Revolving vertebrates

An old idea about the relationship between arthropod and vertebrate body plans has been given new life by studies of the signalling genes controlling dorsal and ventral development in *Drosophila* and *Xenopus*.

In 1822, the early evolutionist Etienne Geoffroy St-Hilaire [1] suggested that arthropods and vertebrates share a common body plan, but that vertebrates turned upside-down during evolution so that ventral structures became dorsal and dorsal structures ventral (Fig. 1). Now, evidence supporting the idea of a dorsoventral inversion of body plan is coming from a comparison of developmental gene expression and function in the fruitfly *Drosophila* and the frog *Xenopus* [2].

In *Drosophila*, the development of dorsal structures is under the control of the gene *decapentaplegic* (*dpp*), which is expressed in dorsal cells of the embryo. The *dpp* product is a secreted protein of the transforming growth factor-β (TGF-β) family. In *Xenopus*, on the other hand, a gene closely related to *dpp* — the gene *BMP-4*, encoding the growth factor bone morphogenetic protein-4, also a member of the TGF-β family — is expressed ventrally and controls ventral development. Intriguing though this observation is, on its own it hardly provides definitive evidence for an inversion of the dorsoventral axis at the time of chordate origin. But supporting evidence is gradually accumulating to show that other genes involved in specifying the dorsoventral axes in *Drosophila* and *Xenopus* are also homologous and expressed in this mutually inverted fashion.

Genetic studies indicate that the action of *dpp* is antagonized by the gene *short gastrulation* (*sog*). A possible counterpart to *sog* in *Xenopus* is the gene *chordin*, which when overexpressed in ventral tissues causes them to develop as dorsal structures. The recent cloning of *sog* and *chordin* [3,4] now allows a comparison of their structure, function and pattern of expression. The *chordin* and *sog* genes do encode related proteins, suggesting that, by analogy with *Drosophila*, Chordin functions in *Xenopus* by inhibiting the action of BMP-4. Thus, not only do the *dpp* gene product (Dpp) and BMP-4 seem to have homologous roles in invertebrate dorsal development and vertebrate ventral development, but the genes that regulate their activities are also conserved.

The dorsoventral pattern of the *Drosophila* embryo is clearly revealed at the blastoderm stage. The dorsal-most cells, which go on to form amnioserosa, express the gene *rhomboid*, as do two lateral stripes of cells which eventually form the neurectoderm. Dorsal-most cells also express *zerknüllt* and *tolloid*, while *twist* and *snail* are expressed in the presumptive mesodermal cells on the ventral side of the embryo. This pattern of gene expression depends on the graded effects of Dpp, with high levels of Dpp activity specifying amnioserosa, intermediate levels specifying dorsal epidermis, and lower levels specifying the border between dorsal epidermis and neurogenic ectoderm (Fig. 2a) [5]. In embryos mutant for *dpp*, dorsal and dorsolateral regions of the embryo adopt the fates of ventral cells, with expression of *rhomboid* in the dorsal stripe greatly reduced or absent. The phenotype of embryos mutant for *sog* is the reciprocal of those mutant for *dpp*, in the sense that the dorsal region of the embryo is expanded. Thus, in *sog* mutants the dorsal domain of *rhomboid* expression is broadened, as is that of *zerknüllt* and *tolloid*.

One of the first clues to the function of *sog* came from the observation that increasing the number of copies of the *dpp* gene enhances the *sog* mutant phenotype, suggesting that the *sog* gene product (Sog) normally blocks Dpp activity in lateral and ventral cells. This, together with the fact that *dpp* is expressed uniformly in the dorsal 40–50 % of the *Drosophila* embryo, caused Ferguson and Anderson [6] to suggest that Sog is involved in creating a gradient of Dpp activity. Further evidence that Sog antagonizes Dpp comes from recent work showing that overexpression of *sog* is able to rescue *dpp* haplo-insufficiency [3]. Most important, however, Bier and colleagues [3] have now cloned *sog*. Expression analysis shows that *sog* transcripts are present in a broad stripe in lateral regions of the early embryo, ventral to *dpp*-expressing cells (Fig. 2b). This suggests that the effects of *sog* on *dpp* activity, and on dorsal cell fates, is non-autonomous, and that the gene product must act over a significant distance.

Back in *Xenopus*, De Robertis and colleagues [4] isolated the gene *chordin* during a search for potential targets of *goosecoid*, a homeobox-containing gene expressed in Spemann's organizer, the most dorsal region of the *Xenopus* embryo [7]. Transplantation of Spemann's organizer to the ventral side of a host embryo causes secondary axis formation, and injection of goosecoid messenger RNA

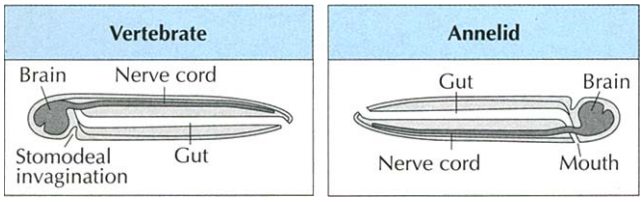

Fig. 1. The tissues within the invertebrate body plan, such as that of an annelid, are inverted with respect to those of a vertebrate. Adapted from [2].

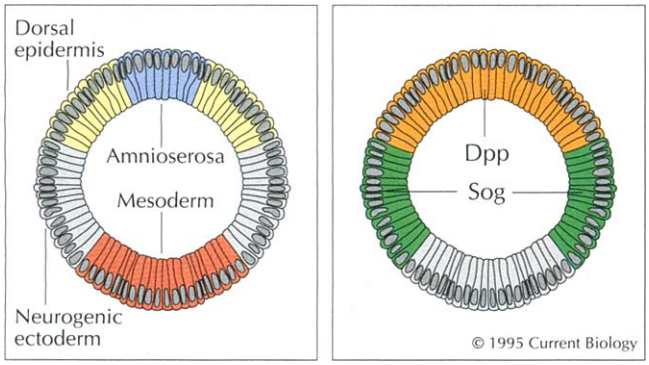

Fig. 2. Specification of cell types in the dorsoventral axis of *Drosophila*. **(a)** Fate map showing amnioserosa, dorsal epidermis, neurogenic ectoderm and mesoderm. Formation of the amnioserosa is specified by high levels of Dpp activity, the dorsal epidermis is specified by intermediate levels of Dpp, and the border between dorsal epidermis and neurogenic ectoderm is specified by low levels of Dpp. Based on [6]. **(b)** Expression domains of Dpp and the *short gastrulation* gene product, Sog. Sog is thought to act non-cell-autonomously to create a functional gradient of Dpp activity; this gradient specifies different cell types in the dorsoventral axis. In both (a) and (b), dorsal is to the top and ventral to the bottom.

has a similar effect [7]. This suggests that *goosecoid* has an important role in dorsoventral patterning in *Xenopus*. In an effort to understand how it functions, De Robertis's group conducted a differential cDNA screen for genes expressed in the same region of the embryo as *goosecoid*.

One gene isolated in this screen was *chordin*. Like *goosecoid*, *chordin* is expressed in Spemann's organizer, although transcription of *chordin* begins later than that of *goosecoid*. This observation suggests that expression of *chordin* could be dependent on *goosecoid*, and two additional observations support this possibility. First, overexpression of *goosecoid* in ventral and lateral cells of the *Xenopus* embryo causes ectopic activation of *chordin*. Second, expression of *goosecoid* is activated by the dorsal mesoderm-inducing factor activin in the absence of protein synthesis, but transcription of *chordin* is highly sensitive to protein synthesis inhibitors [4]. It is possible, therefore, that successful activation of *chordin* depends on translation of *goosecoid* mRNA.

The biological effects of *chordin* are similar to those of *goosecoid*. In particular, overexpression of *chordin* in ventral cells of the *Xenopus* embryo causes secondary axis formation, and *chordin* is also able to rescue normal development of the dorsoventral axis in embryos made radially ventral by ultraviolet irradiation of their vegetal hemispheres shortly after fertilization [4]. In these respects, the effects of *chordin* are also similar to those of other genes expressed in the organizer, such as *noggin* [8]. To see how *chordin* might be involved in the regulation of BMP-4 activity, however, it is illuminating to compare the effects of *chordin* with those of a truncated BMP-4 receptor [9]. It has been known for some time that overexpression of BMP-4 in the *Xenopus* embryo causes dramatic ventralization in a dose-dependent fashion [10,11], and that

Fig. 3. Comparison of the amino-acid sequences encoded by the *Drosophila* gene *sog* (top line) and the *Xenopus* gene *chordin* (bottom line). The proposed transmembrane domain of Sog, the signal peptide of Chordin, and the cysteine repeats of both Sog and Chordin are indicated. Redrawn from [16].

BMP-4 is expressed in ventral and lateral cells of the early gastrula [12] and is absent in dorsal cells that express *goosecoid* [7]. Most recently, work by Melton's [9] and Ueno's [13] groups has shown that inhibition of BMP signalling, by overexpression of a truncated BMP-2/4 receptor, causes dorsalization.

What does this have to do with *chordin*? BMP-4 and Dpp are thought to be homologous proteins, and indeed they can substitute for each other in *Drosophila* early development [14] and in vertebrate bone morphogenesis [15]. Furthermore, levels of BMP-4 are critical in *Xenopus* development [10], as are levels of Dpp in the fly. Might the *chordin* gene product act by antagonizing the action of BMP-4, in the way that Sog inhibits the function of Dpp? Evidence in favour of this idea comes from comparison of the structures of the two proteins encoded by these genes (Fig. 3).

The polypeptides encoded by the *chordin* and *sog* loci have 27 % amino acid identity over 941 amino acids. Both Chordin and Sog proteins are likely to be secreted, although the structure of Sog predicts that it is a type II membrane protein. The highest degree of sequence similarity between the proteins is present in four copies of a motif defined by the conserved spacing of 10 cysteine residues (the so-called CR repeats). Each protein has one CR repeat near its amino terminus and three more near its carboxy-terminal end (Fig. 3). The Chordin and Sog CR repeats are distantly related to similar motifs present in α-procollagen, von Willebrand factor, thrombospondin, laminin, and members of the CEF-10 family [16]. This is significant because collagen, and a region of thrombospondin containing the CR repeat, are known to bind TGF-β [3]. If CR repeats are indeed able to bind to, and sequester, TGF-β-like molecules, this might explain how Sog and Chordin could antagonize the actions of the TGF-β family members Dpp and BMP-4, respectively.

Taken together, these results suggest that there are remarkable similarities between the mechanisms that pattern dorsal structures in *Drosophila* and ventral structures in *Xenopus*. In *Drosophila*, Dpp specifies dorsal structures in a dose-dependent fashion, with functional levels of Dpp being controlled in a non-cell-autonomous fashion by secretion of the Sog protein (see Fig. 2). In *Xenopus*, BMP-4 could specify ventral pattern in a dose-dependent fashion, with Chordin, produced by Spemann's organizer, inhibiting BMP-4 function in dorsal and (to a lesser extent) lateral cells. If this assumption is correct, these conserved mechanisms would provide support for the idea that arthropods and vertebrates share a common body plan, with vertebrates having turned themselves upside-down during evolution. What is now needed is a biochemical study of whether Chordin and Sog interact with, and inhibit the functions of, BMP-4 and Dpp.

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