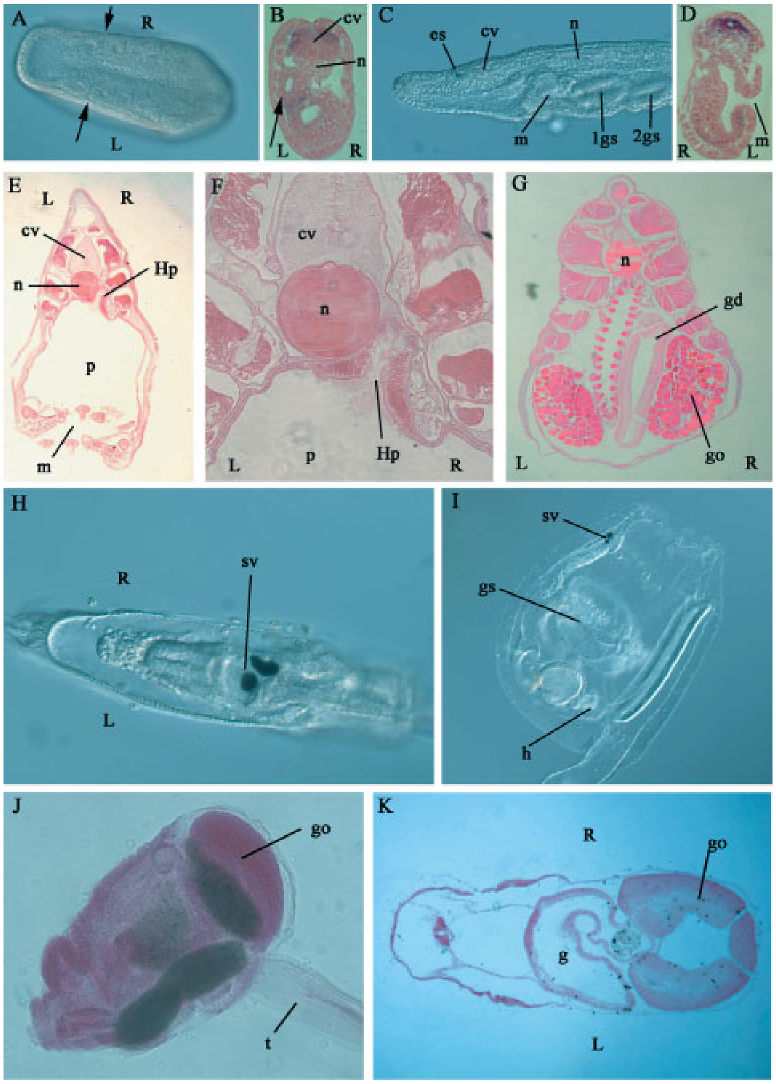

Figure 3. Morphological asymmetry in basal chordates. R and L indicate the right and left sides respectively. **A:** An amphioxus embryo with five somites in dorsal view. The arrows indicate the different relative positioning of somite boundaries on right and left sides. **B:** Transverse section through an older embryo, showing the asymmetric positioning of the left head cavity (arrow). n, notochord; cv, cerebral vesicle, the amphioxus 'brain'. **C:** Left lateral view of an amphioxus larva. Two gill slits (gs) are visible, and these open on the right side of the pharynx while the mouth (m) opens on the left. The eye spot (es) is also visible in the cerebral vesicle. **D:** Transverse section of a larva at the same stage as that shown in C, showing the left-sided opening of the mouth. **E:** Transverse section through the anterior pharynx (p) of an adult amphioxus, showing median positioning of the mouth, but asymmetric positioning of Hatschek's pit (hp), which is also shown magnified in **F**. **G:** Transverse section through the same individual at a more posterior location. Large gonads (go) lie on either side of the body, and an asymmetric gut diverticulum (gd) lies to one side of the pharynx. **H:** Dorsal view of the head of a *Ciona* larva, showing asymmetric positioning of the two pigmented sensory cells in the sensory vesicle (sv). **I:** Lateral view of a recently metamorphosed *Ciona* juvenile, showing asymmetrically folded gut and heart (h). **J:** Left lateral view of the head of the larvacean *Oikopleura*, a member of the most basal group of tunicates. The tail (t), which contains the notochord, is also visible. **K:** Section through the head of *Oikopleura*, showing a predominantly bilateral organisation with an asymmetric gut (g).

living tunicates,(25) suggesting gut morphology is a primitive asymmetry in this clade.

Conservation of asymmetry and its regulation in chordates

As described above, all three chordate Subphyla show clear directional asymmetry. The common site of asymmetry is the internal organs or viscera: the gut in tunicates, gut and pharynx in amphioxus and heart, gut and associated organs in vertebrates. A key question is: are these asymmetries homologous, that is did they evolve from a common ancestor with asymmetry, or are they independently derived? One test of this is to ask if asymmetry in these three taxa is regulated by a common developmental pathway. As outlined above, experiments with vertebrate model systems have suggested a high degree of conservation of a critical regulatory cassette controlling vertebrate left-right asymmetry (Fig. 1). The key components of this cassette are the signalling molecules Nodal and Lefty and the transcription factor Pitx2. Recently, researchers have begun to investigate whether the genes encoding these molecules might also be involved in regulating asymmetry in other chordates.

Molecular asymmetry in amphioxus

Molecular studies of amphioxus development have been underway for over a decade(26) and have yielded numerous insights into the early evolution of vertebrates. Many genes are expressed asymmetrically in the amphioxus head, including members of the Hedgehog, Pax, Nkx and Notch gene families.(27–30) These expression domains typically appear relatively late in development, and likely reflect rather than regulate asymmetric morphology. The vertebrate *Pitx2* gene is a member of a small group of closely related genes known as the *Pitx* family, itself a member of the paired-type homeobox gene superclass. Phylogenetic analysis of vertebrate *Pitx* genes suggests they fall into three well-defined clades, *Pitx1, Pitx2* and *Pitx3*, of which only the *Pitx2* clade has asymmetric expression and function. As well as asymmetric expression in lateral plate mesoderm, *Pitx2* is also expressed in oral ectodermal cells and in the developing pituitary, sites shared with *Pitx1* and inferred to be primitive for vertebrate Pitx genes.(18) *Pitx* genes have been identified in two amphioxus species, *Branchiostoma belcheri*(31) and *Branchiostoma floridae*,(32) and phylogenetics places these basal to the three clades of vertebrate genes, demonstrating that the gene duplications that formed the discrete vertebrate families were specific to the vertebrate lineage. *Pitx* genes in both amphioxus species are expressed in the pre-oral pit, the likely amphioxus homologue of the pituitary. Amphioxus *Pitx* genes are also asymmetrically expressed (Fig. 4) and, importantly, asymmetric expression is observed on the left of the embryo (like vertebrate *Pitx2*) and is earlier in development than the appearance of asymmetric morphology. Unlike vertebrates, where asymmetric *Pitx2* expression is confined to the mesoderm (except in zebrafish, where asymmetric expression is also seen in the brain, Refs, 33,34), asymmetric amphioxus *Pitx* expression is prominent in all three germ layers.

These data strongly suggest that the involvement of the *Pitx* gene family in the control of asymmetry predates the evolution of vertebrates. One potential criticism of this, however, relates to the unusual asymmetric development of amphioxus and, particularly, the left-sided location of the mouth. The oral opening is an ancestral site of Pitx expression in ectoderm, and possibly also in endoderm, of vertebrates.(18) It could thus be argued that the left-sided expression of Pitx in amphioxus reflects derived oral asymmetry, rather than a primitive role of Pitx in regulating asymmetry. Discriminating between these alternate hypotheses is possible in several ways, and two have been undertaken so far. Firstly, outgroup comparison in the form of the analysis of ascidian *Pitx* genes (see below) supports an ancestral role of *Pitx* genes in chordate asymmetry. Secondly, a *nodal* gene has also been found to show left sided expression in amphioxus (J.-K. Yu, L.Z. Holland and N.D. Holland, personal communication). *nodal* expression is not primitively associated with oral structures in vertebrates, so is not subject to the same criticism as *Pitx*. Therefore, in summary, mounting evidence supports the existence of a *nodal/Pitx* pathway regulating asymmetry in amphioxus.

Molecular asymmetries in ascidians

The characterisation of *Pitx* and *nodal* genes in amphioxus shows that the pathway regulating lateral asymmetry predates the origin of vertebrates. Recently, a *Pitx* gene has also been identified in the ascidian *Ciona intestinalis*.(32,35) *Ciona Pitx* is expressed symmetrically in the primordial pharynx (which includes the likely *Ciona* homologue of the pituitary) and brain and, importantly, asymmetrically in left head mesoderm and throughout the ectoderm of the left side, with the exception of dorsal and ventral midline cells (Fig. 4). Asymmetric expression is activated in tail-bud-stage embryos, which have a conspicuous notochord and neural tube, but lack observable morphological asymmetry. Therefore, as in amphioxus, asymmetric expression of *Pitx* in *Ciona* precedes asymmetric morphology. *nodal* genes have also been reported from ascidians, and early indications suggest asymmetric expression.(36,37) Furthermore, the *nodal* gene of the ascidian *Molgula oculata* has been shown to contain a FAST-responsive intronic enhancer element, the position of which is conserved with vertebrate *nodal* genes.(36) FAST (also known as FoxH) transduces Nodal signalling and this enhancer element is probably responsible for *nodal* self-regulation,(38) suggesting this is also conserved in ascidians.

It is also notable that, in the ascidian *Halocynthia roretzi*, the expression of Bmps (factors implicated in the control of asymmetry in several vertebrates) is localised to the dorsal and ventral ectodermal midlines,(39,40) and is mutually exclusive to that observed for *Ciona Pitx*.(32) Thus potential

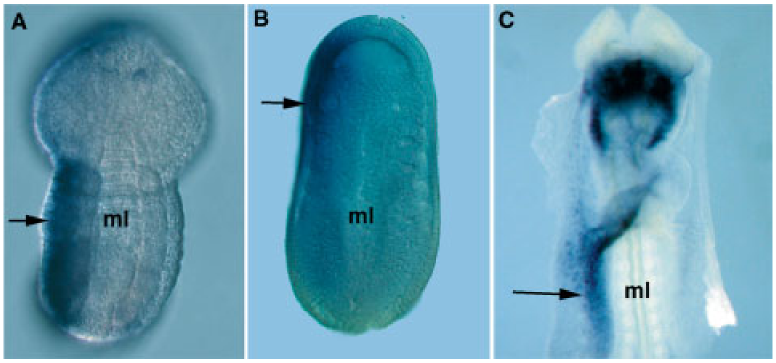

Figure 4. Asymmetric expression of *Pitx* genes in the chordates. **A:** Expression of *Ciona Pitx*; **B:** expression of amphioxus *Pitx* and **C:** expression of chick *pitx2*. All three taxa are in dorsal view with anterior to the top and, in all three, expression (arrowed) is obviously left-sided, as shown by comparison to midline tissues (ml) such as the notochord.

also exists for regulatory interactions between ascidian *nodal/Pitx* and Bmps, and therefore conservation of this part of the pathway. Full interpretation of these observations is complicated by the different ascidian species in which different genes have been analysed, and by conflicting reports on the role of Bmps in regulating vertebrate asymmetry.(41–44) Despite this, however, they confirm the presence and asymmetric expression of *nodal* and *Pitx* in ascidians, and therefore that these and possibly other facets of the pathway are ancient characters of chordates.

Reconstructing the primitive chordate: an asymmetric gut regulated by Nodal and Pitx?

In all three chordate subphyla, *Pitx* genes are asymmetrically expressed, and preliminary results suggest this is also the case for *nodal*. The extent of asymmetric *Pitx* expression varies considerably between these taxa. In vertebrates, it is confined to left lateral plate mesoderm, in amphioxus, it is found on the left in all three germ layers, while in *Ciona*, expression is found in the left mesoderm and throughout the left ectoderm. Functional analysis of gene activity has proven elusive in amphioxus and, although possible in *Ciona*, has yet to undertaken. However we can infer from the expression data that amphioxus and *Ciona Pitx* have a role in regulating asymmetric development, and therefore that regulation of asymmetry is a primitive function of chordate *Pitx* genes that in vertebrates has been maintained only by *Pitx2*. The data also suggest that, while the general left-sided character of *Pitx* genes has been maintained, there has been considerable divergence between these three Subphyla with respect to the precise localisation of expression. The consistent site of expression between all three taxa is mesodermal, suggesting this may be ancestral; however, several patterns of loss or gain in endoderm and ectoderm are possible, making the evolutionary history of asymmetric *Pitx* expression in these tissues hard to infer. The consistent site of gross morphological asymmetry is endodermal, in the orientation of the gut and associated organs. The parsimonious inference therefore is that the ancestral chordate had an asymmetric gut, regulated by a mesodermal pathway including *Pitx* and *nodal* genes.

Outside the chordates: asymmetry in other Deuterostomes and Protostomes

The chordates are members of a higher level taxon known as the Deuterostomata, a position that they share with two other living lineages, the hemichordates and the echinoderms (Fig. 2). Recent molecular phylogenetic analyses robustly place these two Phyla as sister groups and thus, in theory, both are equally well placed as outgroups for examining the evolutionary origins of chordates.(45–48)

Echinoderms develop a bilaterally symmetrical larva, the form of which varies considerably between the five different echinoderm Classes. However, bilateral symmetry is not retained throughout larval development: in sea urchin larvae, the pentaradial adult body plan is laid down in a structure called the adult rudiment, which lies within the larva. This adult rudiment develops on the left of the larva, and not the right, breaking larval bilateral symmetry. Embryological studies have suggested that the asymmetric positioning of the rudiment on the left is regulated by the right side of the echinopleuteus, and can be reversed by surgical manipulation;(49) however, the regulation of this process at the molecular level is unknown. The relationship of the axes of the adult echinoderm to the axes of other bilaterians has been long questioned, and it is even debatable whether the concepts of left and right can be applied to a radially symmetric body plan. Recent evidence from Hox gene expression, however, has gone some way to resolving this conundrum. In many Phyla, expression of Hox genes marks the AP axis. In sea urchins, nested Hox expression has been observed running through the larval somatocoels, mesodermal pockets, which in early larvae are organised in AP series in bilateral pairs that are separated by the gut. During metamorphosis they are incorporated into the adult coelomic system.(50) If we assume that this nested expression marks a primitive AP axis (and does not reflect a derived co-option of Hox patterning, for example as in vertebrate limb development) then we can infer the orientation of the left–right axis in the adult.(51) This interpretation implies a decidedly asymmetric adult left–right axis, although how the development of this is regulated is unknown.

Unlike echinoderms, adult hemichordates have definitive AP, DV and left–right axes.(52) Within this framework, the hemichordate gut can be viewed as coiled asymmetrically, but this shows no evidence of being a directional asymmetry as it varies between individuals. As such it should probably be considered as antisymmetric. Subtle directional asymmetry is, however, present in the form of the opening of the protocoel pore. This connects the protocoel (an anterior coelomic compartment) to the external environment, and is typically located to the left of the midline.

Thus all three Deuterostome Subphyla have directional asymmetry. Is echinoderm/ hemichordate asymmetry regulated by the same molecular pathway as chordate asymmetry? This is a question that we currently have too little data from echinoderms and hemichordates to answer. Neither Pitx nor Nodal family members have been described in echinoderms, and thus we can currently infer little about their role (or lack thereof) in regulating larval or adult asymmetry. Preliminary studies suggest a hemichordate *Pitx* gene is symmetrically expressed, indicating the chordate *Pitx/nodal* cassette might not be involved in asymmetry in this taxon (C. Lowe and J. Gerhart, personal communication). Confirmation of this, however, will require analysis of *Pitx* in echinoderms and of *nodal* in both Phyla. In particular, we need to know if *nodal* and

Pitx play a role in establishing the asymmetric location of the echinoderm adult rudiment or hemichordate protocoel pore. Such studies would allow us to date the origin of the *Pitx/nodal* cassette in asymmetry more accurately.

Conclusions

Regulation of asymmetric development in chordates involves the use of a conserved molecular pathway involving members of the *Pitx* and *nodal* gene families, both of which should be considered as 'left' genes throughout the chordates. Outside the chordates, other Deuterostomes also have directional asymmetries, but we currently know too little about how these develop to know if they are homologous to those in chordates. We should also consider these data in the context of the deeper phylogeny of the bilaterians, as such comparisons can also be informative. While asymmetry of different structures has probably evolved numerous times from a symmetric or antisymmetric state, and therefore at this level is not homologous, the frequent occurrence of such transitions suggests they may be reading off a more conserved molecular asymmetry. Coupled with the wide distribution of directional asymmetries amongst the Bilateria this suggests, by parsimony, that asymmetry of some form may be primitive for these taxa, and therefore at one level homologous. We should therefore separate this question of the similarities and differences between chordates, Deuterostomes and other Bilateria into two parts. Firstly, when did the role of *Pitx* and *nodal* evolve? The lack of involvement of *Pitx* in asymmetry in protostome taxa, and the failure to identify Protostome *nodal* orthologs, suggests these did not form part of a primitive mechanism for generating asymmetry in bilaterians (although the possibility that this represents a lost primitive character in these protostome lineages has not been ruled out). Consequently, we infer an origin of the *Pitx/nodal* cassette in the Deuterostomes, and possibly specifically on the chordate lineage, though this needs confirmation from studies in echinoderms and hemichordates. The second question concerns the possibility that similarities may exist between the mechanisms regulating asymmetry in Deuterostomes and Protostomes at a different level in the pathway. This also remains unknown; however, with the rapid progress of research into the mechanisms regulating asymmetry in diverse taxa, we will probably not have to wait long for answers.

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