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On growth and form: a Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes

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Summary

The regulation of body axis specification in the common ancestor of bilaterians remains controversial. BMP signaling appears to be an ancient program for patterning the secondary, or dorsoventral, body axis, but any such program for the primary, or anteroposterior, body axis is debated. Recent work in invertebrates indicates that posterior Wnt/β-catenin signaling is such a mechanism and that it evolutionarily predates the cnidarian-bilaterian split. Here, I argue that a Cartesian coordinate system of positional information set up by gradients of perpendicular Wnt and BMP signaling is conserved in bilaterians, orchestrates body axis patterning and contributes to both the relative invariance and diversity of body forms.

Key words: Wnt, BMP, Morphogen, Planaria, Echinoderms, Amphioxus, Spemann organizer, Embryonic axis, Urbilateria, Size scaling, Evolution

Introduction

The evolution of animals, and specifically the relationships and lineages between extinct and extant species, is a central problem in biology (Fig. 1). Related to it is a key question of evolutionary developmental biology: the reconstruction of the morphogenetic regulatory programs of common ancestors using the molecular information gained from diverse model organisms. In animals, discerning the molecular regulation of the body axes and the extent to which this regulation is evolutionarily conserved is of fundamental importance to this endeavor, as body axes form the basis of animal body plans (Finnerty, 2003; Martindale, 2005; Gerhart, 2006; De Robertis, 2008; Meinhardt, 2008).

With 32 phyla that encompass over a million described species, it is estimated that more than 99% of all modern animals belong to the subkingdom Bilateria (see Glossary, Box 1) (Collins and Valentine, 2001). Although multiple independent origins for mesoderm, coeloms and throughguts (see Glossary, Box 1), which are distinguishing bilaterian features (Willmer, 1990; Nielsen, 2001), have been proposed, it is now commonly accepted that modern bilaterians are monophyletic (see Glossary, Box 1) (Adoutte et al., 2000) and share a common bilaterian ancestor, called Urbilateria (see Glossary, Box 1) (De Robertis and Sasai, 1996; Erwin and Davidson, 2002; Arendt et al., 2008; De Robertis, 2008). Adult bilaterians have two (imaginary) polar axes of symmetry perpendicular to each other. The primary axis [also referred to as the anteroposterior (AP), cephalo/craniocaudal, apicobasal and oral-aboral axis] extends from front to back (head to tail in chordates) is typically oriented longitudinally, parallel to the direction of movement, and has mouth and gut openings at each end. The secondary axis [also referred to as the dorsoventral (DV), mediolateral, directive and aboral-oral axis in echinoderms] extends from the back to the underside or belly and is perpendicular to the primary axis. Its hallmark in most bilaterians is the unilateral location of the central nervous system (CNS) (Benito-Gutierrez and Arendt, 2009). Although body axis regulation is mainly considered together with early embryonic development, it also occurs in adults: several bilaterians, such as certain annelids, flatworms, echinoderms and colonial ascidians, reproduce agametically (see Glossary, Box 1) and thus establish new body axes in fully grown organisms (Adiyodi et al., 2002). Furthermore, certain invertebrates, such as *Hydra*, earthworms, flatworms and sea squirts, can regenerate an adult body, emphasizing that body axis regulation is not restricted to embryogenesis and is central to understanding bilaterian body plan organization.

The utilization of various homologous developmental regulators in body axis formation, such as the Hox genes in primary axis formation (Butts et al., 2008) and the Chordin-BMP system along the secondary axis (Holley, 1995; De Robertis and Sasai, 1996), argues for a homology (see Glossary, Box 1) of animal body axes. The 'Rosetta Stone' for understanding the evolution of primary/AP axis formation is the Hox gene cluster, which is found in almost all bilaterians analyzed and the expression of which has provided key

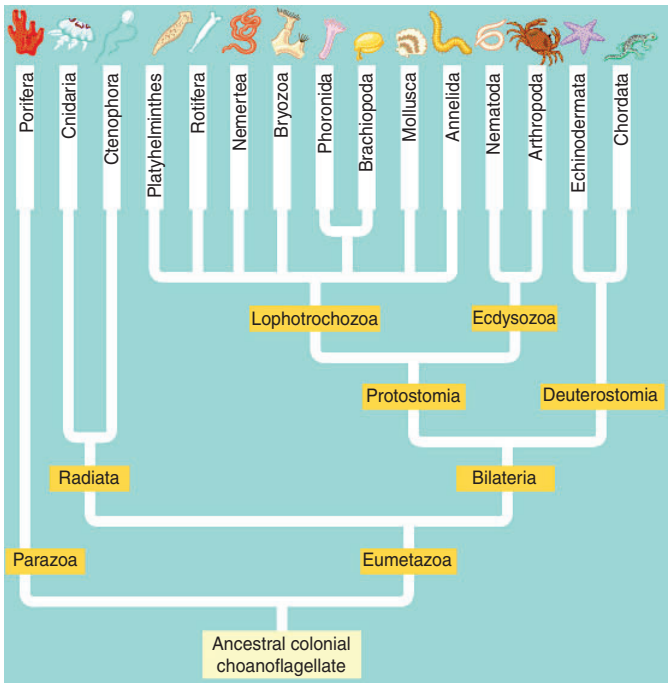

Fig. 1. Evolutionary tree of metazoans. This simplified tree shows lineage relationships between major metazoan clades and is based on DNA sequence phylogenetic relationships. Reproduced, with permission, from Campbell and Reece (Campbell and Reece, 2005).

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Box 1. Glossary

Agametic reproduction. Animal reproduction that does not involve germ cells, but instead occurs by fission or budding of adult individuals.
Bilateria. The clade comprising all major animal groups with bilateral symmetry.
Covariation analysis. A test examining whether under different experimental conditions, changes in two or more characters are correlated.
Coelom. A fluid-filled body cavity derived from the mesoderm during gastrulation that is a characteristic of a large group of animals called the coelomates, which includes most bilaterians, including all vertebrates. The presence of a coelom is strongly correlated with the presence of complex organs.
Derived feature. A morphological or other feature in organisms that has evolved relatively recently. By contrast, so-called primitive characters are more ancient.
Deuterostomes. The animal group in which the first embryonic opening, or blastopore, becomes the anus, such as in echinoderms and chordates.
Dipterans. An order of insects with only two wings, including, for example, all true flies and mosquitoes.
Eumetazoa. The clade comprising all major animal groups, except those animals (e.g. sponges and placozoa) without true tissues organized into germ layers.
Homology. In biology, the term refers to the similarity of a morphological (or other) character of different species based upon their common descent.
Hydranth. An individual polyp of a hydroid colony.
Hydroid. Marine plant-shaped colonial animals closely related to jellyfish and *Hydra*.
Morphogenetic field. A tissue with the ability to repattern and regenerate (see Regulation, below). Cells in a morphogenetic field, e.g. in a sea urchin embryo or a planarian, can regenerate removed parts, and isolated pieces of tissue belonging to a morphogenetic field can form a small, albeit fully patterned, whole organism in culture.
Monophyletic. A term used in cladistics to denote a group of organisms that consists of an ancestor and all its descendants and that therefore share common features, such as the mammals.
Protostomes. The animal group in which the first embryonic opening, or blastopore, becomes the mouth, such as in arthropods and molluscs.
Regulation. The ability of an embryo to develop normally even after injury to, the removal of, or the experimental alteration of a structure.
Ploidy. The number of complete sets of chromosomes in a cell. Ploidy is counted by the number of homologous sets of chromosomes (haploid, n=1; diploid, n=2; tetraploid, n=4).
Throughgut. A gut with a separate entrance and exit (mouth and anus), as opposed to a single opening (bottle- or blind gut) as found, for example, in jellyfish and flatworms.
Urbilateria. The hypothetical last common ancestor of animals with bilateral symmetry. The term was coined by De Robertis and Sasai (De Robertis and Sasai, 1996), who, noticing the remarkable similarities in DV axis formation between *Drosophila* and *Xenopus*, revived the idea of Étienne Geoffroy Saint-Hilaire (1772-1844) that these distant groups of animals share a common body plan. They proposed that this common body plan and the underlying molecular regulatory systems were present in the common ancestor of arthropods and chordates and, hence, of bilaterians.

evidence for the homology of AP patterning in deuterostomes and protostomes (see Glossary, Box 1). The *Hox3* cluster, which regulates cell fate along the body column, is thought to have existed in Urbilateria and to have gradually broken up in some lineages (Butts et al., 2008). Another ancient feature in primary axis formation is the use of posterior Wnt genes. Following the discovery of a Wnt gradient that patterns the AP CNS in frogs, it was proposed that patterning by Wnt gradients has been conserved throughout primary axis evolution (Kiecker and Niehrs, 2001; Niehrs, 2004).

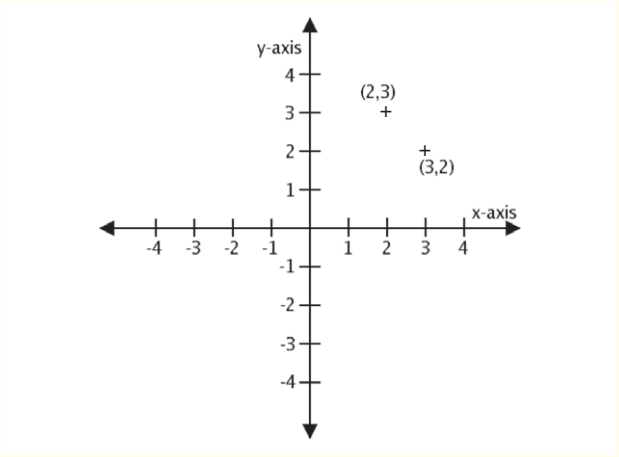
The main argument that I advance here is that the morphogenetic regulatory programs in Urbilateria included perpendicular BMP and Wnt signaling gradients, and that these specify a spatial coordinate system in the body. As discussed below, such a Cartesian coordinate system (see Box 2) could account for certain patterns of growth and body form observed during evolution. This argument is based on the recent discovery that posterior Wnt signaling has a role in body axis formation in a number of invertebrates, notably in cnidarians (Hobmayer et al., 2000; Broun et al., 2005; Kusserow et al., 2005) and planaria (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). This discovery advances our understanding of the evolution of axis formation by indicating that posteriorizing Wnt gradients are not a novelty restricted to chordates or secondarily co-opted in various organisms (Martindale and Hejnol, 2009), but that they probably represent an ancient feature.

Positional information, gradients and axis formation

Before gastrulation, when the primary and secondary axes are established in most bilaterians, the morphological pattern along the body axes varies in a gradual, continuous fashion. For example, during the embryogenesis of many species, features such as yolk content, pigmentation, cell density, cell proliferation, rate of development and metabolic activity tend to vary gradually along the body axes. Similarly, if maternal RNAs or proteins are asymmetrically localized in oocytes, they typically form gradients, for example in *Xenopus* (Smith, 1986; Mariottini et al., 1993). Where cytoplasmic determinants, such as *bicoid* and *nanos* RNAs in *Drosophila* eggs, are specifically localized at the ends of the primary axis, the corresponding proteins diffuse and form gradients (Smith, 1986; Gavis and Lehmann, 1994). Only later in development are continuously changing traits replaced by discrete features, such as differentiated cell types and organs.

Importantly, graded regenerative capacity and reactions to experimental perturbations strongly suggest, as noted over a century ago, that gradients form the basis of axial development. For example, the pioneering embryologist and geneticist Thomas Morgan carried out experiments in hydroids (see Glossary, Box 1), which can regenerate a complete hydranth (see Glossary, Box 1) when ligated at any axial level. The further orally the ligation is placed, the quicker the regeneration (Morgan, 1906). Similarly, the Swedish zoologist John Runnström found that lithium chloride, which is now known to interfere with Wnt signaling by inhibiting Gsk3, a negative regulator of β-catenin, can vegetalize sea urchin embryos in a dose-dependent manner along the animal-vegetal axis (Runnström, 1928). Thus, many pioneering experimentalists, including Boveri, Morgan, Child, Runnström, Dalcq, Huxley and De Beer, proposed that gradients regulate axis development (Gilbert, 1996). Charles M. Child suggested that a two-dimensional (2D) developmental field, like the bilaterian body axes, could be organized by a Cartesian coordinate system of two perpendicular gradients, which would constitute the vectors that regulate the boundaries and the orderly relations in the field (Child, 1941). As elaborated by Wolpert, cells within such a gradient coordinate system are endowed with positional information (Wolpert, 1969). The ability of embryonic cells within the field to differentiate into specific cell types and to give rise to discrete structures depends on both the positional information and the developmental history (or competence) of the cell that is interpreting the gradient.

Box 2. Cartesian coordinate systems



A two-dimensional (2D) Cartesian coordinate system, as introduced by René Descartes (1596-1650). Cartesian coordinate systems are based on orthogonal coordinates that specify each point in a plane or volume by 2D numerical coordinates. The coordinate values correspond to the distances from two fixed perpendicular lines or axes (x, y). Two points with their x, y coordinates are shown. D'Arcy Thompson projected morphologies of biological specimens into Cartesian coordinate systems and applied simple Euclidian transformations (e.g. $y'=y$; $x'=x^2$) to landmark points, which distorted the specimens' morphology, such that they resembled the morphologies of other species (see Fig. 8).

The 2D gradient field was a formally attractive concept that could explain gradual feature changes in developing bilaterians, but for decades corresponding molecular mechanisms remained elusive. The discovery of perpendicular gradients of the Bicoid homeodomain protein, which determines the AP axis, and of the nuclear localization gradient of the transcription factor Dorsal (a NF-κB protein) along the DV axis in *Drosophila* embryos, were crucial for the concept of gradients as body axis determinants being accepted (Driever and Nüsslein-Volhard, 1988; Steward et al., 1988). However, neither of these gradients is evolutionarily conserved. Bicoid does not exist outside insects, and its protein gradient appears to be a specific adaptation for the evolutionarily derived (see Glossary, Box 1) syncytium stage of *Drosophila* embryos, which allows diffusion through the embryo (Davis and Patel, 2002). Similarly, except for insects, NF-κB does not appear to be important for DV axis formation. So, which additional gradients provide positional information in bilaterians, and are they evolutionarily conserved?

Perpendicular Wnt and BMP signaling gradients determine body axes in chordates

In chordates, primary/AP and secondary/DV body axis formation is intimately linked to a small group of cells in the gastrula stage embryo that is pivotal for early body plan establishment: the Spemann organizer (De Robertis, 2008). In amphibian embryos, the organizer corresponds to the upper dorsal blastopore lip. When the dorsal lip is grafted to the ventral side of a host gastrula, it induces a twinned embryo, i.e. a secondary body column with AP and DV axes (Fig. 2). The transplanted cells only form a small part of the conjoined twin. Most cells are recruited from the host under the inductive influence of the dorsal lip and change their fate to produce a secondary embryo. The organizer establishes the AP and DV axes in all three germ layers (ectoderm, mesoderm and endoderm). The most prominent feature of the AP axis is the patterning of the CNS, which, in chordates, is placed dorsally but is patterned along the AP axis into forebrain, midbrain, hindbrain and spinal cord. The DV mesodermal axis is patterned to form notochord, somite, pronephric and coelomic mesoderm. Tissues that correspond to the Spemann organizer have been identified in chicken and mice (in which they are known as Hensen's node) and in fish (in which they are called the shield).

In the premolecular era, Saxén and Toivonen dissected embryonic induction in amphibia and identified two inducers that act in a graded and perpendicular fashion. A neuralizing gradient was highest dorsally in the embryo, evenly distributed along the AP axis, and lowest ventrally. A perpendicular mesodermalizing gradient was highest posteriorly and diminished anteriorly (Saxén and Toivonen, 1961; Toivonen and Saxén, 1968). These findings inspired subsequent molecular studies, which revealed that TGFβ and Wnt family growth factors, as well as their antagonists, are at the heart of a 2D coordinate system of positional information that functions during axial patterning (reviewed by Niehrs, 2004; Meinhardt, 2006; De Robertis, 2008) (Fig. 3A). This is particularly well understood in *Xenopus*, in which the TGFβ family member Nodal is crucial in initiating this process by inducing both Wnt and BMP growth factors, as well as their antagonists. BMP4 is predominately ventrally expressed in the gastrula and neurula and acts as a morphogen, patterning the DV mesoderm and neuroectoderm in a concentration-dependent manner (Table 1). The Spemann organizer and its derivatives secrete BMP antagonists, such as Chordin, Noggin and Follistatin, which are expressed in the dorsal midline. A BMP4 activity gradient is established through the diffusion of BMP antagonists and the action of the Chordin protease Tolloid, which regulate gradient polarity and shape by establishing and maintaining a dorsal BMP sink (De Robertis and Kuroda, 2004).

Perpendicular to the *Xenopus* DV BMP gradient is an AP Wnt/β-catenin gradient (Fig. 4), which patterns the CNS during gastrulation (Kiecker and Niehrs, 2001) and might also pattern mesoderm (Schneider and Mercola, 2001; In der Rieden et al., 2009) and endoderm (McLin et al., 2007). Multiple Wnts are expressed in the gastrulating embryo and probably cooperate in establishing the gradient. The Wnt signaling gradient is high posteriorly and low anteriorly owing to the anterior expression of Wnt antagonists, including Dkk1, Cerberus and sFRP/Frzb.

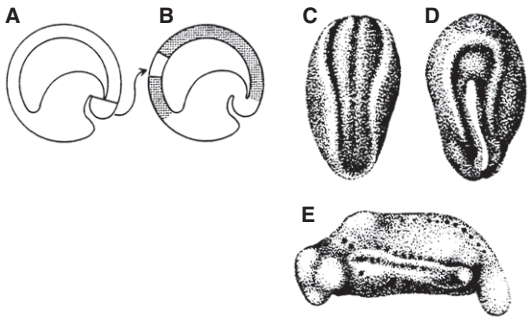


Fig. 2. The Spemann organizer experiment. (**A**,**B**) Transplantation of the upper blastopore lip of a gastrula of *Triturus cristatus* (A) to the ventral side of a gastrula of *T. taeniatus* (B). (**C**) Neural plate of host embryo. (**D**) Secondary induced neural plate. Note the white donor cells in the midline. (**E**) Secondary embryo on the flank of the host embryo. From Spemann and Mangold (Spemann and Mangold, 1924).

Table 1. Wnt/β-catenin and BMP signaling in anteroposterior (AP) and dorsoventral (DV) axis formation

	Wnt/β-catenin in AP patterning	References	BMP in DV patterning	References
Deuterostomes				
Amphimedon (Porifera)	Wnt expressed at the posterior pole of larvae	Adamska et al., 2007		
Hydra (Cnidaria)	Regulates regenerating adult body column	Hobmayer et al., 2000; Broun et al., 2005; Guder et al., 2006b		
Nematostella (Cnidaria)	Regulates oral-aboral patterning in embryos and planula larva	Kusserow et al., 2005; Wikramanayake et al., 2003	BMP expressed asymmetrically in the gastrulating embryo	Finnerty et al., 2004; Hayward et al., 2002; Saina et al., 2009
Clytia (Cnidaria)	Regulates oral-aboral patterning in embryos and planula larva	Momose et al., 2008; Momose and Houliston, 2007		
Sea urchin (*Lytechinus* and *Paracentrotus*; echinoderm)	Regulates animal-vegetal embryonic patterning	Logan et al., 1999; Wikramanayake et al., 1998	Regulates oral (ventral)-aboral (dorsal) axis	Duboc et al., 2004; Lapraz et al., 2009
Ciona (ascidian)	Regulates endodermal (vegetal) cell fate	Imai et al., 2000	Regulates DV neural tube patterning	Darras and Nishida, 2001
Saccoglossus (hemichordate)			Regulates DV axis	Lowe et al., 2006
Amphioxus (cephalochordate)	Posterior Wnt, anterior Wnt-antagonist expression; LiCl posteriorizes embryos	Holland et al., 2005; Yu et al., 2007; Onai et al., 2009	Regulates DV axis	Yu et al., 2007
Zebrafish (vertebrate)	Regulates AP CNS and mesodermal patterning	Agathon et al., 2003; Erter et al., 2001; Kim et al., 2000; Kim et al., 2002; Lekven et al., 2001	Gradient regulates DV mesodermal and ectodermal patterning	Neave et al., 1997; Nguyen et al., 1998
Xenopus (vertebrate)	Gradient regulates early AP CNS patterning	Kiecker and Niehrs, 2001	Gradient regulates DV mesodermal and ectodermal patterning	Dosch et al., 1997; Jones and Smith, 1998; Knecht and Harland, 1997; Tribulo et al., 2003
Chicken (vertebrate)	Regulates CNS patterning and anterior mesoderm (heart) specification	Marvin et al., 2001; Nordstrom et al., 2002	Regulates DV mesodermal patterning	Joubin and Stern, 1999; Streit et al., 1998
Mouse (vertebrate)	Regulates AP axis before and during gastrulation; evidence for gradient from allelic series in LRP6 and Wnt mutants	Huelsken et al., 2000; Kelly et al., 2004; Liu et al., 1999; Yamaguchi et al., 1999	BMP antagonists expressed in node	Klingensmith et al., 1999
Protostomes				
Schmidtea (planaria)	Requirement for β-catenin in the AP axis of intact and regenerating animals	Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008	Dorsal BMP signaling specifies DV adult body pattern	Molina et al., 2007
Cerebratulus (nemertean worm)	Requirement for β-catenin in animal-vegetal embryonic patterning	Henry et al., 2008		
C. elegans (nematode)	Specifies global embryonic AP cell fates	Kaletta et al., 1997; Lin et al., 1998		
Platynereis (annelid)	β-catenin asymmetries regulate global animal-vegetal development	Schneider and Bowerman, 2007		
Loligo (mollusc)	LiCl affects animal vegetal axis	Crawford, 2003		
Drosophila (arthropod)			Regulates embryonic DV patterning	Holley et al., 1995
Achaearanea (arthropod)	Required for posterior development	McGregor et al., 2008	Regulates embryonic DV patterning	Akiyama-Oda and Oda, 2006
Gryllus (arthropod)	β-catenin regulates embryonic AP patterning	Miyawaki et al., 2004		

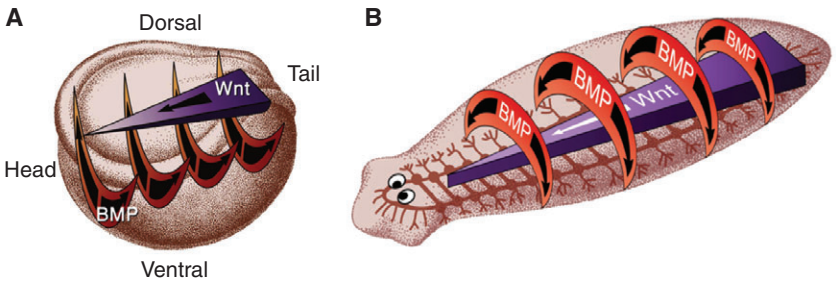


Fig. 3. Evolutionary conservation of double-gradient axial patterning in Bilateria. (**A**) Double-gradient model of embryonic axis formation in vertebrates. The model shows how perpendicular activity gradients of Wnts and Bone morphogenetic proteins (BMPs) regulate head-to-tail and dorsal-ventral patterning in an amphibian embryo, shown at the neurula stage. Adapted, with permission, from Kiecker and Niehrs (Kiecker and Niehrs, 2001). (**B**) Double-gradient model of axis formation in protostomes. The model shows how perpendicular signaling gradients of Wnts and BMPs regulate AP and DV patterning in a planarian (see also Fig. 5).

The expression of Wnts, BMPs and their antagonists, as well as their respective roles in AP and DV axis formation, are conserved in other vertebrates, including zebrafish, chicken and mice (Table 1), as is their dose-dependent mode of action. For example, a BMP gradient also patterns the DV axis in the zebrafish CNS and mesoderm (Neave et al., 1997; Nguyen et al., 1998), whereas a Wnt gradient functions in chicken and mice during embryonic CNS and mesoderm AP patterning (Aulehla et al., 2003; Nagano et al., 2006; Nordstrom et al., 2002). Studying the cephalochordate amphioxus, a basal chordate, can provide insights into the origin of vertebrate developmental mechanisms, and the analysis of amphioxus axial patterning has shown that perpendicular BMP and Wnt signaling gradients along the DV and AP axes probably reflect the ancestral state of chordate axial patterning (Yu et al., 2007). For example, amphioxus embryos are ventralized in a dose-dependent manner by recombinant BMP4, consistent with a role in DV patterning, and, as in vertebrates, Wnt antagonists are expressed predominately anteriorly.

The analysis of Wnt and BMP signaling in the axial patterning of the gastrula is hampered by the fact that these growth factors also function before gastrulation in processes that are required indirectly for subsequent AP and DV patterning. For example, maternal Wnt/β-catenin signaling specifies the organizer in *Xenopus*, chicken and zebrafish (Heasman et al., 1994; Roeser et al., 1999; Kelly et al., 2000; Oelgeschläger et al., 2003), whereas BMP signaling is required in mouse extraembryonic tissues for gastrulation and mesoderm formation (Kishigami and Mishina, 2005). Furthermore, roles for Wnt signaling are conserved in the regulation of the gastrulation process itself (Caneparo et al., 2007; Tahinci et al., 2007) and during specific cell and organ differentiation events (Nejak-Bowen and Monga, 2008; Thomas and Erickson, 2008). Thus, it is difficult to untangle the roles of Wnt and BMP signaling in axis specification from those in other processes. Furthermore, axis formation can extend well into organogenesis, and Wnts and BMPs also continue to act during later stages; for example, *Xenopus* and zebrafish tail formation requires BMP signaling (Gont et al., 1993; Agathon et al., 2003). Similarly, Wnt signaling continues to regulate CNS AP patterning even after the broad subdivision of the CNS (Houart et al., 2002).

Wnt signaling specifies the primary axis

The involvement of Wnt and BMP signaling in axis determination is not limited to chordates, but extends to invertebrates. Wnt- and BMP-related genes are evolutionarily highly conserved and are found in all animals analyzed to date (Nichols et al., 2006; Adamska et al., 2007; Lapebie et al., 2009), but not in choanoflagellates, the closest known relatives of animals (King et al., 2008).

The use of posterior Wnt signaling and anterior Wnt inhibition during invertebrate primary axis formation is now well documented in most major clades (Table 1) and has been reviewed elsewhere (see Guder et al., 2006a; Lee et al., 2006; De Robertis, 2008; Petersen and Reddien, 2009). In sea urchin and nemertean worm embryos, Wnt signaling specifies the animal-vegetal axis during gastrulation, and evidence in support of graded Wnt signaling (Table 1) has been obtained from dose-response experiments with lithium chloride. For example, the extent of vegetalization in sea urchin embryos and their final morphology vary with the concentration of lithium used, which led Runnström to propose one of the first classic gradient models (Runnström, 1928).

A striking, recently discovered example of Wnt signaling in axis specification is planarian regeneration, in which β-catenin regulates head versus tail identity (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). Freshwater planaria regenerate a head or tail when amputated, depending on the position of the cut. However, when β-catenin is depleted by RNA interference (RNAi), a head is generated instead of a tail at posterior amputations (Fig. 5A). Conversely, RNAi-mediated knockdown of the β-catenin antagonist adenomatous polyposis coli causes the regeneration of a tail at anterior wounds. β-catenin silencing is also sufficient to transform the tail into a head in uncut adult animals. Consistent with this, Wnts and Wnt antagonists are expressed along the AP axis.

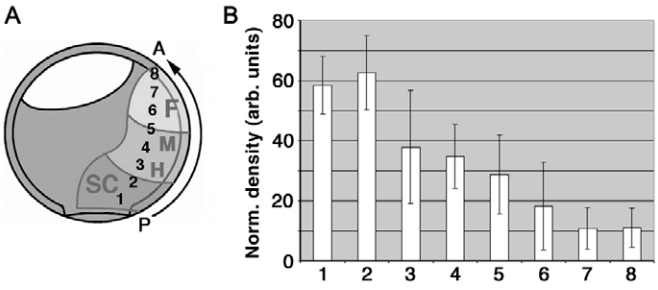


Fig. 4. An AP gradient of nuclear β-catenin in the presumptive neural plate. (**A**) Mid-gastrula *Xenopus* embryos were cut sagittally and analyzed by immunostaining for β-catenin. Eight consecutive regions of the presumptive neuroectoderm of immunostained halves were selected from posterior-to-anterior positions. The AP axis is indicated (P→A). (**B**) Mean normalized nuclear β-catenin labeling densities of all cells within the eight regions. Reproduced, with permission, from Kiecker and Niehrs (Kiecker and Niehrs, 2001). F, presumptive forebrain; M, presumptive midbrain; H, presumptive hindbrain; SC, presumptive spinal cord.

Taken together, this is compelling evidence for Wnt/β-catenin signaling specifying the AP axis throughout the bilaterian subkingdom (Fig. 3).

In the radially symmetric cnidarian *Hydra*, Wnts are expressed at the oral terminus, the hypostome, whereas a single *Dkk1/2/4* ortholog encoding a Wnt antagonist is oppositely expressed in the body column. Overactivating canonical Wnt signaling in *Hydra* induces ectopic tentacles and hypostomes, rescues regeneration-deficient mutants and suppresses *Dkk1/2/4* expression, indicating that the Wnt-Dkk axis represents an ancestral axial patterning system (Hobmayer et al., 2000; Broun et al., 2005; Kusserow et al., 2005; Guder et al., 2006b; Lengfeld et al., 2009; Philipp et al., 2009). Recently, it has been suggested that in cnidarians, Wnts became involved in AP patterning only secondarily, based on the proposition that the oral-aboral axis was inverted in relation to the embryonic animal-vegetal axis during evolution (Martindale and Hejnol, 2009). However, it appears that, based on the expression of various marker genes, the so-called head in *Hydra* is in fact the posterior end of the body column (reviewed by Meinhardt, 2002; Guder et al., 2006a). Most prominently, the *Hydra* peduncle, the lower stalk that attaches the animal to substrates, is a pumping organ that shares a common origin with the heart of higher organisms (Shimizu and Fujisawa, 2003). As hearts are anterior organs, Shimizu and Fujisawa posit, and I agree, that the expression of anterior markers and Wnts at opposite ends of the body column in adult *Hydra*, as well as in chordate embryos, reflects the evolutionary conservation of primary body axis patterning throughout the eumetazoan clade.

Wnt signaling affects the axial patterning of very different tissues and cell types in different species, ranging from CNS patterning in vertebrates to germ layer specification in sea urchins (Table 1). Axial Wnt signaling is not limited to gastrulating embryos, but also occurs in adults, as highlighted by the aforementioned findings in planaria and *Hydra*. Furthermore, not only is the involvement of Wnts in primary axis determination conserved, but so is its directionality (posterior/vegetal/oral). Thus, just as for Hox genes, what is evolutionarily conserved is the involvement of Wnts in the patterning of the long axis per se, rather than a role in gastrulation, germ layer specification, specific cell-type or organ specification or a particular differentiation process (Erwin and Davidson, 2002). Incidentally, Wnt signaling might regulate Hox gene expression in AP axis formation (Nordstrom et al., 2006; Pilon et al., 2006; Iimura et al., 2009), and the functional coupling of these two mechanisms in primary axis formation might also represent an ancient feature. Taken together, there is a strong case for Wnt signaling in primary axis patterning being an ancestral property that is already found in Urbilateria.

Why, then, is there no evidence for Wnt signaling in AP axis determination in the well-characterized *Drosophila* embryo? This is probably because *Drosophila* axis formation is highly derived compared with that of ancestral insects (Riechmann and Ephrussi, 2001). In *Drosophila*, Wnt/Wingless (Wg) signaling is involved in the AP patterning of embryonic segments, but there is no evidence for any global function. Instead, *bicoid* specifies global AP patterning; this, however, is not conserved outside dipterans (see Glossary, Box 1) (Lemke et al., 2008). Furthermore, the AP and DV axes are established well before fertilization, during oogenesis, possibly obviating the need for a Wnt-Dkk axis (Riechmann and Ephrussi, 2001). Intriguingly, in more ancestral insect species, such as crickets and spiders, RNAi-mediated depletion of β-catenin or Wnt8 progressively deletes posterior, middle and anterior segments, leaving the head unaffected (Miyawaki et al., 2004; McGregor et al., 2008) (Fig. 6A,B). In spiders, Wnt8 regulates posterior growth zone formation, a process similar to vertebrate segmentation, in which Wnt signaling is important during somitogenesis (Aulehla et al., 2003; Nagano et al., 2006). This supports the idea that a Wnt signaling gradient is used for posterior specification in these more basal insects.

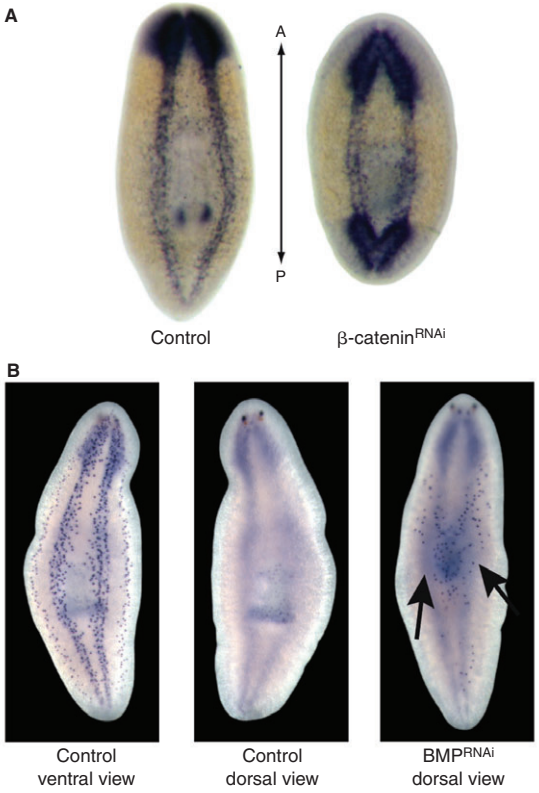


Fig. 5. Signaling through β-catenin and BMP defines AP and DV axes, respectively, in planaria. (**A**) Wnt signaling patterns the AP axis. Individuals of the planarian *Schmidtea mediterranea* were treated with control or *Smed-β-catenin* RNAi and their tail amputated to assess the role of the silenced genes during regeneration. In situ hybridization of the anterior CNS marker *prohormone convertase 2* shows duplication of the head in the β-catenin RNAi-treated animals. The experiment demonstrates the requirement for β-catenin in AP patterning. See Gurley et al. (Gurley et al., 2008). (**B**) Signaling through BMP specifies dorsal fates in *S. mediterranea*. Individuals were treated with control or *Smed-BMP* RNAi. In situ hybridization of the ventral CNS marker *eye53* shows ectopic dorsal expression in the *Smed-BMP* RNAi-treated animals (arrows). The experiment demonstrates the requirement for BMP signaling in DV patterning. See Molina et al. (Molina et al., 2007).

BMP signaling specifies the secondary axis

Bilaterians have various unilateral features that define their secondary/DV axis, such as a nerve chord, musculature, major blood vessels and the intestine. However, the nervous system runs along the dorsal side in vertebrates, but is located ventrally in invertebrates; this situation is reversed for the heart in the two groups. With the advent of information on BMP and BMP antagonist distribution, Arendt and Nübler-Jung (Arendt and Nübler-Jung, 1994), as well as De Robertis and Sasai (De Robertis and Sasai, 1996) revived the idea of Geoffroy Saint-Hilaire (1822) that the DV axis is in fact homologous among animals, but has inverted during evolution. Consistent with this view, the DV axis is patterned by a conserved system that involves BMP and its antagonist Chordin being expressed at opposite sides of the embryo, e.g. in *Drosophila*

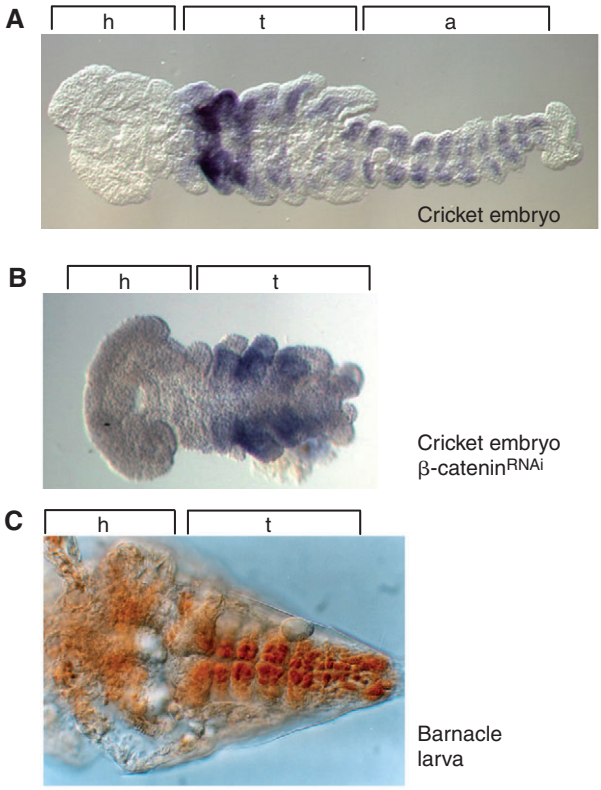


Fig. 6. Evolution of the arthropod body plan by modulation of Wnt signaling? (**A**,**B**) Wild-type and β-*catenin* RNAi-treated cricket embryos stained for the expression of the segmentation marker *scr*, showing that abdominal segments are lost in the RNAi-treated animal. See Miyawaki et al. (Miyawaki et al., 2004). (**C**) Barnacle (*Sacculina carcini*) larva stained for *distalless* (Mouchel-Vielh et al., 2002). β-catenin inhibition in cricket embryos phenocopies the truncated body plan of barnacle larvae. h, head; t, thoracic segments; a, abdominal segments.

(Holley et al., 1995). There is good evidence for the involvement of the BMP-Chordin system in patterning the secondary axis in other protostomes, such as spiders (Akiyama-Oda and Oda, 2006), in planaria (Molina et al., 2007) and in lower deuterostomes, such as the hemichordates (Lowe et al., 2006) (Table 1). For example, in planaria, the CNS develops on the ventral side, but RNAi-mediated knockdown of either BMP or the BMP mediator *Smad1* leads to duplicated dorsal CNS differentiation, both in intact and regenerating animals (Fig. 5B). This suggests that BMP signaling is conserved in planaria and that it plays a key role in DV axis regeneration and maintenance (Fig. 3B).

Although low BMP signaling and high *chordin* expression are often associated with CNS specification (Bier, 1997), the role of BMP signaling is not restricted to the specification of a particular cell type or organ. Rather, all three germ layers are patterned by BMP signaling across different species (Table 1). In the hemichordate *Saccoglossus kowalevskii*, for example, *BMP* and *chordin* are expressed in opposing domains, and BMP signaling does not suppress neural development but instead patterns ectoderm, mesoderm and endoderm (Lowe et al., 2006). Not only is the BMP-Chordin axis conserved, but also its ability to act as a signaling gradient that specifies different cell types in a dose-dependent manner. For example, in *Xenopus* mesoderm, increasing BMP4 concentrations induce muscle, pronephros and blood (Dosch et al., 1997), whereas in *Drosophila* different Decapentaplegic (Dpp)/BMP concentrations specify CNS, epidermis and amnioserosa (Sutherland et al., 2003) and generate DV patterns within the CNS (Mizutani et al., 2006).

Recently, the process of body axis specification has been intensively studied in cnidarians, the sister group of the bilaterians (Fig. 1), which are regarded as radially symmetric animals with little overt dorsoventrality, notably in freshwater *Hydra*. Yet, marine cnidarians, such as the Starlet sea anemone *Nematostella vectensis* or the stone coral *Acropora millepora*, display certain internal asymmetries reminiscent of bilaterality. These define a second axis, termed the directive axis, which is orthogonal to the primary oral-aboral axis. Intriguingly, asymmetric BMP expression has been documented in *Acropora* and *Nematostella* in gastrulating embryos at one side of the blastopore, prefiguring the directive axis (Hayward et al., 2002; Finnerty et al., 2004; Saina et al., 2009). Surprisingly, however, a *chordin* homolog is expressed on the same side as BMPs in *Nematostella* (Matus et al., 2006; Rentzsch et al., 2006), raising the question of whether this BMP-Chordin system acts in a fashion homologous to that in chordates or *Drosophila* in secondary body axis specification. Recent elegant antisense morpholino experiments by Technau and colleagues show that, as in *Xenopus* (De Robertis, 2009), BMP and Chordin interact in a double-negative feedback loop in *Nematostella* (Saina et al., 2009); despite being expressed on the same side as BMP, *chordin* could still generate a BMP signaling gradient. In *Xenopus*, it has been shown that Chordin has different diffusion rates to BMP, and that the ability of Chordin to bind and inhibit BMPs can be regulated by Crossveinless 2 and Tolloid proteases (De Robertis, 2009a; Umulis et al., 2009); both these features can generate graded BMP signaling. Similarly, in echinoderms, BMP and Chordin generate a DV signaling gradient despite being coexpressed ventrally, because Chordin provides a ventral sink (Lapraz, 2009).

In summary, as for the Wnt-Dkk axis, the occurrence of a BMP-Chordin axis is probably not due to evolutionary convergence, but instead represents an ancient, conserved molecular module for bilaterian secondary axis specification (Martindale, 2005; Gerhart, 2006; Rentzsch et al., 2007; Mizutani and Bier, 2008). The fact that the well-characterized nematode *C. elegans* apparently does without a BMP system for axial patterning is probably due to a secondary loss of this feature, which would be consistent with the poor conservation of the *C. elegans* genome (compared with, for example, the genome of the more primitive sea anemone *Nematostella*) and with *C. elegans* having lost genes, introns and gene linkages (Putnam et al., 2007).

In our opinion, the most parsimonious view is, therefore, that perpendicular Wnt and BMP signaling gradients represent a pan-bilaterian program that has been conserved from an ancestral form (Fig. 3).

Regulation of Wnt-BMP signaling in axis formation

Whereas polarized Wnt and BMP signaling in body axis formation is a conserved feature throughout animal evolution, the mechanisms by which this polarity and the gradients are set up are diverse, ranging from the sperm entry point in amphibian embryos determining the DV axis, to gravity in chick embryos determining the AP axis, and to cytoplasmic determinants in the *Drosophila* egg determining the AP and DV axes (Eyal-Giladi, 1997; Riechmann and Ephrussi, 2001). In vertebrates, the BMP and Wnt gradients are both dependent on yet another signaling gradient, namely that of Nodal. High Nodal signaling specifies dorsal mesoderm, the organizer and dorsoanterior development, whereas low Nodal levels

are required for ventral mesoderm and ventroposterior specification (Schier and Shen, 2000). The involvement of a Nodal signaling gradient in axis formation is conserved among vertebrates and echinoderms (Duboc et al., 2004; Lapraz, 2009). The mechanisms involved in setting up the DV gradient of BMP signaling are well characterized and involve a sophisticated network of BMP-binding proteins and proteases (De Robertis, 2009b).

The primary and secondary body axes are not fully independent and cross-regulate each other, which ensures proportional growth and body axis patterning in development and throughout evolution. In *Xenopus* embryos, for example, the AP and DV axes are both specified by the Spemann organizer, and enhanced or reduced organizer activity will lead to dorsoanteriorized or ventroposteriorized embryos, respectively (Niehrs, 2004); this integration partly stems from molecular cross-talk between Wnt and BMP signaling. For example, BMP overexpression inhibits head formation, and this phenotype can be rescued by inhibiting Wnt signaling (Kazanskaya et al., 2000). This cross-talk is partly mediated by Smad1, a downstream BMP signaling transducer that is phosphorylated and negatively regulated by the Wnt pathway kinase Gsk3. Wnt signaling inhibits Gsk3, thereby also stabilizing Smad1 and thus promoting BMP signaling (Fuentealba et al., 2007). Primary and secondary axis regulation is also linked in *Nematostella*, as the RNAi-mediated BMP depletion affects both the directive and the oral-aboral axis (Saina et al., 2009). Interestingly, the Wnt-BMP double gradient also seems to have been evolutionarily co-opted for organ formation, for example in DV and AP patterning in the *Drosophila* wing disc (Strigini and Cohen, 1999).

Cartesian coordinate axial patterning and the evolution of growth and form

If perpendicular Wnt and BMP signaling gradients represent a pan-bilaterian program conserved from an ancestral form, what are the features that might render this morphogenetic program adaptive? What properties can it convey, and how does it constrain the evolution of body form? The main feature of this program is that it might generate a Cartesian coordinate system of positional information (see Box 2), which could be used to orchestrate development and to determine the growth, polarity, robust patterning and differentiation of cells as an integrated whole, and which might underlie the evolution of size and form.

Growth

The concept that growth is regulated by morphogen gradients as an explanation for why organs and organisms grow until they reach their characteristic size and shape, and then stop, has a long history (Day and Lawrence, 2000). Some embryos, animals and organs can regulate (see Glossary, Box 1) after their cell number or cell size has been manipulated experimentally. For example, when the ploidy (see Glossary, Box 1) of newts is manipulated, the animals grow to the normal (diploid) size, but contain very different cell numbers (Fankhauser, 1945). Similarly, in *Drosophila*, the growth and final size of diploid/haploid mosaic animals is relatively normal, but their haploid regions contain more, but smaller, cells (Santamaria, 1983). This indicates that animals measure absolute dimensions rather than counting cell divisions during development. One way for cells to do so is to compare the concentration of a morphogen to which they are exposed with that to which their neighbors are exposed; if the concentration differences exceed a certain threshold, cells would proliferate (Day and Lawrence, 2000) and would keep dividing as long as the gradient is sufficiently steep. Once a field of cells had grown sufficiently, the gradient steepness would fall below the threshold and cell proliferation would stop. This model could account for the regenerative growth observed in developing organisms and explain why growth regulation is independent of cell size and number. A good example for such a growth regulatory mechanism appears to be the Wg-Dpp double gradient in the *Drosophila* wing (Day and Lawrence, 2000), where not absolute levels, but the slope of the Dpp morphogen gradient, determines cell proliferation (Rogulja and Irvine, 2005). A mitogenic gradient of Wnts also organizes growth in the mouse spinal cord, where dorsally expressed Wnt1 and Wnt3a promote high cell proliferation dorsally and high differentiation rates ventrally (Megason and McMahon, 2002).

The conservation of perpendicular Wnt and BMP gradients leads me to predict that these gradients regulate body axis growth along the AP and DV axes. This prediction could be tested by analyzing the effects of the specific knockdown or overexpression of effectors of these pathways on growth and by analyzing cell proliferation; in doing so, an experimental challenge will be to distinguish any simultaneous effects on cell fate that these effectors might have.

Polarity

An important property of morphogen gradients is that they can specify cell polarity, which manifests itself in the direction and beating of cilia, the outgrowth of bristles and hairs, the direction of cell motility, mitotic spindle orientation and asymmetric cell division. Wnt and BMP signaling are both known to regulate cell polarity in different contexts. During zebrafish gastrulation, for example, a BMP gradient establishes a gradient of cell-cell adhesiveness and thereby specifies the direction of lateral mesoderm cell migration, a read-out of cell polarity (von der Hardt et al., 2007). On the other hand, β-catenin regulates global AP cell polarity that spans the entire animal during the early development of *C. elegans* and of the annelid *Platynereis*. In *C. elegans*, β-catenin controls the binary fate specification of posterior sister cells after nearly every AP-oriented cell division in embryonic and postembryonic development (Kaletta et al., 1997; Lin et al., 1998). Similarly, in *Platynereis*, there are sister-cell asymmetries in nuclear β-catenin levels after animal/vegetal-oriented cell divisions throughout embryogenesis (Schneider and Bowerman, 2007). In addition, Wnt signaling might control apicobasal cell polarity (Karner et al., 2006). However, the interpretation of these results is complicated by the fact that the β-catenin and the planar cell polarity (PCP) branches of Wnt signaling share several components involved in cell polarity regulation, including the kinase PAR and the scaffold protein Dishevelled. In primary axis formation, Wnt signaling through β-catenin regulates patterning, whereas the PCP pathway is involved in morphogenetic movements, such as gastrulation (Simons and Mlodzik, 2008). Hence, it remains to be determined whether Wnt/β-catenin or Wnt/PCP signaling, or both, drive apicobasal cell polarity.

The regulation of cell polarity by Wnt or BMP gradients could be functionally important for body axis formation. This could be tested by the specific knockdown or overexpression of effectors of these pathways and analyzing landmarks of polarity, such as the direction of bristle or cilia growth, or by analyzing molecular markers of cell polarity, such as PAR, which show asymmetric distribution in polarized cells.

Robust patterning and morphogenetic fields

Morphogen gradients can explain a universal feature in both vertebrate and invertebrate embryos: the morphogenetic field (see Glossary, Box 1) (Gilbert, 1996). Morphogenetic fields enable certain tissues to scale, develop or regenerate correctly. In amphibian

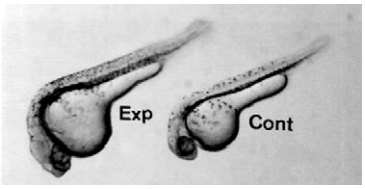


Fig. 7. Size regulation during axis development. The electrical fusion of fertilized goldfish eggs produces enlarged larvae (Exp) with normal axial pattern, as compared with a control (Cont) embryo. Reproduced, with permission, from Yamaha and Yamazaki (Yamaha and Yamazaki, 1993). The underlying principle for size regulation might be the regulative system properties of three-dimensional morphogen gradient fields, as discussed in the text.

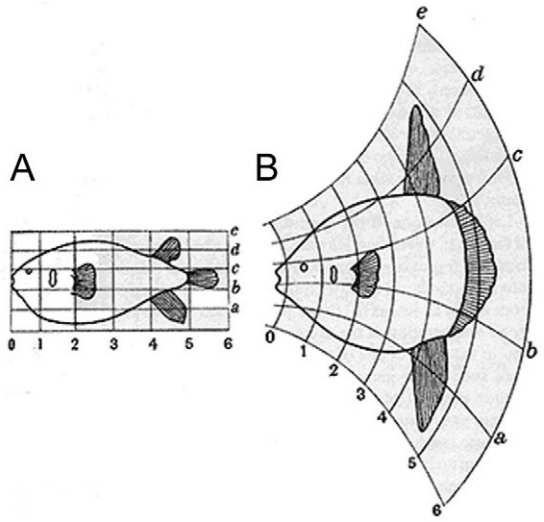


Fig. 8. Evolution of body form. The transformation of Cartesian coordinates from (**A**) the body plan of the fish *Diodon* to (**B**) the closely related fish *Orthagoricus*. As suggested by C. M. Child (Child, 1941), the underlying principle for such transformations might be changes in the shape of the morphogen gradients that establish the body plan. From Thompson (Thompson, 1961).

embryos, for example, dorsal halves isolated before gastrulation develop into normal, but smaller, tadpoles (Cooke, 1981). Somehow, the dorsal-half embryos 'recognize' that their ventral half is missing and scale their pattern accordingly. Conversely, the fusion of two fields, e.g. of two fish eggs, results in giant normal embryos (Fig. 7) (Yamaha and Yamazaki, 1993), which is indicative of self-organization into a single new field. In frog and fish embryos, unlike in mammals, cell proliferation does not play a major role in early body patterning (Harris and Hartenstein, 1991; Ikegami et al., 1997), so regulation is probably not due to increased proliferation (Cooke, 1981). Instead, the cell descendants of the dorsal-half embryo and of the fused eggs are probably repatterned.

The system properties that underpin robust patterning and size scaling are particularly well understood for the BMP gradient in DV axis formation. In *Drosophila* and *Xenopus*, one conserved mechanism involves BMP ligand shuttling to the dorsal (*Drosophila*) or ventral (*Xenopus*) side by the BMP antagonist Short gastrulation/Chordin. This involves positive- and negative-feedback loops that stabilize patterning upon perturbation (Eldar et al., 2002; Wang and Ferguson, 2005; Ben-Zvi et al., 2008). In *Xenopus*, another mechanism uses the BMP Anti-dorsalizing morphogenic protein (Admp), which is, paradoxically, expressed on the dorsal side of the Spemann organizer, where BMP antagonists normally restrict BMP signaling. Intriguingly, Admp depletion abolishes repatterning in dorsal-half embryos, and embryos have greatly expanded neural tissue. Furthermore, when ventral BMP signals are depleted, Admp expression increases, which promotes the autoregulation of DV patterning (Reversade and De Robertis, 2005). Thus, the DV BMP gradient in insects and vertebrates features a combination of negative- and positive-feedback loops that establish self-organizing properties and buffer axial patterning against perturbations (Lander, 2007). Therefore, the morphogenetic field appears to allow the individual organism to adapt to perturbations and to promote robust development when embryos are challenged by intrinsic or extrinsic biological noise. This could be tested by challenging embryos, for example with high temperature or high salt, and monitoring the frequency of malformations that result from perturbed development. The prediction is that sub-threshold manipulations of effectors of BMP or Wnt pathways, which on their own do not induce defects, would enhance such malformations because the morphogenetic field properties are compromised.

Evolution of body size

Morphogenetic gradient fields might not only promote robust development in individual organisms, but also constrain their evolution. Natural selection acting on variations in body size is important for diversification and specialization in animal evolution. Numerous factors can influence body size selection, including food requirements, number of offspring and predation (Schmidt-Nielsen, 1983). The relative constancy of morphological proportions between larger and smaller organisms of the same or of related species, however, is remarkable. The volume of amphibian eggs varies 1000-fold between species, and yet their development produces morphologically similar, if differently sized, larvae. Even within a single species there can be great natural variation in egg size: for example, more than 3-fold intra- and interclutch variation in amphibian egg volume (Dziminski and Alford, 2005; Dziminski and Roberts, 2006). Likewise, under experimental conditions, sea urchin embryos show size invariance over an at least 8-fold range: an isolated four-cell stage blastomere gives rise to a patterned larva, as does an embryo formed by fusion of two eggs (Hörstadius, 1973). Thus, the phenomenon that morphological form scales proportionally with body size occurs not only in experimental embryology, but also in intra- and interspecies variation. Morphogenetic fields could account for such pattern size invariance between species. Although there are no examples to date that involve BMP or Wnt gradients, the scaling of segmentation gene expression has its origins in the scaling of the Bicoid gradient in different Drosophilids (Gregor et al., 2005; Lott et al., 2007). Similarly, I suggest that the ability of the Wnt-BMP double-gradient system to scale pattern with size might not only promote robust development, but also confer scaling between species, an important evolutionary feature. To investigate whether Wnt or BMP signaling is involved in body size evolution, one could, for example, analyze whether their gradient shapes scale with body size in related species.

Evolution of body form

The evolution of morphological form is, to a great extent, a quantitative change in pattern. This phenomenon intrigued the mathematician D'Arcy Thompson 90 years ago, who suggested that many body forms in related species vary as if quantitatively transformed within a Cartesian coordinate system (Thompson,

1917). The application of a transformation constant on the *x*- or *y*-axis, which is mapped onto the body pattern, matches the morphologies of different species surprisingly well (Fig. 8). Importantly, in these examples, body form is quantitatively transformed not just locally, but in its entirety. Thompson realized that body form changes as if "the living body is one integral and indivisible whole", suggesting that globally acting parameters, like morphogenetic gradients, underlie such evolutionary transformations (Child, 1941).

The underlying principle for these transformations is probably the genetic integration of distant morphological characters. Integration is an important biological concept and arises from the pleiotropic effects of genes on multiple traits. For example, β-catenin is ubiquitously expressed, and depleting β-catenin through RNAi in planaria induces global posterior defects (Fig. 5A). Thus, cells in the planarian posterior are genetically integrated by β-catenin.

The genetic linkage of morphological characters leads to covariation within and between different species and, via coselection, to coevolution (Altenberg, 1994; Cheverud, 1996; Wagner and Altenberg, 1996; Schlichting and Pigliucci, 1998). For example, morphometric covariation analysis (see Glossary, Box 1) in the *Drosophila* wing, the patterning of which is governed by a Wnt-BMP double gradient, indicates the pervasive integration of distant morphological landmarks. Specifically, wing vein sizes tend to vary simultaneously in the anterior and posterior wing compartment, indicating that they are genetically coupled (Klingenberg and Zaklan, 2000). It is therefore tempting to predict that during body axis evolution, pleiotropic changes in the Wnt-BMP double-gradient profiles also orchestrate morphological transformations that span the entire body. For example, when treated with lithium to disrupt Wnt signaling, sea star embryos resemble sea urchin gastrulae (Child, 1941). Barnacles are marine crustaceans that lack an abdomen. They have thus lost the posterior body part that was present in their ancestor's body plan, which indicates that they might have undergone a systemic mutation (Geant et al., 2006). Barnacle larvae indeed resemble cricket embryos that have been treated with β-catenin RNAi (Miyawaki et al., 2004) (Fig. 6C). This suggests that body plan differences might be due to modifications of Wnt-BMP gradient shapes. The availability of molecular tools to manipulate Wnt and BMP signaling makes such scenarios testable. Do barnacles lack posterior Wnt signaling, and would forced expression of β-catenin restore part of their lost abdomen? Along these lines, a comparative analysis in different Darwin finches has shown that *Bmp4* expression in beak mesenchyme strongly correlates with deep and broad beak morphology. When misexpressed in chicken embryos, BMP4 causes morphological transformations that recall the beak morphology of the large ground finch (Abzhanov et al., 2004).

Conclusions

In chordates, experimental evidence indicates that perpendicular morphogen gradients of Wnt-Dkk and BMP-Chordin set up a Cartesian coordinate system of positional information, which orchestrates development along the primary and secondary body axes. Work in a variety of invertebrates, most recently in planaria and cnidarians, strongly indicates that the Wnt-BMP double gradient is an ancestral morphogenetic regulatory program present in Urbilateria. As is the case for the Hox cluster, which is ancestral but broken up in some extant species, the Wnt-BMP double gradient is not conserved in all bilaterians. Given that RNAi knockdown and transgenesis techniques are now available in non-standard model organisms, manipulating Wnt and BMP signaling has become feasible. It will be interesting to analyze whether perpendicular morphogen gradients function in less-studied invertebrate phyla, such as molluscs, tardigrades or chaetognaths, and also to what extend they are involved in more primitive arthropods.

The system properties of these morphogen gradients might contribute to the robustness and the fidelity of development and underlie regulation upon experimental or environmental perturbation. Morphogen gradients can scale patterning proportionally with body size and can account for body plan invariance between related species of different sizes. Conversely, evolutionary changes in the shape of morphogen gradients might generate quantitative changes of body form between species. Taken together, these findings suggest that axial Wnt-BMP gradients can contribute to both the relative invariance and the diversity of body form in evolution. The evolution of this Cartesian coordinate system could thus contribute to answering a central question in evolutionary developmental biology: how morphological novelty comes about in organisms that share most of their gene repertoire.

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Competing interests statement

The authors declare no competing financial interests.

References

Abzhanov, A., Protas, M., Grant, B. R., Grant, P. R. and Tabin, C. J. (2004). Bmp4 and morphological variation of beaks in Darwin's finches. *Science* **305**, 1462-1465.

Adamska, M., Degnan, S. M., Green, K. M., Adamski, M., Craigie, A., Larroux, C. and Degnan, B. M. (2007). Wnt and TGF-beta expression in the sponge Amphimedon queenslandica and the origin of metazoan embryonic patterning. *PLoS ONE* **2**, e1031.

Adiyodi, K. G., Raikhel, A. and Sappington, T. (2002). Reproductive biology of invertebrates. In *Progress in Asexual Reproduction*, vol. XI (ed. R. N. Hughes). New York: Wiley.

Adoutte, A., Balavoine, G., Lartillot, N., Lespinet, O., Prud'homme, B. and de Rosa, R. (2000). The new animal phylogeny: reliability and implications. *Proc. Natl. Acad. Sci. USA* **97**, 4453-4456.

Agathon, A., Thisse, C. and Thisse, B. (2003). The molecular nature of the zebrafish tail organizer. *Nature* **424**, 448-452.

Akiyama-Oda, Y. and Oda, H. (2006). Axis specification in the spider embryo: dpp is required for radial-to-axial symmetry transformation and sog for ventral patterning. *Development* **133**, 2347-2357.

Altenberg, L. (1994). The evolution of evolvability. In *Advances in Genetic Programming* (ed. J. K. E. Kinnear). Cambridge: MIT Press.

Arendt, D. and Nübler-Jung, K. (1994). Inversion of dorsoventral axis? *Nature* **371**, 26.

Arendt, D., Denes, A. S., Jekely, G. and Tessmar-Raible, K. (2008). The evolution of nervous system centralization. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 1523-1528.

Aulehla, A., Wehrle, C., Brand-Saberi, B., Kemler, R., Gossler, A., Kanzler, B. and Herrmann, B. G. (2003). Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev. Cell* **4**, 395-406.

Benito-Gutierrez, E. and Arendt, D. (2009). CNS evolution: new insight from the mud. *Curr. Biol.* **19**, R640-R642.

Ben-Zvi, D., Shilo, B. Z., Fainsod, A. and Barkai, N. (2008). Scaling of the BMP activation gradient in Xenopus embryos. *Nature* **453**, 1205-1211.

Bier, E. (1997). Anti-neural-inhibition: a conserved mechanism for neural induction. *Cell* **89**, 681-684.

Broun, M., Gee, L., Reinhardt, B. and Bode, H. R. (2005). Formation of the head organizer in hydra involves the canonical Wnt pathway. *Development* **132**, 2907-2916.

Butts, T., Holland, P. W. and Ferrier, D. E. (2008). The urbilaterian Super-Hox cluster. *Trends Genet.* **24**, 259-262.

Campbell, N. A. and Reece, J. B. (2005). *Biology* (7th edition). New Jersey: Benjamin Cummings.

Caneparo, L., Huang, Y. L., Staudt, N., Tada, M., Ahrendt, R., Kazanskaya, O., Niehrs, C. and Houart, C. (2007). Dickkopf-1 regulates gastrulation movements

by coordinated modulation of Wnt/beta catenin and Wnt/PCP activities, through interaction with the Dallylike homolog Knypek. *Genes Dev.* **21**, 465-480.
Cheverud, J. M. (1996). Developmental integration and the evolution of pleiotropy. *Amer. Zool.* **36**, 44-50.
Child, C. M. (1941). *Patterns and Problems of Development*. Chicago: University of Chicago Press.
Collins, A. G. and Valentine, J. W. (2001). Defining phyla: evolutionary pathways to metazoan body plans. *Evol. Dev.* **3**, 432-442.
Cooke, J. (1981). Scale of body pattern adjusts to available cell number in amphibian embryos. *Nature* **290**, 775-778.
Crawford, K. (2003). Lithium chloride inhibits development along the animal vegetal axis and anterior midline of the squid embryo. *Biol. Bull.* **205**, 181-182.
Darras, S. and Nishida, H. (2001). The BMP/CHORDIN antagonism controls sensory pigment cell specification and differentiation in the ascidian embryo. *Dev. Biol.* **236**, 271-288.
Davis, G. K. and Patel, N. H. (2002). Short, long, and beyond: molecular and embryological approaches to insect segmentation. *Annu. Rev. Entomol.* **47**, 669-699.
Day, S. J. and Lawrence, P. A. (2000). Measuring dimensions: the regulation of size and shape. *Development* **127**, 2977-2987.
De Robertis, E. M. (2008). Evo-devo: variations on ancestral themes. *Cell* **132**, 185-195.
De Robertis, E. M. (2009). Spemann's organizer and the self-regulation of embryonic fields. *Mech. Dev.* **126**, 925-941.
De Robertis, E. M. and Sasai, Y. (1996). A common plan for dorsoventral patterning in Bilateria. *Nature* **380**, 37-40.
De Robertis, E. M. and Kuroda, H. (2004). Dorsal-ventral patterning and neural induction in Xenopus embryos. *Annu. Rev. Cell Dev. Biol.* **20**, 285-308.
Dosch, R., Gawantka, V., Delius, H., Blumenstock, C. and Niehrs, C. (1997). Bmp-4 acts as a morphogen in dorsoventral mesoderm patterning in Xenopus. *Development* **124**, 2325-2334.
Driever, W. and Nusslein-Volhard, C. (1988). A gradient of bicoid protein in Drosophila embryos. *Cell* **54**, 83-93.
Duboc, V., Rottinger, E., Besnardeau, L. and Lepage, T. (2004). Nodal and BMP2/4 signaling organizes the oral-aboral axis of the sea urchin embryo. *Dev. Cell* **6**, 397-410.
Dziminski, M. A. and Alford, R. A. (2005). Patterns and fitness consequences of intraclutch variation in egg provisioning in tropical Australian frogs. *Oecologia* **146**, 98-109.
Dziminski, M. A. and Roberts, J. D. (2006). Fitness consequences of variable maternal provisioning in quacking frogs (Crinia georgiana). *J. Evol. Biol.* **19**, 144-155.
Eldar, A., Dorfman, R., Weiss, D., Ashe, H., Shilo, B. Z. and Barkai, N. (2002). Robustness of the BMP morphogen gradient in Drosophila embryonic patterning. *Nature* **419**, 304-308.
Erter, C. E., Wilm, T. P., Basler, N., Wright, C. V. and Solnica-Krezel, L. (2001). Wnt8 is required in lateral mesendodermal precursors for neural posteriorization in vivo. *Development* **128**, 3571-3583.
Erwin, D. H. and Davidson, E. H. (2002). The last common bilaterian ancestor. *Development* **129**, 3021-3032.
Eyal-Giladi, H. (1997). Establishment of the axis in chordates: facts and speculations. *Development* **124**, 2285-2296.
Fankhauser, G. (1945). The effects of changes in chromosome number on amphibian development. *Q. Rev. Biol.* **20**, 20-78.
Finnerty, J. R. (2003). The origins of axial patterning in the metazoa: how old is bilateral symmetry? *Int. J. Dev. Biol.* **47**, 523-529.
Finnerty, J. R., Pang, K., Burton, P., Paulson, D. and Martindale, M. Q. (2004). Origins of bilateral symmetry: Hox and dpp expression in a sea anemone. *Science* **304**, 1335-1337.
Fuentealba, L. C., Eivers, E., Ikeda, A., Hurtado, C., Kuroda, H., Pera, E. M. and De Robertis, E. M. (2007). Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal. *Cell* **131**, 980-993.
Gavis, E. R. and Lehmann, R. (1994). Translational regulation of nanos by RNA localization. *Nature* **369**, 315-318.
Geant, E., Mouchel-Vielh, E., Coutanceau, J. P., Ozouf-Costaz, C. and Deutsch, J. S. (2006). Are Cirripedia hopeful monsters? Cytogenetic approach and evidence for a Hox gene cluster in the cirripede crustacean Sacculina carcini. *Dev. Genes Evol.* **216**, 443-449.
Geoffroy Saint-Hilaire, E. (1822). Mém, du Mus. *Hist. Nat.* **9**, 89-119.
Gerhart, J. (2006). The deuterostome ancestor. *J. Cell. Physiol.* **209**, 677-685.
Gilbert, S. F. (1996). A brief history of premolecular induction studies. *Semin. Cell Dev. Biol.* **7**, 67-76.
Gilbert, S. F., Opitz, J. M. and Raff, R. A. (1996). Resynthesizing evolutionary and developmental biology. *Dev. Biol.* **173**, 357-372.
Gont, L. K., Steinbeisser, H., Blumberg, B. and De Robertis, E. M. (1993). Tail formation as a continuation of gastrulation: the multiple cell populations of the Xenopus tailbud derive from the late blastopore lip. *Development* **119**, 991-1004.
Gregor, T., Bialek, W., de Ruyter van Steveninck, R. R., Tank, D. W. and Wieschaus, E. F. (2005). Diffusion and scaling during early embryonic pattern formation. *Proc. Natl. Acad. Sci. USA* **102**, 18403-18407.
Guder, C., Philipp, I., Lengfeld, T., Watanabe, H., Hobmayer, B. and Holstein, T. W. (2006a). The Wnt code: cnidarians signal the way. *Oncogene* **25**, 7450-7460.
Guder, C., Pinho, S., Nacak, T. G., Schmidt, H. A., Hobmayer, B., Niehrs, C. and Holstein, T. W. (2006b). An ancient Wnt-Dickkopf antagonism in Hydra. *Development* **133**, 901-911.
Gurley, K. A., Rink, J. C. and Sanchez Alvarado, A. (2008). Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science* **319**, 323-327.
Harris, W. A. and Hartenstein, V. (1991). Neuronal determination without cell division in Xenopus embryos. *Neuron* **6**, 499-515.
Hayward, D. C., Samuel, G., Pontynen, P. C., Catmull, J., Saint, R., Miller, D. J. and Ball, E. E. (2002). Localized expression of a dpp/BMP2/4 ortholog in a coral embryo. *Proc. Natl. Acad. Sci. USA* **99**, 8106-8111.
Heasman, J., Crawford, A., Goldstone, K., Garner, H. P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y. and Wylie, C. (1994). Overexpression of cadherins and underexpression of beta-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell* **79**, 791-803.
Henry, J. Q., Perry, K. J., Wever, J., Seaver, E. and Martindale, M. Q. (2008). Beta-catenin is required for the establishment of vegetal embryonic fates in the nemertean, Cerebratulus lacteus. *Dev. Biol.* **317**, 368-379.
Hobmayer, B., Rentzsch, F., Kuhn, K., Happel, C. M., von Laue, C. C., Snyder, P., Rothbacher, U. and Holstein, T. W. (2000). WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* **407**, 186-189.
Holland, L. Z., Panfilio, K. A., Chastain, R., Schubert, M. and Holland, N. D. (2005). Nuclear beta-catenin promotes non-neural ectoderm and posterior cell fates in amphioxus embryos. *Dev. Dyn.* **233**, 1430-1443.
Holley, S. A., Jackson, P. D., Sasai, Y., Lu, B., De Robertis, E. M., Hoffmann, F. M. and Ferguson, E. L. (1995). A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and chordin. *Nature* **376**, 249-253.
Hörstadius, S. (1973). *Experimental Embryology of Echinoderms*. Oxford: Clarendon Press.
Houart, C., Caneparo, L., Heisenberg, C., Barth, K., Take-Uchi, M. and Wilson, S. (2002). Establishment of the telencephalon during gastrulation by local antagonism of Wnt signaling. *Neuron* **35**, 255-265.
Huelsken, J., Vogel, R., Brinkmann, V., Erdmann, B., Birchmeier, C. and Birchmeier, W. (2000). Requirement for beta-catenin in anterior-posterior axis formation in mice. *J. Cell Biol.* **148**, 567-578.
Iglesias, M., Gomez-Skarmeta, J. L., Salo, E. and Adell, T. (2008). Silencing of Smedbetacatenin1 generates radial-like hypercephalized planarians. *Development* **135**, 1215-1221.
Iimura, T., Denans, N. and Pourquie, O. (2009). Establishment of Hox vertebral identities in the embryonic spine precursors. *Curr. Top. Dev. Biol.* **88**, 201-234.
Ikegami, R., Rivera-Bennetts, A. K., Brooker, D. L. and Yager, T. D. (1997). Effect of inhibitors of DNA replication on early zebrafish embryos: evidence for coordinate activation of multiple intrinsic cell-cycle checkpoints at the mid-blastula transition. *Zygote* **5**, 153-175.
Imai, K., Takada, N., Satoh, N. and Satou, Y. (2000). (beta)-catenin mediates the specification of endoderm cells in ascidian embryos. *Development* **127**, 3009-3020.
In der Rieden, P. M., Vilaspasa, F. L. and Durston, A. J. (2009). Xwnt8 directly initiates expression of labial Hox genes. *Dev. Dyn.* **239**, 126-139.
Jones, C. M. and Smith, J. C. (1998). Establishment of a BMP-4 morphogen gradient by longrange inhibition. *Dev. Biol.* **194**, 12-17.
Joubin, K. and Stern, C. (1999). Molecular interactions continuously define the organizer during the cell movements of gastrulation. *Cell* **98**, 559-571.
Kaletta, T., Schnabel, H. and Schnabel, R. (1997). Binary specification of the embryonic lineage in Caenorhabditis elegans. *Nature* **390**, 294-298.
Karner, C., Wharton, K. A. and Carroll, T. J. (2006). Apical-basal polarity, Wnt signaling and vertebrate organogenesis. *Semin. Cell Dev. Biol.* **17**, 214-222.
Kazanskaya, O., Glinka, A. and Niehrs, C. (2000). The role of Xenopus dickkopf1 in prechordal plate specification and neural patterning. *Development* **127**, 4981-4992.
Kelly, C., Chin, A. J., Leatherman, J. L., Kozlowski, D. J. and Weinberg, E. S. (2000). Maternally controlled (beta)-catenin-mediated signaling is required for organizer formation in the zebrafish. *Development* **127**, 3899-3911.
Kelly, O. G., Pinson, K. I. and Skarnes, W. C. (2004). The Wnt co-receptors Lrp5 and Lrp6 are essential for gastrulation in mice. *Development* **131**, 2803-2815.
Kiecker, C. and Niehrs, C. (2001). A morphogen gradient of Wnt/beta-catenin signalling regulates anteroposterior neural patterning in Xenopus. *Development* **128**, 4189-4201.
Kim, C. H., Oda, T., Itoh, M., Jiang, D., Artinger, K. B., Chandrasekharappa, S. C., Driever, W. and Chitnis, A. B. (2000). Repressor activity of Headless/Tcf3 is essential for vertebrate head formation. *Nature* **407**, 913-916.
Kim, S. H., Shin, J., Park, H. C., Yeo, S. Y., Hong, S. K., Han, S., Rhee, M., Kim, C. H., Chitnis, A. B. and Huh, T. L. (2002). Specification of an anterior neuroectoderm patterning by Frizzled8a-mediated Wnt8b signalling during late gastrulation in zebrafish. *Development* **129**, 4443-4455.

King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I. et al. (2008). The genome of the choanoflagellate Monosiga brevicollis and the origin of metazoans. *Nature* **451**, 783-788.
Kishigami, S. and Mishina, Y. (2005). BMP signaling and early embryonic patterning. *Cytokine Growth Factor Rev.* **16**, 265-278.
Klingenberg, C. P. and Zaklan, S. D. (2000). Morphological integration between development compartments in the Drosophila wing. *Evolution* **54**, 1273-1285.
Klingensmith, J., Ang, S. L., Bachiller, D. and Rossant, J. (1999). Neural induction and patterning in the mouse in the absence of the node and its derivatives. *Dev. Biol.* **216**, 535-549.
Knecht, A. and Harland, R. M. (1997). Mechanisms of dorsal-ventral patterning in noggin-induced neural tissue. *Development* **124**, 2477-2488.
Kusserow, A., Pang, K., Sturm, C., Hrouda, M., Lentfer, J., Schmidt, H. A., Technau, U., von Haeseler, A., Hobmayer, B., Martindale, M. Q. et al. (2005). Unexpected complexity of the Wnt gene family in a sea anemone. *Nature* **433**, 156-160.
Lander, A. D. (2007). Morpheus unbound: reimagining the morphogen gradient. *Cell* **128**, 245-256.
Lapebie, P., Gazave, E., Ereskovsky, A., Derelle, R., Bezac, C., Renard, E., Houliston, E. and Borchiellini, C. (2009). WNT/beta-catenin signalling and epithelial patterning in the homoscleromorph sponge Oscarella. *PLoS ONE* **4**, e5823.
Lapraz, F., Besnardeau, L. and Lepage, T. (2009). Patterning of the dorsal-ventral axis in echinoderms: insights into the evolution of the BMP-chordin signaling network. *PLoS Biol.* **7**, e1000248.
Lee, P. N., Pang, K., Matus, D. Q. and Martindale, M. Q. (2006). A WNT of things to come: evolution of Wnt signaling and polarity in cnidarians. *Semin. Cell Dev. Biol.* **17**, 157-167.
Lekven, A. C., Thorpe, C. J., Waxman, J. S. and Moon, R. T. (2001). Zebrafish wnt8 encodes two wnt8 proteins on a bicistronic transcript and is required for mesoderm and neurectoderm patterning. *Dev. Cell* **1**, 103-114.
Lemke, S., Stauber, M., Shaw, P. J., Rafiqi, A. M., Prell, A. and Schmidt-Ott, U. (2008). Bicoid occurrence and Bicoid-dependent hunchback regulation in lower cyclorrhaphan flies. *Evol. Dev.* **10**, 413-420.
Lengfeld, T., Watanabe, H., Simakov, O., Lindgens, D., Gee, L., Law, L., Schmidt, H. A., Ozbek, S., Bode, H. and Holstein, T. W. (2009). Multiple Wnts are involved in Hydra organizer formation and regeneration. *Dev. Biol.* **330**, 186-199.
Lin, R., Hill, R. J. and Priess, J. R. (1998). POP-1 and anterior-posterior fate decisions in C. elegans embryos. *Cell* **92**, 229-239.
Liu, P., Wakamiya, M., Shea, M. J., Albrecht, U., Behringer, R. R. and Bradley, A. (1999). Requirement for Wnt3 in vertebrate axis formation. *Nat. Genet.* **22**, 361-365.
Logan, C. Y., Miller, J. R., Ferkowicz, M. J. and McClay, D. R. (1999). Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* **126**, 345-357.
Lott, S. E., Kreitman, M., Palsson, A., Alekseeva, E. and Ludwig, M. Z. (2007). Canalization of segmentation and its evolution in Drosophila. *Proc. Natl. Acad. Sci. USA* **104**, 10926-10931.
Lowe, C. J., Terasaki, M., Wu, M., Freeman, R. M., Jr, Runft, L., Kwan, K., Haigo, S., Aronowicz, J., Lander, E., Gruber, C. et al. (2006). Dorsoventral patterning in hemichordates: insights into early chordate evolution. *PLoS Biol.* **4**, e291.
Mariottini, P., Bagni, C., Francesconi, A., Cecconi, F., Serra, M. J., Chen, Q. M., Loreni, F., Annesi, F. and Amaldi, F. (1993). Sequence of the gene coding for ribosomal protein S8 of Xenopus laevis. *Gene* **132**, 255-260.
Martindale, M. Q. (2005). The evolution of metazoan axial properties. *Nat. Rev. Genet.* **6**, 917-927.
Martindale, M. Q. and Hejnol, A. (2009). A developmental perspective: changes in the position of the blastopore during bilaterian evolution. *Dev. Cell* **17**, 162-174.
Marvin, M. J., Di Rocco, G., Gardiner, A., Bush, S. M. and Lassar, A. B. (2001). Inhibition of Wnt activity induces heart formation from posterior mesoderm. *Genes Dev.* **15**, 316-327.
Matus, D. Q., Pang, K., Marlow, H., Dunn, C. W., Thomsen, G. H. and Martindale, M. Q. (2006). Molecular evidence for deep evolutionary roots of bilaterality in animal development. *Proc. Natl. Acad. Sci. USA* **103**, 11195-11200.
McGregor, A. P., Pechmann, M., Schwager, E. E., Feitosa, N. M., Kruck, S., Aranda, M. and Damen, W. G. (2008). Wnt8 is required for growth-zone establishment and development of opisthosomal segments in a spider. *Curr. Biol.* **18**, 1619-1623.
McLin, V. A., Rankin, S. A. and Zorn, A. M. (2007). Repression of Wnt/beta-catenin signaling in the anterior endoderm is essential for liver and pancreas development. *Development* **134**, 2207-2217.
Megason, S. G. and McMahon, A. P. (2002). A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development* **129**, 2087-2098.
Meinhardt, H. (2002). The radial-symmetric hydra and the evolution of the bilateral body plan: an old body became a young brain. *BioEssays* **24**, 185-191.
Meinhardt, H. (2006). Primary body axes of vertebrates: generation of a near-Cartesian coordinate system and the role of Spemann-type organizer. *Dev. Dyn.* **235**, 2907-2919.
Meinhardt, H. (2008). Models of biological pattern formation: from elementary steps to the organization of embryonic axes. *Curr. Top. Dev. Biol.* **81**, 1-63.
Miyawaki, K., Mito, T., Sarashina, I., Zhang, H., Shinmyo, Y., Ohuchi, H. and Noji, S. (2004). Involvement of Wingless/Armadillo signaling in the posterior sequential segmentation in the cricket, Gryllus bimaculatus (Orthoptera), as revealed by RNAi analysis. *Mech. Dev.* **121**, 119-130.
Mizutani, C. M. and Bier, E. (2008). EvoD/Vo: the origins of BMP signalling in the neuroectoderm. *Nat. Rev. Genet.* **9**, 663-677.
Mizutani, C. M., Meyer, N., Roelink, H. and Bier, E. (2006). Threshold-dependent BMP-mediated repression: a model for a conserved mechanism that patterns the neuroectoderm. *PLoS Biol.* **4**, e313.
Molina, M. D., Salo, E. and Cebria, F. (2007). The BMP pathway is essential for re-specification and maintenance of the dorsoventral axis in regenerating and intact planarians. *Dev. Biol.* **311**, 79-94.
Momose, T. and Houliston, E. (2007). Two oppositely localised frizzled RNAs as axis determinants in a cnidarian embryo. *PLoS Biol.* **5**, e70.
Momose, T., Derelle, R. and Houliston, E. (2008). A maternally localised Wnt ligand required for axial patterning in the cnidarian Clytia hemisphaerica. *Development* **135**, 2105-2113.
Morgan, T. H. (1906). Hydranth formation and polarity in Tubularia. *J. Exp. Zool.* **3**, 501-515.
Mouchel-Vielh, E., Blin, M., Rigolot, C. and Deutsch, J. S. (2002). Expression of a homologue of the fushi tarazu (ftz) gene in a cirripede crustacean. *Evol. Dev.* **4**, 76-85.
Nagano, T., Takehara, S., Takahashi, M., Aizawa, S. and Yamamoto, A. (2006). Shisa2 promotes the maturation of somitic precursors and transition to the segmental fate in Xenopus embryos. *Development* **133**, 4643-4654.
Neave, B., Holder, N. and Patient, R. (1997). A graded response to BMP-4 spatially coordinates patterning of the mesoderm and ectoderm in the zebrafish. *Mech. Dev.* **62**, 183-195.
Nejak-Bowen, K. and Monga, S. P. (2008). Wnt/beta-catenin signaling in hepatic organogenesis. *Organogenesis* **4**, 92-99.
Nguyen, V. H., Schmid, B., Trout, J., Connors, S. A., Ekker, M. and Mullins, M. C. (1998). Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a bmp2b/swirl pathway of genes. *Dev. Biol.* **199**, 93-110.
Nichols, S. A., Dirks, W., Pearse, J. S. and King, N. (2006). Early evolution of animal cell signaling and adhesion genes. *Proc. Natl. Acad. Sci. USA* **103**, 12451-12456.
Niehrs, C. (2004). Regionally specific induction by the Spemann-Mangold organizer. *Nat. Rev. Genet.* **5**, 425-434.
Nielsen, C. (2001). *Animal Evolution-Interrelationships of the Living Phyla*. Oxford: Oxford University Press.
Nordstrom, U., Jessell, T. M. and Edlund, T. (2002). Progressive induction of caudal neural character by graded Wnt signaling. *Nat. Neurosci.* **5**, 525-532.
Nordstrom, U., Maier, E., Jessell, T. M. and Edlund, T. (2006). An early role for WNT signaling in specifying neural patterns of Cdx and Hox gene expression and motor neuron subtype identity. *PLoS Biol.* **4**, e252.
Oelgeschläger, M., Kuroda, H., Reversade, B. and Robertis, E. M. D. (2003). Chordin is required for the Spemann organizer transplantation phenomenon in Xenopus embryos. *Dev. Cell* **4**, 219-230.
Onai, T., Lin, H. C., Schubert, M., Koop, D., Osborne, P. W., Alvarez, S., Alvarez, R., Holland, N. D. and Holland, L. Z. (2009). Retinoic acid and Wnt/beta-catenin have complementary roles in anterior/posterior patterning embryos of the basal chordate amphioxus. *Dev. Biol.* **332**, 223-233.
Petersen, C. P. and Reddien, P. W. (2008). Smed-betacatenin-1 is required for anteroposterior blastema polarity in planarian regeneration. *Science* **319**, 327-330.
Petersen, C. P. and Reddien, P. W. (2009). Wnt signaling and the polarity of the primary body axis. *Cell* **139**, 1056-1068.
Philipp, I., Aufschnaiter, R., Ozbek, S., Pontasch, S., Jenewein, M., Watanabe, H., Rentzsch, F., Holstein, T. W. and Hobmayer, B. (2009). Wnt/beta-catenin and noncanonical Wnt signaling interact in tissue evagination in the simple eumetazoan Hydra. *Proc. Natl. Acad. Sci. USA* **106**, 4290-4295.
Pilon, N., Oh, K., Sylvestre, J. R., Bouchard, N., Savory, J. and Lohnes, D. (2006). Cdx4 is a direct target of the canonical Wnt pathway. *Dev. Biol.* **289**, 55-63.
Putnam, N. H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V. V. et al. (2007). Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317**, 86-94.
Rentzsch, F., Anton, R., Saina, M., Hammerschmidt, M., Holstein, T. W. and Technau, U. (2006). Asymmetric expression of the BMP antagonists chordin and gremlin in the sea anemone Nematostella vectensis: implications for the evolution of axial patterning. *Dev. Biol.* **296**, 375-387.

Rentzsch, F., Guder, C., Vocke, D., Hobmayer, B. and Holstein, T. W. (2007). An ancient chordin-like gene in organizer formation of Hydra. *Proc. Natl. Acad. Sci. USA* **104**, 3249-3254.
Reversade, B. and De Robertis, E. M. (2005). Regulation of ADMP and BMP2/4/7 at opposite embryonic poles generates a self-regulating morphogenetic field. *Cell* **123**, 1147-1160.
Riechmann, V. and Ephrussi, A. (2001). Axis formation during Drosophila oogenesis. *Curr. Opin. Genet. Dev.* **11**, 374-383.
Roeser, T., Stein, S. and Kessel, M. (1999). Nuclear beta-catenin and the development of bilateral symmetry in normal and LiCl-exposed chick embryos. *Development* **126**, 2955-2965.
Rogulja, D. and Irvine, K. D. (2005). Regulation of cell proliferation by a morphogen gradient. *Cell* **123**, 449-461.
Runnström, J. (1928). Plasmabau und Determination bei dem Ei von Paracentrotus lividus. *Wilhelm Roux Arch. Entw. Mech. Org.* **113**, 556-581.
Saina, M., Genikhovich, G., Renfer, E. and Technau, U. (2009). BMPs and chordin regulate patterning of the directive axis in a sea anemone. *Proc. Natl. Acad. Sci. USA* **106**, 18592-18597.
Santamaria, P. (1983). Analysis of haploid mosaics in Drosophila. *Dev. Biol.* **96**, 285-295.
Saxen, L. and Toivonen, S. (1961). The two-gradient hypothesis in primary induction: the combined effect of two types of inducers mixed in different ratios. *J. Embryol. Exp. Morph.* **9**, 514-533.
Schier, A. F. and Shen, M. M. (2000). Nodal signalling in vertebrate development. *Nature* **403**, 385-389.
Schlichting, C. D. and Pigliucci, M. (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland: Sinauer Associates.
Schmidt-Nielsen, K. (1983). *Scaling: Why Is Animal Size So Important?*. Cambridge: Cambridge University Press.
Schneider, S. Q. and Bowerman, B. (2007). beta-Catenin asymmetries after all animal/vegetal-oriented cell divisions in Platynereis dumerilii embryos mediate binary cell-fate specification. *Dev. Cell* **13**, 73-86.
Schneider, V. A. and Mercola, M. (2001). Wnt antagonism initiates cardiogenesis in Xenopus laevis. *Genes Dev.* **15**, 304-315.
Shimizu, H. and Fujisawa, T. (2003). Peduncle of Hydra and the heart of higher organisms share a common ancestral origin. *Genesis* **36**, 182-186.
Simons, M. and Mlodzik, M. (2008). Planar cell polarity signaling: from fly development to human disease. *Annu. Rev. Genet.* **42**, 517-540.
Smith, R. C. (1986). Protein synthesis and messenger RNA levels along the animal-vegetal axis during early Xenopus development. *J. Embryol. Exp. Morphol.* **95**, 15-35.
Spemann, H. and Mangold, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *Arch. Mikrosk. Anat. Entwicklungsmechan.* **100**, 599-638.
Steward, R., Zusman, S. B., Huang, L. H. and Schedl, P. (1988). The dorsal protein is distributed in a gradient in early Drosophila embryos. *Cell* **55**, 487-495.
Streit, A., Lee, K. J., Woo, I., Roberts, C., Jessell, T. M. and Stern, C. D. (1998). Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. *Development* **125**, 507-519.
Strigini, M. and Cohen, S. M. (1999). Formation of morphogen gradients in the Drosophila wing. *Semin. Cell Dev. Biol.* **10**, 335-344.
Sutherland, D. J., Li, M., Liu, X. Q., Stefancsik, R. and Raftery, L. A. (2003). Stepwise formation of a SMAD activity gradient during dorsal-ventral patterning of the Drosophila embryo. *Development* **130**, 5705-5716.
Tahinci, E., Thorne, C. A., Franklin, J. L., Salic, A., Christian, K. M., Lee, L. A., Coffey, R. J. and Lee, E. (2007). Lrp6 is required for convergent extension during Xenopus gastrulation. *Development* **134**, 4095-4106.
Thomas, A. J. and Erickson, C. A. (2008). The making of a melanocyte: the specification of melanoblasts from the neural crest. *Pigment Cell Melanoma Res.* **21**, 598-610.
Thompson, D. (1917). *On Growth and Form*. Cambridge: Cambridge University Press.
Toivonen, S. and Saxen, L. (1968). Morphogenetic interaction of presumptive neural and mesodermal cells mixed in different ratios. *Science* **159**, 539-540.
Tribulo, C., Aybar, M. J., Nguyen, V. H., Mullins, M. C. and Mayor, R. (2003). Regulation of Msx genes by a Bmp gradient is essential for neural crest specification. *Development* **130**, 6441-6452.
Umulis, D., O'Connor, M. B. and Blair, S. S. (2009). The extracellular regulation of bone morphogenetic protein signaling. *Development* **136**, 3715-3728.
von der Hardt, S., Bakkers, J., Inbal, A., Carvalho, L., Solnica-Krezel, L., Heisenberg, C. P. and Hammerschmidt, M. (2007). The Bmp gradient of the zebrafish gastrula guides migrating lateral cells by regulating cell-cell adhesion. *Curr. Biol.* **17**, 475-487.
Wagner, G. P. and Altenberg, L. (1996). Perspective: complex adaptations and the evolution of evolvability. *Evolution* **50**, 967-976.
Wang, Y. C. and Ferguson, E. L. (2005). Spatial bistability of Dpp-receptor interactions during Drosophila dorsal-ventral patterning. *Nature* **434**, 229-234.
Wikramanayake, A. H., Huang, L. and Klein, W. H. (1998). beta-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. *Proc. Natl. Acad. Sci. USA* **95**, 9343-9348.
Wikramanayake, A. H., Hong, M., Lee, P. N., Pang, K., Byrum, C. A., Bince, J. M., Xu, R. and Martindale, M. Q. (2003). An ancient role for nuclear beta-catenin in the evolution of axial polarity and germ layer segregation. *Nature* **426**, 446-450.
Willmer, P. (1990). *Invertebrate Relationships*. Cambridge: Cambridge University Press.
Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1-47.
Yamaguchi, T. P., Takada, S., Yoshikawa, Y., Wu, N. and McMahon, A. P. (1999). T (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev.* **13**, 3185-3190.
Yamaha, E. and Yamazaki, F. (1993). Electrically fused-egg induction and its development in the goldfish, Carassius auratus. *Int. J. Dev. Biol.* **37**, 291-298.
Yu, J. K., Satou, Y., Holland, N. D., Shin, I. T., Kohara, Y., Satoh, N., Bronner-Fraser, M. and Holland, L. Z. (2007). Axial patterning in cephalochordates and the evolution of the organizer. *Nature* **445**, 613-617.