A common plan for dorsoventral patterning in Bilateria

E. M. De Robertis & Yoshiki Sasai

Functional studies seem now to confirm, as first suggested by E. Geoffroy Saint-Hilaire in 1822, that there was an inversion of the dorsoventral axis during animal evolution. A conserved system of extracellular signals provides positional information for the allocation of embryonic cells to specific tissue types both in *Drosophila* and vertebrates; the ventral region of *Drosophila* is homologous to the dorsal side of the vertebrate. Developmental studies are now revealing some of the characteristics of the ancestral animal that gave rise to the arthropod and mammalian lineages, for which we propose the name *Urbilateria*.

One of the challenges in evolutionary biology is to ascertain to what extent animals have homologous structures that have been derived by descent from a common ancestor (as in the case of the pectoral fin of fishes, forelimbs of tetrapods, and wings of birds and bats) or whether any similarities are due to convergent evolution resulting from the need to perform similar functions (as in the case of wings of flies and birds). Fifty years before Darwin, French naturalist E. Geoffroy Saint-Hilaire proposed that the ventral side of the arthropods was homologous to the dorsal side of the vertebrates[1]. He dissected a lobster (Fig. 1), but instead of placing it in its usual orientation with respect to the ground, he placed it upside down. In this orientation the lobster's central nervous system (CNS) was located above the digestive tract, which in turn was located above the heart. In his own words (our translation): "What was my surprise, and I add, my admiration, in perceiving an ordering that placed under my eyes all the organic systems of this lobster in the order in which they are arranged in mammals?" This idea of a dorsoventral inversion between arthropods and mammals led to a dispute with Georges Cuvier[2] in the French Academy, and has been revived[3–6] and contested[3,7–9] several times. Here we re-examine the issue of homology versus convergence in the development of animal species from the perspective of recent results in molecular embryology.

Dorsoventral patterning by *chd* and *sog*

In vertebrates the formation of the dorsal mesoderm and of the CNS are induced by a region of the embryo called the organizer. *Xenopus chordin* (*chd*) is an organizer-specific secreted protein that can mimic the activity of the organizer, resulting in a twinned body axis[10]. A *Drosophila* complementary DNA of related structure (Fig. 2), was reported[11,12] and, interestingly, it encoded *short gastrulation* (*sog*), a gene long known to play an important role in *Drosophila* dorsoventral patterning[13,14]. As shown in Fig. 3, a number of zygotic genes are required for dorsoventral patterning of the *Drosophila* embryo. The regions in which these genes are expressed in the embryo are controlled by the activity of the maternal gene *dorsal*, which can repress or activate their transcription. Most are expressed in the dorsal 40% of the embryo, and are required to produce active *decapentaplegic* (*dpp*) gene product, which is a growth factor of the transforming growth factor-β (TGF-β) family. Dpp diffuses, generating a morphogen gradient that patterns the ectoderm[15,16]. Conversely, *sog* is expressed ventrally and its protein diffuses dorsally, where it antagonizes the activity of *dpp* (Fig. 3).

When the embryonic expression patterns of *chd* in the vertebrate[10] and *sog* in the fly[11] are compared, it can be observed that they are inverted with respect to each other (Fig. 4). *sog* is initially expressed in the ventral 60% of the blastoderm, and by the end of gastrulation it becomes localized to two rows of cells, called the mesectodermal cells, along the ventral midline. *chd* is expressed initially in the dorsal side of the blastopore (that is, in the organizer region), and by the end of gastrulation it is restricted to the dorsal midline (Fig. 4).

The case of *sog* and *chd* allows us to test whether ventral in the fly corresponds to dorsal in the vertebrate, because the gene products (in the form of messenger RNAs) have biological activity when injected into embryos. Injected *sog* mRNA ventralizes fly embryos, leading to formation of ventral denticle belts and ectopic patches of CNS[17]. In *Xenopus*, *sog* mRNA causes dorsal development (for example, formation of dorsal tissues such as notochord and CNS)[17]. The vertebrate homologue, *chd*, behaves functionally much in the same way as does *sog*: injection of *chd* mRNA, a dorsalizing factor in *Xenopus*[10], promotes ventralization of cell fates in *Drosophila*[17]. Thus, the function of *sog* and *chd* is reversed in insects and vertebrates; in both cases injection of the gene product promotes the development of the side of the embryo that contains the CNS.

dpp and *Bmp-4* antagonize *sog/chd*

In *Drosophila*, *dpp* is expressed dorsally and promotes dorsal development[15,16], that is, it is the opposite of *sog*. Microinjection of increasing amounts of *dpp* mRNA leads to threshold responses in the pattern of tissue differentiation in *Drosophila* ectoderm, shifting the balance in the dorsal direction until at high doses ventral development is prevented[15,16]. In the vertebrate, Bmp-4 (bone morphogenetic protein 4) and Bmp-2 share extensive sequence similarity with Dpp. In the *Xenopus* gastrula *Bmp-4* is expressed in the ventral and lateral marginal zone (which gives rise to ventral mesoderm) as well as in the animal cap (which gives rise to skin ectoderm when cultured in explants)[18]. In *Xenopus* embryos *Bmp-4* has potent activity, changing the fate of dorsal mesoderm into ventral cell fates (blood and mesenchyme)[19,20]. *Drosophila dpp* can mimic the ventralizing activity of *Bmp-4* in *Xenopus* and this activity can be antagonized by injected *sog* mRNA[17]. The gradient of *dpp* activity observed in *Drosophila* may result, at least in part, from the antagonism between the Sog and Dpp diffusible factors[21]. It is not yet known whether Sog antagonizes by binding directly to Dpp or its receptor, or whether it acts through its own receptor. A similar antagonism has been reported for *chd* and *Bmp-4* (ref. 22).

The suggestion that *Bmp-4* is the vertebrate homologue of *dpp* sparked the recent interest in the inversion of the dorsoventral axis[4]. The functional experiments discussed above indicate that dorsoventral patterning in both *Drosophila* and *Xenopus* is dependent upon a system of antagonistic extracellular signals provided by *dpp/Bmp-4* and *sog/chd*. Despite the morphological differences

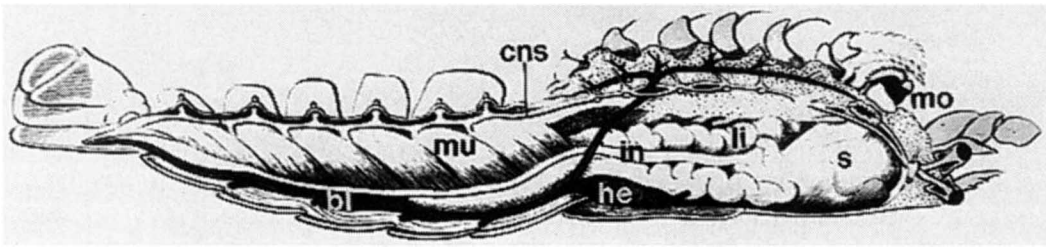

FIG. 1 Geoffroy Saint-Hilaire's famous lobster. In this dissection the animal is presented in the orientation opposite to that it would normally have with respect to the ground. The central nervous system (cns or nerve cord) is above, and is traversed by the mouth (mo). Underneath is the digestive tract, with the stomach (s), liver (li) and intestine (in). Below the gut are the heart (he) and main blood vessels (bl). Muscles (mu) flank the CNS. In this orientation the body plan of the arthropods resembles that of the vertebrate. From ref. 1, by courtesy of the History and Special Collection Division, Louise M. Darling Biomedical Library, UCLA.

between embryos of the two species, the *sog/chd* gene is expressed on the side from which the CNS arises while the *dpp/Bmp-4* gene is expressed on the opposite side of the embryo. The functional conservation of the *sog/chd* and the *dpp/Bmp-4* secreted proteins suggests a homologous mechanism of dorsoventral patterning that must have existed in the common ancestor from which insects and vertebrates diverged. The results support the view that a reversal of the dorsoventral axis occurred during the course of evolution.

The inversion challenged

The recent revival of Geoffroy Saint-Hilaire's inversion hypothesis[4] was opposed[7–9] and this merits some discussion in light of recent findings. Three main objections have been raised.

First, it has been argued that the use of marker genes, such as those of the *achaete-scute* complex, that are conserved in vertebrates and insects[4] is inappropriate because they mark the presence of similar tissues, in this case nerve cells, and not homologous topology[7,8]. This objection does not apply to the case of *sog/chd* and *dpp/Bmp-4* because these molecules are upstream regulators providing patterning information that determines tissue differentiation according to specific dorsoventral fates. The importance of identifying the upstream regulatory switches is highlighted by the recent discovery that eye development is controlled by a conserved gene, *Pax-6/eyeless*, in both vertebrates and *Drosophila*[23,24]. This is a good example because previously the formation of eyes in insects and mammals was one of the classic cases of convergent evolution, despite the presence of common molecular elements in the light-detection system, such as conserved opsin proteins[25]. Because eye development is controlled by the same genetic switch in flies and mice it is now considered that eyes are homologous structures related by descent from an ancestral photoreceptor[26].

Second, the homology between *dpp* and *Bmp-4* has been challenged on the basis of the similarities between *Bmp-4* and its close relative *Bmp-2* (refs 7, 8). Indeed, *Bmp-2* and *Bmp-4* are very similar in sequence, and both can functionally substitute for *dpp* in *Drosophila*[27]. However, recent loss-of-function studies

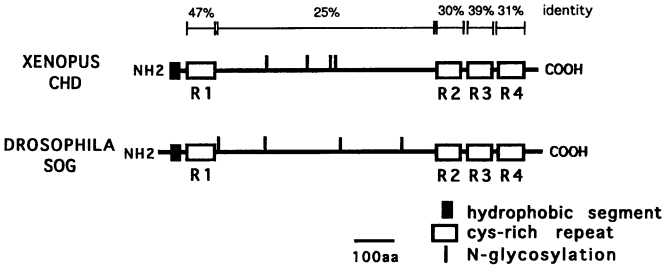

FIG. 2 The amino-acid sequences of *Xenopus chd* and *Drosophila sog* share similarities. Both proteins have a secretory signal sequence, or hydrophobic segment, at the amino (NH2) end (dark box), several putative *N*-glycosylation sites (vertical lines) and four cysteine-rich repeats. The spacing of the nine cysteines contained in the cys-rich repeats is conserved in other secreted proteins such as thrombospondin, von Willebrand factor and procollagen. The first repeat (R1) of chd is more similar to R1 of sog than to any of the other repeats in chd. The same is true for R4, indicating that both genes are derived from a common ancestor. The percentage of amino-acid identity between the two proteins is indicated. The amino-acid identity is only 28% along the entire protein, so it was especially important to show that sog and *chd* were functionally homologous. Because of its homology of *sog*, and for other reasons[49], we propose the designation *s-chordin* to refer to the vertebrate homologue (*chd*) in future.

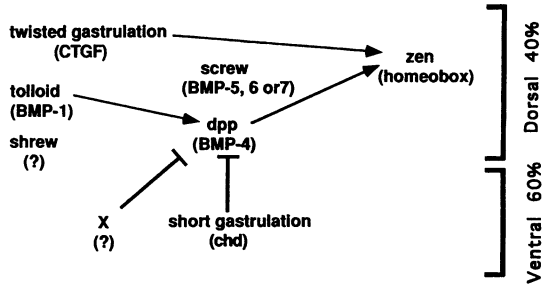

FIG. 3 Zygotic genes involved in dorsoventral patterning of the ectoderm in *Drosophila* embryos, and their vertebrate relatives. Several genes are expressed in the dorsal 40% of the embryo; *tolloid* encodes a metalloprotease required for *dpp* activity, and is similar in sequence to vertebrate *Bmp-1*; *twisted-gastrulation* encodes a protein related to vertebrate connective tissue growth factor; *screw* is a secreted growth factor related to either *Bmp-5*, *6* or *7* that might form heterodimers with *dpp*; *zen* encodes a homeobox gene for which a vertebrate counterpart has not been isolated. Injection of *short-gastrulation* (*sog*) product in wild-type *Drosophila* embryos leads to formation of ectopic ventral tissues and CNS, but in embryos mutant for *dorsal* it can only produce a partial ventralization[17]; this led to the proposal that a second ventralizing gene (indicated as 'X'), that cooperates with *sog* and is activated by *dorsal*, should also exist[17].

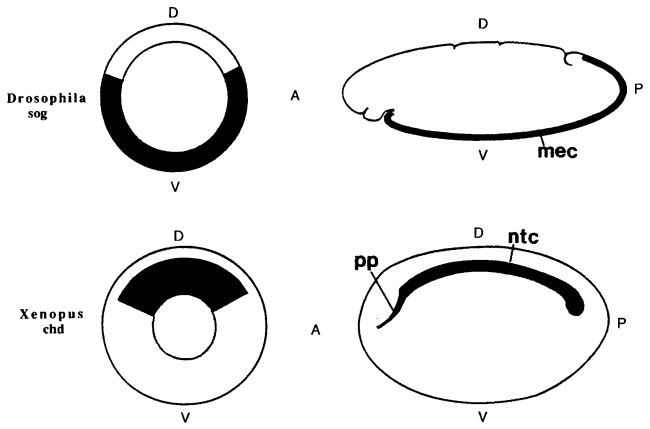

FIG. 4 The expression of *Drosophila sog* (top) and *Xenopus chd* (bottom) is reversed with respect to the dorsoventral axis. In *Drosophila sog* is first activated in the ventral 60% of the blastoderm, is then switched off in the mesoderm and persists in the neurogenic ectoderm, and by late gastrulation the expression resolves into two rows of cells located in the ventral midline, the mesectodermal cells (*mec*)[11]. In *Xenopus*, *chd* is expressed initially in the dorsal side of the blastopore, whereas by the end of gastrulation it is resolved to the mesoderm of the dorsal midline, occupying the head mesoderm or prechordal plate (pp) and the notochord (ntc)[10]. *sog* is ventral in *Drosophila* whereas *chd* is dorsal in *Xenopus*.

using antisense RNA in *Xenopus*[28] and gene disruption in mice[5,29] indicate that Bmp-4, but not Bmp-2, plays an essential role in early dorsoventral patterning of the vertebrates, supporting the view[4] that *Bmp-4*, rather than *Bmp-2*, is the homologue of *dpp* that functions in early embryos.

Third, it has been argued that the displacement of the CNS from ventral to dorsal could have resulted from the appearance of a novel region of ectoderm around the mouth region[7]. In this view, first proposed by Garstang, the region corresponding to the ventral midline of the ventral CNS would become displaced, forming the lateral edges of the dorsal neural plate in vertebrates[7]. Recent molecular findings strongly argue that this is not the case, because the midline of the CNS in *Drosophila* and in the vertebrate share a conserved signalling molecule called *netrin*. The vertebrate *netrin-1* gene encodes a secreted protein expressed in the midline of the CNS (the floor plate) that guides commissural axons to the opposite side of the developing spinal cord[30]. A *netrin* homologue is also expressed in the midline of the *Drosophila* CNS (C. Goodman, personal communication) and in the *Caenorhabditis elegans* ventral nerve cord[31]. Similarly, in the case of enteropneusts[8,9] and other vermiform animals with multiple nerve cords the region homologous to the CNS will ultimately have to be defined molecularly by the expression of *chd* and *netrin* homologues.

Thus, although arguments opposing the dorso-ventral inversion have been raised, the recent results from molecular biology tend to vindicate the hypothesis of Geoffroy Saint-Hilaire.

Common signals

An unexpected conclusion from recent studies on dorsoventral patterning is that the ectoderm and the mesoderm appear to be patterned by a common set of signalling molecules involving *dpp*/*Bmp-4* and *sog*/*chd*[22,32]. In *Drosophila*, there is strong genetic evidence that the amount of ectoderm allocated to the neurogenic region is controlled by positional information provided by this system. Both loss-of-function and gain-of-function studies show that *dpp* has an antineurogenic activity[15,16,21], promoting the formation of dorsal epidermis, whereas *sog* promotes neurogenesis in the ectoderm[11,17,21]. Dpp is widely recognized as the main morphogen patterning the ectoderm, but recent evidence indicates that *dpp* patterns the *Drosophila* mesoderm as well. The mesoderm expresses a Dpp receptor, *thickveins*[33], and Dpp produced by the ectoderm activates the expression of dorsal mesodermal genes (*bagpipe* and *tinman*) and represses the expression of a ventral mesodermal marker (pox meso)[34,35].

In *Xenopus*, CNS formation is thought to be different to *Drosophila*. The vertebrate neural plate is considered to be induced by signals secreted by the organizer. The organizer is also considered to be responsible for the release of signals that promote dorsal differentiation of mesoderm[36]. It was generally assumed that these signals would be different, but it now appears that the same molecules that pattern mesoderm also regulate CNS formation, as depicted in Fig. 5. Microinjection of *chd* (or *sog*) mRNA changes the fate of gastrula animal cap explants from ventral ectoderm (epidermis) to dorsal ectoderm (neural tissue)[22]. In ventral marginal zone explants (which form ventral mesoderm such as blood and mesenchyme) *chd* induces dorsal mesoderm (notochord and muscle)[10]. A similar situation has been observed for two other organizer-specific secreted factors, noggin and follistatin, which are neural inducers as well as dorsalizing agents[22,37–39]. This indicates that the signalling molecules used for dorsal differentiation of both ectoderm and mesoderm are the same, and that the differences must reside in the responding tissues (Fig. 5).

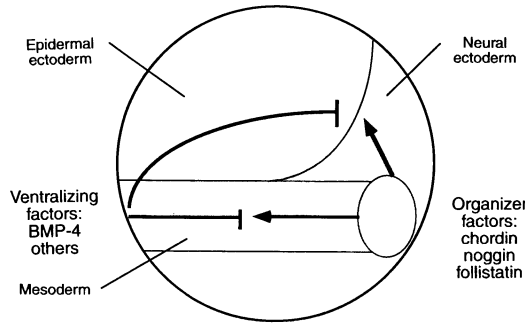

FIG. 5 Model indicating that the same set of regulatory signals may provide the positional information that patterns both ectoderm and mesoderm in *Xenopus*. On the dorsal side (right), the organizer (oval) provides dorsal positional values to ectodermal (animal cap, top) and mesodermal (marginal zone, middle) tissues by secreting organizer factors such as *chd*, *noggin* and *follistatin*. On the opposite side (left), ventralizing factors such as *Bmp-4* and presumably other signals give ventral positional values to the tissues, antagonizing organizer signals. High dorsal values promote neural differentiation in the ectoderm and formation of notochord and muscle in mesoderm, whereas high ventral values lead to epidermogenesis in ectoderm and the differentiation of blood island and mesenchyme in the mesoderm. In this model, a default status of tissues does not exist and the balance between the dorsal and ventral signals decides dorsoventral fates.

Studies on animal cap explants suggest that Bmp-4 has potent antineurogenic effects in *Xenopus*. Animal cap cells contain endogenous *Bmp-4* mRNA[18] which is required to prevent neural differentiation[22]. In addition, a recent experiment suggests that the neuralizing effect of cell dissociation on animal caps[40,41] may be due to the dilution of the Bmp-4 signal by diffusion into the culture medium. Indeed, when Bmp-4 was added to dissociated cells, they differentiated into epidermis (ventral ectoderm) and neural differentiation was prevented[32]. Overexpression of *Bmp-4* can suppress neural induction caused by *chd* and, intriguingly, also neuralization by *noggin* and *follistatin*[22]. The antagonism between *Bmp-4* and *chd* in neural induction is consistent with the roles of their homologues, *dpp* and *sog*, in *Drosophila* ectodermal patterning. It is not known whether *Drosophila* has homologues for *noggin* and *follistatin*, but this is an attractive area for future research because it has been proposed that additional factors should

cooperate with *sog* in *Drosophila* neurogenesis (see 'X' in Fig. 3)[17].

Although *Drosophila* neurogenesis and *Xenopus* neural induction have traditionally been considered to be different, the molecular mechanisms underlying the patterning of two distinct germ layers, ectoderm and mesoderm, seem to be regulated by the conserved *sog/chd* and *dpp/Bmp-4* system. The concept of dorsoventral patterning by positional information, which has been so useful in *Drosophila*, might also be applicable to vertebrates. In this view, the organizer would be the source of dorsal positional values which are counteracted by ventral values provided by Bmp-4 and presumably other ventralizing factors (Fig. 5). Thus, the unity of plan revealed by the dorsoventral axial reversal issue can be extended to detailed tissue patterns within individual germ layers.

The *Urbilateria* or common ancestor

In 1874 Ernst Haeckel proposed a sweeping homology: that the ectoderm and endoderm in all metazoans was related by descent from a hypothetical animal called the *Gastrea*[42]. The *Gastrea* consisted of two cell layers, the ectoderm and the endoderm, forming a primitive gut cavity opening to the outside. Although simplistic, the *Gastrea* theory historically was very useful because it proposed that all multicellular animals were monophyletic.

A large amount of data have now become available on genes controlling development from *Drosophila* and the vertebrates. The hypothetical ancestral animal, for which we propose the name *Urbilateria* (primitive bilateral animal), from which the arthropod and the chordate lineages diverged 600 million years ago may have presented the following characteristics. (1) It had anteroposterior polarity determined by the Hox gene complexes[43,44]. Additional genes present in *Drosophila* and vertebrates, for example homologues of *orthodenticle*, *empty spiracles* and *caudal* formed a network that cooperated with Hox genes in the generation of anteroposterior pattern[45]. (2) It had a conserved system of dorsoventral patterning provided by the antagonistic *sog/chd* and *dpp/Bmp-4* extracellular signals, as described above. (3) It presumably had a subepidermal longitudinal CNS that had well defined midline structures secreting axon guidance molecules such as netrin-1. (4) The function of *Pax-6/eyeless*[23,24], as well as the conservation of opsins[25], suggests that the common ancestor had primitive photoreceptor cells from which the eyes of *Drosophila* and vertebrates evolved. (5) It presumably also had a circulatory system with a contractile blood vessel. *Drosophila* has a contractile dorsal vessel whose differentiation is controlled the homeobox gene *tinman* as well as by the transcription factor DMEF2, both of which have vertebrate homologues expressed in the developing heart tissue[46]. In addition, other characteristics for which present evidence suggests independent evolutionary origins and convergent evolutionary solutions in insects and vertebrates, such as segmentation (metamerism) and the formation of appendages, may require revisions if new upstream regulatory systems are identified in future.

An important question is whether all deuterostomes evolved from the same ancestral animal. Evidence from 18S ribosomal RNA sequence comparisons is consistent with a common grouping for all deuterostomes[47]. Thus it is possible that the inversion event may have occurred concomitantly with a great innovation in gastrulation mechanisms that led to the two main groups of metazoans: the protostomes and the deuterostomes[4,48]. Most of the evidence on dorsoventral inversion of the axis reviewed here comes from comparisons of developmental genes in two very distantly related groups of organisms, the arthropods and the vertebrates. In future it will be useful to extend the comparisons of the *sog/chd* and *dpp/Bmp-4* signalling systems to other phyla in order to fill in the gaps, as has been so fruitful in the case of the Hox genes[44].

Note added in proof: Since this manuscript was first submitted, several papers have appeared that further substantiate the idea of a conserved ventral specification pathway in vertebrates. These include: (1) the expression patterns of *Bmp-4* and *Bmp-2* in *Xenopus*[50–52]; (2) additional support for the requirement of Bmp signalling to repress neutralization of animal cap explants[53–55]; (3) a role for Bmp-7, in collaboration with Bmp-4, as a ventralizing factor[55]; (4) evidence for an inhibitory interaction between the ventralizing factor *Bmp-7* and follistatin; and (5) further evidence for the dorsalizing activity of *sog* in *Xenopus* embryos[57]. □

E. M. De Robertis and Yoshiki Sasai are at the Howard Hughes Medical Institute, Department of Biological Chemistry, University of California, Los Angeles, California 90095-1737, USA.

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