

MIT Open Access Articles

Wnt Signaling and the Polarity of the Primary Body Axis

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

Citation: Petersen, Christian P., and Peter W. Reddien. "Wnt Signaling and the Polarity of the Primary Body Axis." *Cell* 139, no. 6 (December 2009): 1056–1068. © 2009 Elsevier Inc.

As Published: <http://dx.doi.org/10.1016/j.cell.2009.11.035>

Publisher: Elsevier

Persistent URL: <http://hdl.handle.net/1721.1/96176>

Version: Final published version: final published article, as it appeared in a journal, conference proceedings, or other formally published context

Terms of Use: Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.



Wnt Signaling and the Polarity of the Primary Body Axis

Christian P. Petersen¹ and Peter W. Reddien^{1,2,3,*}

¹Whitehead Institute for Biomedical Research, 9 Cambridge Center

²Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue
Cambridge, MA 02142, USA

³Howard Hughes Medical Institute

*Correspondence: reddien@wi.mit.edu

DOI 10.1016/j.cell.2009.11.035

How animals establish and pattern the primary body axis is one of the most fundamental problems in biology. Data from diverse deuterostomes (frog, fish, mouse, and amphioxus) and from planarians (protostomes) suggest that Wnt signaling through β -catenin controls posterior identity during body plan formation in most bilaterally symmetric animals. Wnt signaling also influences primary axis polarity of pre-bilaterian animals, indicating that an axial patterning role for Wnt signaling predates the evolution of bilaterally symmetric animals. The use of posterior Wnt signaling and anterior Wnt inhibition might be a unifying principle of body plan development in most animals.

Introduction

How do animals develop regionalized features on an axis that runs from head to tail? Addressing this question is fundamental to understanding how animal form emerges from a fertilized egg. An appealing model involves the molecular polarization of embryos along two axes, anteroposterior (for head-to-tail) and dorsoventral (for back-to-belly), to provide an orthogonal coordinate system for regional patterning (Nüsslein-Volhard and Wieschaus, 1980; Niehrs, 2004).

The search for mechanisms underlying axis formation and patterning has yielded some of the biggest surprises of developmental biology. For example, the discovery of conservation of positional roles for *Hox* genes suggested the existence of a primary axis patterning system used by most if not all bilaterally symmetric animals (Slack et al., 1993). The ubiquity of the positional role for *Hox* genes indicates that as animals diversified, some features of primary axis patterning were not readily subjected to evolutionary change. However, the physical events of early embryogenesis can vary dramatically across animals, making it difficult to define general models that explain how animal axes emerge. Molecular methods and resources, such as RNA interference, genome sequencing, and expression profiling, are permitting the study of primary axis patterning in an unprecedented range of animals. This enables a comparative approach that could allow the identification of common principles of axis formation.

Wnt signaling is extensively studied for its roles in a myriad of developmental events in both familiar model organisms as well as representatives of many phyla across the animal kingdom. In this Review we analyze this rich dataset to compare the impact of Wnt signaling on primary axis development that occurs in these phyla. The Review is organized into three main sections that correspond to major groupings of animal phyla: the deuterostomes, the protostomes, and pre-bilaterians (Figure 1). Remarkably, Wnt genes are expressed in the posterior

of most bilaterians and are expressed in a polarized manner along the primary axis in pre-bilaterians (Figure 1). Additionally, perturbation of Wnt signaling has generated some of the most dramatic primary axis phenotypes yet uncovered (Figure 2). Taken together, the data demonstrate that Wnt signaling is broadly used in promoting posteriorly polarized features of the head-to-tail axis.

A Comparative Approach Reveals Central Features of Axis Development

To compare and contrast axis development across animals requires some description of terms for axial features. The primary axis typically coincides with the rostrocaudal (head-tail) and the anteroposterior (A-P, front-back) axes, with “anterior” and “posterior” most frequently used to describe primary axis polarity in bilaterally symmetric animals. In most species, before larval or adult forms are established, the embryo itself has an axis called the animal-vegetal axis. The animal pole is marked by polar bodies, whereas the vegetal pole often contains yolk, and germ layers (ectoderm, mesoderm, and endoderm) can be specified along the animal-vegetal axis. The relationship of the animal-vegetal axis to the primary axis of larvae or adults can vary across species, although in general the head forms from somewhere corresponding to the animal pole and the posterior/tail typically forms in an orientation corresponding to the vegetal pole (Martindale, 2005). Therefore the polarization of embryos along the animal-vegetal axis can influence the development of the anteroposterior axis.

The early physical events of axis development can differ dramatically among animals; this is probably because early embryonic development can be under very different selective conditions (e.g., for external or internal development). A brief survey of some of the commonly studied model organisms can illustrate this point. Extraembryonic tissues in the mouse can influence the polarity of the embryo itself, whereas many other

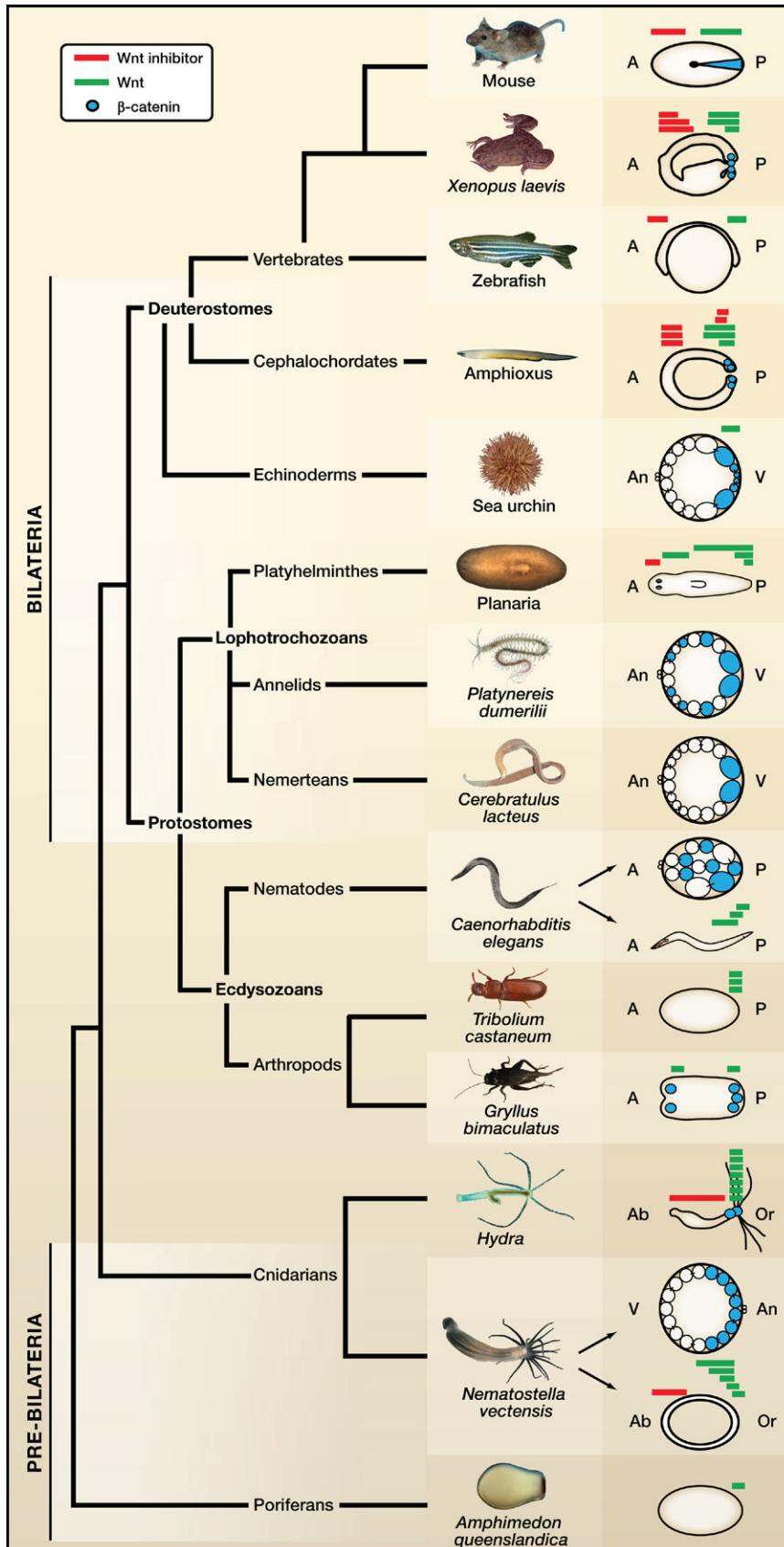


Figure 1. Posterior Wnt Signaling and Anterior Wnt Inhibition in the Metazoa

Animals are divided into pre-bilaterians that lack overt bilateral symmetry and bilaterians. Bilaterians are divided into deuterostomes and protostomes, and the latter are further divided into ecdysozoans and lophotrochozoans (Aoutte et al., 2000). Abundant nuclear β -catenin protein (blue) and Wnt gene expression domains (green) tend to be posterior, whereas Wnt inhibitor gene expression domains (red) tend to be anterior. For simplicity, in most cases only a single developmental stage is shown. (A) Anterior pole, (P) posterior pole, (An) animal pole, (V) vegetal pole, (Ab) aboral pole, (Or) oral pole. Note that in some cases, only the expression of a representative Wnt gene is shown. Early gastrulation stage mouse: cylinder-shaped embryo is depicted as a flattened projection onto two dimensions; β -catenin protein (blue triangle) and *Wnt3* expression are at the prospective primitive streak, and *Dkk1* is expressed in the anterior visceral endoderm (Glinka et al., 1998; Liu et al., 1999; Mohamed et al., 2004). Late gastrula stage *Xenopus*: *Xwnt3a*, *Xwnt8*, and *Xwnt11* and nuclear β -catenin protein are posterior and *Dkk1/2/4* and *sFRP3/4/frzb* anterior (Christian and Moon, 1993; McGrew et al., 1997; Glinka et al., 1998; Kiecker and Niehrs, 2001; Schohl and Fagotto, 2002). Zebrafish tailbud stage: *wnt8* and *wnt3a* are posterior and *sFRP3/frzb* is anterior (Lekven et al., 2001; Shimizu et al., 2005; Thorpe et al., 2005; Tendeng and Houart, 2006). Late gastrula stage amphioxus: *Wnt3a* and *Wnt8* are posterior and *Dkk3*, *Dkk1/2/4*, *sFRP2-like* are anterior. *sFRP3/4* and *Dkk1/2/4* are also posterior (Yu et al., 2007). Blastula stage sea urchin: vegetal β -catenin protein and *Wnt8* (Logan et al., 1999; Wikramanayake et al., 2004). Planaria: *Smed-wntP-1*, *Smed-wntP-2*, *Smed-wnt11-1* are posterior, *Smed-wnt2-1* is in the pre-pharyngeal region, and *Smed-sFRP-1* is anterior (Petersen and Reddien, 2008). Cleavage stage *Platynereis dumerilii*: β -catenin localizes to the vegetal cell of each axial division (Schneider and Bowerman, 2007). Blastula stage *Cerebratulus lacteus*: vegetal β -catenin protein (Henry et al., 2008). Cleavage stage *C. elegans* (above): asymmetric localization of WRM-1/ β -catenin and SYS-1/ β -catenin to the posterior daughters of each axial cell division (Rocheleau et al., 1997; Thorpe et al., 1997; Nakamura et al., 2005; Takeshita and Sawa, 2005; Huang et al., 2007). Larval stage *C. elegans* (below): *lin-44/Wnt*, *egl-20/Wnt*, and *cwn-1/Wnt* are posterior (Herman et al., 1995; Coudreuse et al., 2006; Pan et al., 2006). Blastoderm stage *Tribolium castaneum*: *WntA*, *Wnt1*, and *Wnt8* are posterior (Bolognesi et al., 2008a). Early segmentation stage *Gryllus bimaculatus*: *wingless/Wnt* and β -catenin protein are in the posterior growth zone and also in the anterior eye-forming zones (36 hr) (Miyawaki et al., 2004). Adult *Hydra*: nuclear β -catenin protein and expression of *Wnt3*, *Wnt9/10a*, *Wnt11*, *Wnt9/10c*, *Wnt1*, *Wnt16*, and *Wnt7* near the oral pole; *Dkk1/2/4* expression in body column (Hobmayer et al., 2000; Broun et al., 2005; Guder et al., 2006; Lengfeld et al., 2009). Blastula stage *Nematostella vectensis* (above): animal pole β -catenin protein (Wikramanayake et al., 2003). Planula larva stage *Nematostella vectensis* (below): expression of *WntA*, *Wnt1*, *Wnt4*, *Wnt7* orally, *Wnt2* mid-axially, and *Dkk1/2/4* aborally (Kusserow et al., 2005; Lee et al., 2006). Larval *Amphimedon queenslandica*: *Wnt* expression at pole (Adamska et al., 2007). Photo and drawing credits: C. Brown, N. Putnam, K. Tessmar-Raible, N. Meinke, P. Greb, M. Adamska, Southeastern Regional Taxonomic Center, Henry et al., 2008; Schneider and Bowerman, 2007; Yu et al., 2007.

Species and system	Wild-type	Decrease in Wnt signaling activity	Increase in Wnt signaling activity
A Mouse head-tail patterning			
B Chick lateral mesoderm			
C Frog neurectoderm			
D Zebrafish head-tail patterning			
E Planarian regeneration			
F Nemertean animal-vegetal patterning			
G <i>C. elegans</i> axial asymmetric cell division			
H Cnidarian oral-aboral patterning			

Figure 2. Perturbation of Wnt Signaling Causes Polarized Axial Defects

Regional markers or fates are depicted in orange and purple. A decrease in Wnt activity generally causes expansion of anterior markers and loss of posterior markers. Increase in Wnt activity has the opposite effect. Representative example systems are depicted.

(A–H) Anterior pole (A), bilateral animal pole (An), and cnidarian aboral pole (Ab), left. (A) Lateral view. Left, mouse embryo at 8.5 days post-coitus. Markers, *Otx2* (orange, forebrain) and *brachyury* (purple, primitive streak and notochord). Middle, *Wnt3* knockout mice fail to gastrulate and remain as a single epithelial sheet at 7.5 days post-coitus (Liu et al., 1999). Right, *APC* hypomorphic mutant (Ishikawa et al., 2003). (B) Dorsal view. Left, chick embryo mesoderm at stage 5 from which tissues were removed (small boxes within mesoderm) and grown as tissue explants (larger boxes above). Markers, *Nkx-2.5* (orange, heart muscle) or *globin* (purple, embryonic blood); primitive streak, black line. Middle, overexpression of *Dkk1* in posterior lateral mesoderm explants. Right, overexpression of *Wnt3a* and *BMP4* in anterior lateral mesoderm explants (Marvin et al., 2001). (C) Dorsal view. Left, neurula stage frog. Markers, *Bf1* (orange, prospective forebrain) and *Krox20* (purple, prospective hindbrain). Middle, *XDkk1* or *Xfrzb1* overexpression. Right, *Xwnt8* overexpression (Kiecker and Niehrs, 2001). (D) Lateral view. Left, zebrafish embryo from 90% epiboly to tailbud stages. Markers, *Otx2* or *anfl/Hesx1* (orange, forebrain), *cdx1a* (purple, posterior mesoderm) (Kim et al., 2000; Shimizu et al., 2005). Middle, injection of morpholinos targeting *wnt3a* and *wnt8* (Shimizu et al., 2005). Right, injection of *wnt3a* mRNA caused an increase in *cdx1a* expression (Shimizu et al., 2005) and the *headless/Tcf* loss-of-function mutation caused loss of *anfl/Hesx1* expression (Kim et al., 2000). (E) Dorsal view. Left, planarian after 14 days of regeneration following removal of head and tail. Markers, *sFRP-1* (orange, anterior pole), *frizzled-4* (purple, posterior pole), regenerated tissue (white), and pre-existing tissue (gray). Middle, RNA interference (RNAi) knockdown of β -catenin-1 (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). Right, RNAi knockdown of *APC-1* (Gurley et al., 2008). (F) Left, nemertean worm embryo at late blastula stage. Markers, prospective ectoderm (orange) and prospective endoderm (purple) as determined by lineage observation. Middle, morpholino-mediated knockdown of β -catenin. Right, overexpression of dominant-negative *GSK-3 β* (Henry et al., 2008). (G) Left, *C. elegans* progeny of EMS cell at the 4-cell stage. Markers, presumptive mesoderm daughter cell (orange, MS) and presumptive endoderm cell (purple, E) as determined by lineage observation. Middle, loss-of-function mutations in *mom-2/Wnt* or *wrm-1/ β -catenin* (Rocheleau et al., 1997; Thorpe et al., 1997). Right, loss-of-function mutation in *pop-1/Tcf* (Lin et al., 1995). (H) Left, blastula stage cnidarian *Clytia hemisphaerica*. Markers, *CheBra* (purple, prospective oral pole) and *CheFoxQ2a* (orange, prospective aboral pole). Middle, inhibition of *Wnt3* by morpholino injection. Right, overexpression of *Wnt3* by mRNA injection (Momose et al., 2008).

animals lack extraembryonic tissues altogether. Embryos of the frog *Xenopus laevis* have a large amount of embryonic yolk and undergo complex tissue movements during gastrulation (involution). By contrast, during gastrulation in many other embryos, tissue movements can involve invagination (as in sea urchins and amphioxus) or cell ingression (as in mice). The main axis is patterned all at once in the fruit fly *Drosophila*, but in most other animals the axis is formed gradually by patterning and growth. Furthermore, in *Drosophila*, transcription factor gradients in a syncytium pattern the A-P axis, whereas this mechanism does not occur in cellularized embryos. Development of the nematode *Caenorhabditis elegans* follows a reproducible cell lineage, whereas many other animals display more regulative development, in which cells can be replaced by neighboring cells and large regions of cells are patterned. Therefore, study of the molecular mechanisms controlling embryogenesis in these and other organisms is almost certain to reveal differences in the modes of creating and patterning the primary axis.

How can the different events of early animal embryogenesis be reconciled with seemingly similar head-to-tail body architectures and *Hox* expression domains? It is probable that clade-specific processes promote the emergence in development of conserved molecular processes. By comparing axial development in diverse species, conserved mechanisms can emerge (De Robertis, 2008). Identification of conserved mechanisms can reveal something fundamental about how most if not all animals are patterned. Additionally, interpretation of complex phenotypic data is facilitated by identification of unifying principles obtained from the study of different organisms. For example, diverse phenotypic consequences of *Hox* gene perturbation share a common principle that *Hox* genes control positional patterning.

The identification of a conserved role for signaling by bone morphogenetic protein (BMP) in dorsal-ventral (D-V) polarity in *Drosophila* and *Xenopus* provides an important and informative example of the distinction between conserved and clade-specific mechanisms of axis patterning (De Robertis and Sasai, 1996). The broad use of BMP signaling in dorsal-ventral patterning in animals, ranging from vertebrates (Khokha et al., 2005) to planarians (Molina et al., 2007; Orii and Watanabe, 2007; Reddien et al., 2007), is a striking example of similarity in body plan development. By contrast, the mechanisms used to establish dorsoventral asymmetry of BMP activity can be very different. In frogs, fertilization of the oocyte causes a cortical rotation and activation of β -catenin opposite the sperm entry point, resulting in dorsal establishment of Spemann's organizer. The organizer emits BMP inhibitory proteins (De Robertis, 2006). In contrast to how BMP signaling becomes polarized in *Xenopus*, in *Drosophila* a Toll-Dorsal signaling pathway results in a dorsal activity gradient of the BMP-like Decapentaplegic (Dpp) protein (Moussian and Roth, 2005). A Toll-Dorsal system is not found to regulate D-V polarity in other phyla. Therefore, although a BMP activity gradient along the D-V axis appears to be highly conserved, the mechanisms that establish BMP gradients along the D-V axis can be highly divergent. In this Review, we similarly compare data from different organisms related to Wnt signaling and axial development.

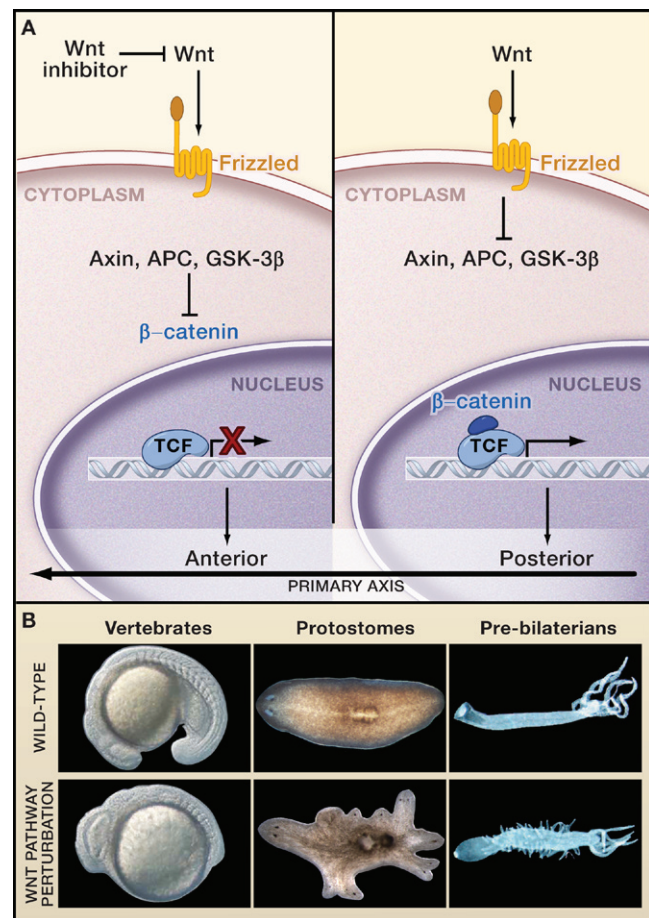


Figure 3. Wnt Signaling Controls Polarized Aspects of the Primary Axis

(A) The primary axis is typically polarized such that a head (in the anterior) and a tail (in the posterior) are found at opposite poles. Posterior canonical Wnt signaling and frequently anterior Wnt inhibition occur in animals throughout the Bilateria. Wnt signaling is also polarized along the primary axis of pre-bilaterians. In some animals, posterior pathway activation controls the patterning of large regions and in others it controls other polarized events such as asymmetric cell division.

(B) Wnt pathway perturbation causes primary axis defects throughout the Metazoa. Left, *wnt3a* and *wnt8* inhibition caused head enlargement and tail development failure in zebrafish (Shimizu et al., 2005, reprinted with permission from Elsevier). Middle, *Smed-βcatenin-1* RNA interference causes ectopic head formation in planarians (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). Right, β -catenin overactivation with GSK-3 β inhibition caused ectopic tentacle formation in *Hydra* (Broun et al., 2005, reproduced with permission of the Company of Biologists). Anterior or aboral pole, left.

Wnt Signal Transduction

In the canonical pathway, secreted Wnt proteins activate β -catenin proteins (Figure 3A; reviewed in Logan and Nusse, 2004). Wnts bind Frizzled receptors, resulting in alleviation of pathway inhibition caused by GSK-3 β , APC, and Axin proteins. This stabilizes β -catenin and promotes its nuclear translocation where it regulates target gene transcription together with Tcf/Lef proteins. Lithium chloride inhibits GSK-3 β and consequently is commonly used to ectopically activate the pathway in organisms lacking alternative means of genetic perturbation. Conversely, extracellular secreted molecules,

including members of the secreted Frizzled-related protein family (sFRPs) and Dickkopf-like proteins (Dkk), can inhibit Wnt signaling.

Animal genomes contain many genes encoding Wnts and Wnt antagonists, which can be expressed in various times and places during development. This complexity complicates analysis in multiple ways. For instance, the site of activity for β -catenin cannot always be predicted by the site of expression of particular Wnts. Reporter constructs for pathway activity could be a good way to refine knowledge of the site of Wnt action in many of the examples cited below. In addition, phenotypes caused by overactivation or inhibition of β -catenin can be complex and pleiotropic. Finally, comparing the role of Wnts in different organisms is complicated if orthology is unclear. For example, some Wnt proteins act in a β -catenin-independent manner, referred to as noncanonical Wnt signaling. We aim to discuss only canonical, β -catenin-dependent Wnt signaling in this Review. However, whether a particular Wnt acts in a β -catenin-dependent or -independent manner has not been determined in all cases.

Wnt Signaling Controls A-P Patterning in Diverse Deuterostomes

Observations in diverse deuterostomes (including frogs, fish, mammals, birds, amphioxus, and echinoderms) reveal effects of Wnt signaling or β -catenin activity on numerous aspects of primary axis polarity (Kiecker and Niehrs, 2001; Holland, 2002; Niehrs, 2004). Experiments on *Xenopus* neurectoderm illustrate a role for posterior Wnt signaling and anterior Wnt inhibition in A-P patterning and raise the possibility that Wnts may be important general posteriorizing factors (Kiecker and Niehrs, 2001).

A-P Patterning of Neurectoderm

The *Xenopus* neurectoderm extends across the length of the developing embryonic primary axis and gives rise to forebrain, midbrain, hindbrain, and spinal cord precursor tissues by the late gastrula stage. The elaboration of spinal cord, trunk, and tail occurs later during growth from the caudal end of the embryo. Increasing Wnt signaling can push neurectoderm cells toward a posterior identity (Figure 2C) (Christian and Moon, 1993; Fredieu et al., 1997; Kiecker and Niehrs, 2001), and decreasing Wnt signaling can push cells toward an anterior identity (Figure 2C) (Leyns et al., 1997; Wang et al., 1997; Glinka et al., 1998; Kiecker and Niehrs, 2001). Because of the early role of β -catenin in formation of the organizer, some key experiments involved Wnt perturbation after organizer formation but during neurectoderm patterning. At this late gastrula stage, Wnts are produced in the posterior, Wnt inhibitors are produced in the anterior, and there is a posterior to anterior gradient of nuclear β -catenin and Tcf-reporter activity (Figure 1) (Christian and Moon, 1993; McGrew et al., 1995; Leyns et al., 1997; McGrew et al., 1997, 1999; Glinka et al., 1998; Kiecker and Niehrs, 2001; Schohl and Fagotto, 2002).

Data in zebrafish indicate that neurectoderm patterning by Wnts does not reflect a frog-specific innovation. Wnts are expressed posteriorly in early gastrula, tailbud, and somite stages and a Wnt inhibitor is expressed in the anterior neurectoderm from the tailbud stage (Figure 1) (Kelly et al., 1995; Lekven et al., 2001; Shimizu et al., 2005; Tendeng and Houart, 2006). Overactivation of Wnt signaling in the zebrafish *headless* mutant, which

lacks Tcf3 function, results in anterior defects—an absence of eyes, forebrain, and midbrain (Figure 2D) (Kim et al., 2000). By contrast, inhibition of Wnt genes causes posterior defects (such as head enlargement and failure to form posterior mesoderm and maintain the growing tail bud) (Erter et al., 2001; Lekven et al., 2001; Rhinn et al., 2005; Shimizu et al., 2005; Thorpe et al., 2005). Taken together, this suggests a model for neurectoderm patterning in frog and fish in which domains within a field of cells acquire anterior versus posterior identity according to the local level of Wnt signaling (Kiecker and Niehrs, 2001).

An Early β -Catenin Function Impacts Later Axis Development in Frogs and Fish

What is the significance of the orientation of Wnt signaling in neurectoderm patterning? In addition to the A-P role for Wnt signaling for neurectoderm development, β -catenin also has a well-documented role earlier in embryogenesis that impacts development of the D-V axis in *Xenopus* and zebrafish. β -catenin is maternally provided and has an important role in establishment of Spemann's organizer in *Xenopus*. Following fertilization, cortical rotation results in localization of maternal β -catenin protein to the nuclei of cells that are on the prospective dorsal side of the embryo, opposite the point of sperm entry (Schneider et al., 1996; Larabell et al., 1997). Within this region, β -catenin promotes the formation of Spemann's organizer. Thus, any experiment that perturbs β -catenin activity at these early stages (before gastrulation has occurred) is anticipated to cause defects associated with altered activity of the Spemann's organizer. The outcome of such perturbation includes effects on the D-V axis. For example, early inhibition of β -catenin ultimately impairs dorsal tissue formation because of a requirement for Spemann's organizer in the generation of dorsal tissue (Heasman et al., 1994). Conversely, overactivation of β -catenin activity early in development (for example, with lithium chloride) results in dorsal-anteriorized embryos because of an expanded organizer (Kao and Elinson, 1988). It is later, after the mid-blastula transition when zygotic gene expression begins, that the role of β -catenin (in canonical Wnt signaling) in A-P tissue patterning is evident. Therefore, depending upon developmental stage, experiments that disrupt β -catenin activity can affect both D-V and A-P axis development in *Xenopus*. There is also evidence in *Xenopus* of signaling integration between Wnts and BMPs for coordinate axial patterning of the orthogonal A-P and D-V axes (Fuentealba et al., 2007).

Which aspects of Wnt signaling and β -catenin activity are conserved in other organisms? Is it orientation of signaling with respect to one or the other animal axis, neurectoderm regionalization, or simply general polarization of regions of an embryo? A role in influencing D-V polarization for β -catenin, or Wnt signaling, has not been broadly observed in animals. The simplest view is that the early role of β -catenin in *Xenopus* does not reflect an evolutionarily conserved role in dorsalization. To further understand the evolutionary implications of the orientation of Wnt signaling observed in the different stages of *Xenopus* development, data from additional organisms and tissue types are required (described below).

A-P Axial Patterning of Multiple Tissues

Does Wnt signaling affect the A-P patterning of multiple tissues? In the early mesoderm following gastrulation, the heart is derived from anterior lateral mesoderm, and embryonic

blood is made from posterior lateral mesoderm. Wnt inhibition in chicks promotes heart formation in the anterior and Wnt activation promotes embryonic blood formation in the posterior (Figure 2B) (Marvin et al., 2001). Furthermore, inhibition of β -catenin in the posterior endoderm in *Xenopus* causes ectopic organ buds that express early foregut, liver, and pancreas markers. Conversely, activation of Wnt signaling in the anterior endoderm eliminates foregut marker expression and expands hindgut marker expression (McLin, et al., 2007; Li et al., 2008). Therefore, posteriorizing Wnt activity and anteriorizing Wnt inhibition affect polarization of multiple types of tissues.

Primary Axis Development in Mice

Wnt signaling has multiple roles in the production and patterning of the mouse primary axis, some of which share similarity to A-P patterning of the frog and fish neurectoderm. Two embryo features correspond to future anterior and posterior ends: (1) the anterior visceral endoderm (formed 5.5 days post-coitum) marks the future anterior pole and (2) the primitive streak (formed beginning 6.5 days post-coitum) is the site of gastrulation and marks the future posterior pole. The anterior visceral endoderm expresses the Wnt inhibitor *Dkk1* (Glinka et al., 1998). Conversely, *Wnt3* expression, high levels of β -catenin protein, and TCF-responsive promoter expression are detected at the site of primitive streak formation in the embryo posterior (Figure 1) (Liu et al., 1999; Mohamed et al., 2004).

Wnt3 and β -catenin knockout mice fail to form the primitive streak (Figure 2A) (Haegel et al., 1995; Liu et al., 1999; Huelsken et al., 2000), whereas knockout of the Wnt inhibitor *Dkk1* results in an anterior truncation (Mukhopadhyay et al., 2001). Numerous experiments indicate that ectopic Wnt signaling activation causes anterior truncation and expansion or bifurcations of the primitive streak, resulting in partial axial structure duplications (Pöpperl et al., 1997; Zeng et al., 1997; Ishikawa et al., 2003; Merrill et al., 2004). These studies suggest that formation of a head in the anterior and a primitive streak in the posterior is regulated by the local level of Wnt signaling. In this way, the role for Wnt in early head-tail development within the mouse embryo could be viewed as similar to that for A-P patterning in frogs. However, because there are numerous differences in the mechanisms of gastrulation between mammals and amphibians, it is difficult to conclude from these two clades alone that the similarity in positional use of Wnt signaling represents true conservation of axial control mechanisms.

Primary Axis Polarity in Basal Deuterostomes

The study of Wnt signaling in basal deuterostomes (Figure 1) can help determine whether the axial roles of Wnt signaling, as seen in vertebrates, are the result of conservation from the ancestral condition or are independently derived. β -catenin controls polarization along the animal-vegetal axis of sea urchins (echinoderms), where it is active in the vegetal pole (Wikramanayake et al., 1998, 2004; Logan et al., 1999). Ectoderm arises from the sea urchin animal pole, and endoderm and mesoderm arise from the vegetal pole. Overactivation of β -catenin results in vegetalized embryos with ectopic endoderm, whereas inhibition of Wnt signaling prevents endoderm formation. Sea urchins lack a recognizable head-tail axis, making it difficult to compare the orientation of Wnt signaling in these organisms with other deuterostomes; however, the head

of other animals frequently emerges from the animal pole (Martindale, 2005). Ascidians (urochordates) and starfish (echinoderms) are other basal deuterostomes that have nuclear-localized β -catenin in the vegetal pole (Imai et al., 2000; Miyawaki et al., 2003). A role for β -catenin proteins in early animal-vegetal polarization is seen in multiple organisms, with β -catenin vegetally localized in at least some basal deuterostomes and protostomes and localized to the animal pole of the pre-bilaterian cnidarians (Figure 1). However, some deuterostomes do not clearly display this animal-vegetal role for β -catenin. For example, following cortical rotation in *Xenopus*, β -catenin is dorsally localized at the site of gastrulation initiation and is required for dorsalizing the mesoderm, but it is not required for endoderm formation, mesoderm formation, or gastrulation (Heasman et al., 1994; Schneider et al., 1996).

Cephalochordates (amphioxus) are basal chordates. In this species, the blastopore (localized to the vegetal pole) marks the prospective posterior end of the embryo. Nuclear β -catenin is present and several Wnts are expressed posteriorly around the blastopore, although weaker staining of nuclear β -catenin is detected in the anterior ectoderm (Figure 1) (Holland et al., 2005; Yu et al., 2007). In addition, Wnt antagonists are expressed anteriorly, although two are also expressed in the posterior (Yu et al., 2007). Overactivation of β -catenin in amphioxus (with lithium chloride) caused apparent loss of the neural plate and posteriorization (Holland et al., 2005; Onai et al., 2009). These data suggest that β -catenin has a conserved role in promoting A-P axis development rather than in controlling the D-V axis (Holland, 2002). Together, the data from basal deuterostomes suggest the intriguing possibility that β -catenin activity and Wnt signaling might generally act to polarize early embryos and pattern the primary axis (Holland, 2002; Yu et al., 2007).

Trunk and Tail Outgrowth

In many animals, initial axis formation and patterning are followed by a period of trunk and tail development in the posterior. Wnts act subsequent to initiation of gastrulation in the posterior of the mouse embryo to form and pattern trunk and tail mesoderm. At 7.5 days post-coitum, *Wnt3* expression subsides and *Wnt3a* becomes expressed in the primitive streak. A spontaneous hypomorphic allele of *Wnt3a*, *vestigial tail*, lacks a tail and *Wnt3a* null animals lack a tail bud and somites posterior to the forelimb (Takada et al., 1994). As the embryo grows posteriorly, a complex mechanism controls the timing and patterning of somite formation in the trunk and tail (Dubrulle and Pourquié, 2004). There is a posterior-to-anterior gradient of nuclear β -catenin in the growing tail, and *Wnt3a* determines the anterior-posterior position of the somite determination front (Aulehla et al., 2008; Dunty et al., 2008).

The continued involvement of Wnt signaling in the posterior for trunk and tail formation appears widespread (Martin and Kimelman, 2009). For instance, Wnt signaling is involved in tail development in zebrafish (Agathon et al., 2003; Shimizu et al., 2005; Thorpe et al., 2005), and some *Wnt* genes are expressed during posterior growth at the amphioxus tail bud during larval development (Schubert et al., 2001). These data indicate that many animals may utilize Wnt signaling at the posterior in a phase of trunk/tail outgrowth and patterning following initial establishment of head-to-tail axis polarity.

Wnt Signaling Controls A-P Tissue Identity in Protostomes

Although data from deuterostomes suggest that Wnt signaling has an important general role in promoting polarized A-P features, there are multiple possible interpretations for the data. For example, it is possible that some defects caused by Wnt perturbation reflect a role for Wnt signaling/ β -catenin in a process occurring at a polarized location on the prospective A-P axis (such as endoderm specification, gastrulation, or growth), rather than a role in A-P polarity regulation per se. Additionally, Wnt signaling may have been recruited independently to accomplish embryonic patterning tasks in the examined deuterostomes. Further investigation of the cases described above will be important for clarifying the roles of Wnts in each organism. However, examination of Wnt signaling in distantly related protostomes (which diverged from deuterostomes over 500 million years ago) allows assessment of alternative explanations for phenotypes associated with Wnt perturbation. Because particular physical processes will differ in nature and position, or even not occur at all in distantly related animals, similarities and differences in the role of Wnt signaling can help distinguish between models for Wnt signaling roles in axis development. Ubiquitous positional aspects of Wnt phenotypes, for instance, support the model of Wnt signaling in control of A-P polarity. Below, relevant data from the two major groupings of protostome phyla, the Lophotrochozoa and Ecdysozoa (Figure 1), are described.

A-P Polarity in Planarians

Planarians are freshwater flatworms, members of the Lophotrochozoa, and are famous for their ability to regenerate a head or a tail following transection (Reddien and Sánchez Alvarado, 2004). Because of their ability to re-establish axial identities in regeneration, planarians provide a system with which to uncover properties of axis polarization that are independent of the complicated tissue movements and developmental events that coordinately occur during gastrulation. Five of the nine planarian Wnt genes are expressed in domains along the A-P axis, predominantly in the posterior, and a Wnt inhibitor-like gene is expressed at the anterior pole (Figure 1) (Petersen and Reddien, 2008).

RNA interference of a β -catenin gene in the planarian *S. mediterranea* results in regeneration of posterior-facing heads rather than tails, indicating that planarian β -catenin controls the tail-versus-head decision at posterior-facing wounds (Figure 2E) (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). Intact planarians constantly maintain their bodies during homeostasis. Inhibition of β -catenin-1 causes intact animals to eventually develop ectopic heads, indicating a requirement for β -catenin-1 in maintaining A-P axial identity (Figure 3B). Inhibition of the β -catenin-inhibitory APC-like gene results in regeneration of tails from anterior-facing wounds (Gurley et al., 2008), suggesting that the head-versus-tail identity is a binary choice controlled by high β -catenin activity at posterior-facing wounds and low β -catenin activity at anterior-facing wounds. Wnt genes are required for this polarity process (Adell et al., 2009; Petersen and Reddien, 2009). Together, these results are consistent with a hypothesis that posterior Wnt signaling and anterior Wnt inhibition were present in the ancestor of bilaterally symmetric animals and are conserved features of protos-

tome and deuterostome axial patterning. The fact that planarians use this pathway in A-P axis regeneration independent of the complexities of early development further suggests the possible existence of a common role of Wnt signaling in regulating positional features rather than solely a common role in regulating any particular process.

β -Catenin Can Control Animal-Vegetal Identity in Protostomes

Does β -catenin control early embryonic aspects of axis development in protostomes? In the indirectly developing nemertean worm *Cerebratulus lacteus* (lophotrochozoans, Figure 1), the larval animal-vegetal axis corresponds in direction of movement to the head-tail axis of most other animals (Henry et al., 2008). Green fluorescent protein-tagged β -catenin has highest abundance in the four vegetal-most cells at the 64-cell stage (Figure 1). Morpholino knockdown of β -catenin causes animalization of the early embryo (vegetal fates of gastrulation and endoderm formation are impaired, and animal pole structures are expanded). By contrast, gain of function of β -catenin causes vegetalization (Figure 2F) (Henry et al., 2008). Therefore, β -catenin appears to promote vegetal as opposed to animal fates. This observation is reminiscent of the observed role for β -catenin in the vegetal pole of sea urchins. After metamorphosis, the main body axis of the adult *Cerebratulus* is orthogonal to the larval animal-vegetal axis, and because the larva lacks a brain, it is unknown whether this truly corresponds to the bilaterian A-P axis. However, these data illustrate that in a distantly related protostome, β -catenin is likely involved in early embryonic axial polarization.

Axial Asymmetric Cell Divisions

Unlike many other species, development in the nematode *C. elegans* (a member of the ecdysozoans; Figure 1) occurs via highly reproducible cell lineages rather than patterning of tissue fields. In spite of this difference in development, posteriorly expressed Wnt genes control posterior versus anterior developmental decisions throughout *C. elegans* embryonic and postembryonic development. During most embryonic A-P asymmetric cell divisions, two β -catenins (WRM-1 and SYS-1) are localized to the posterior daughter cell (Figure 1) (Nakamura et al., 2005; Takeshita and Sawa, 2005; Huang et al., 2007). In one well-studied cell division at the 4-cell stage, the posterior P2 cell secretes MOM-2/Wnt to control asymmetric division of the neighboring EMS cell, resulting in an anterior daughter cell (MS) that forms mesoderm and a posterior daughter cell (E) that forms endoderm. Loss of function of *mom-2/Wnt* or *wrm-1/ β -catenin* causes the posterior E cell to adopt the anterior fate of mesoderm formation (Figure 2G) (Rocheleau et al., 1997; Thorpe et al., 1997). Conversely, loss of function of the *pop-1/Tcf* repressor causes the anterior MS cell to adopt the posterior fate of endoderm formation (Lin et al., 1995). Therefore, a posteriorly expressed Wnt gene controls posterior-versus-anterior cell fate at the 4-cell stage.

Posteriorly expressed Wnt genes continue to regulate A-P asymmetric cell division during postembryonic development as well. Strikingly, expression of three of the five *C. elegans* Wnt homologs—*lin-44/Wnt*, *egl-20/Wnt*, and *cwn-1/Wnt*—is localized to the posterior end of the animal from late embryogenesis throughout larval development (Figure 1) (Herman et

al., 1995; Coudreuse et al., 2006; Pan et al., 2006) and can control the A-P polarity of axial asymmetric cell divisions (Herman and Horvitz, 1994; Goldstein et al., 2006). Posteriorly expressed Wnt genes also control other cellular processes that are polarized along the *C. elegans* primary axis, including posterior neuroblast migration, A-P axon outgrowth (reviewed in Silhankova and Korswagen, 2007), and posterior inhibition of synapse formation in a motoneuron (Klassen and Shen, 2007). These data show that Wnt genes expressed in the posterior control multiple A-P polarized cellular events in *C. elegans*.

Wnt signaling also controls axial asymmetric cell division in the annelid *Platynereis dumerilii* (members of the Lophotrochozoa; Figure 1). β -catenin is asymmetrically localized along the animal-vegetal axis, such that higher levels are present in the vegetal daughter cell of each early division (Figure 1) (Schneider and Bowerman, 2007). Overactivation of β -catenin causes animal daughters of axial cell divisions to adopt vegetal fates, indicating that β -catenin promotes non-animal-pole identities during axial asymmetric cell divisions. *Platynereis* development is indirect, with embryogenesis producing a trochophore larva that later undergoes metamorphosis to form the adult worm. Because the animal pole in most animals is aligned with head formation and because head-like cells form in the animal end of *Platynereis* larvae (Schneider and Bowerman, 2007), the role of β -catenin in A-V cell division polarity might be similar to its role in A-P cell-division polarity in *C. elegans*.

The role of Wnt signaling in *C. elegans* and *Platynereis* appears to differ from other examined species in that it involves control of the polarity of individual cell divisions as opposed to broadly controlling posterior identity of tissue regions. One possibility is that both *C. elegans* and *Platynereis* have evolved a derived version of the polarizing role of β -catenin, in which it is active in vegetal or posterior cells in many asymmetric divisions as opposed to patterning a large region of cells (Schneider and Bowerman, 2007). Despite this difference, the similar role in control of polarized events on the primary axis in general, and in promoting posterior in particular, is conspicuous. At least in the case of *C. elegans*, other processes, such as axon outgrowth, cell migration, and synapse formation, are influenced by a later posterior source of Wnts. Together, these data support the view that during evolution Wnt signaling has come to be used in a diverse manner in different metazoans to control posterior positional identity of A-P polarized events.

Posterior Growth in Protostomes

At first glance, insects might appear to be an exception to the hypothesized ubiquity of Wnt regulation of A-P axis patterning because of lack of clear evidence for such a role in *Drosophila*. The best-characterized role for Wnt signaling in primary axis development in *Drosophila* involves Wingless/Wnt and the reiterated A-P tissue pattern during segmentation along the primary axis. *wingless* is expressed in the posterior of each parasegment and *wingless* mutants have segment polarity defects (Nüsslein-Volhard and Wieschaus, 1980). *Drosophila* is a long-germ developing insect, in which all body segments are formed simultaneously, and undergoes an unusual mode of axis formation initiated by transcription factor gradients within

a syncytial blastoderm. Many other arthropods, such as the beetle *Tribolium castaneum*, the cricket *Gryllus bimaculatus*, or the spider *Achaearanea tepidariorum*, undergo short or intermediate-germ development, in which the anterior segments are specified before gastrulation and the posterior segments are specified during subsequent posterior outgrowth.

Data in multiple arthropods indicate a role for Wnt in posterior growth and development. Three *Tribolium* Wnt genes are expressed at the posterior pole in the early blastoderm stage (Bolognesi et al., 2008a). In addition, Wnt genes are expressed in the posterior growth zone in *Gryllus*, *Tribolium*, and *Achaearanea* (Figure 1). Knockdown of *armadillo*/ β -catenin in *Gryllus*, *Wnt8/D* in *Tribolium*, or *Wnt8* in the *Achaearanea*, cause defects in posterior growth (Miyawaki et al., 2004; Bolognesi et al., 2008b; McGregor et al., 2008). Therefore, Wnt signaling can control posterior growth in arthropods. There exists similar gene expression for *caudal*, *even-skipped*, and *brachyury* in a posterior growth zone of the annelid *Platynereis* and vertebrates (de Rosa et al., 2005). It has been argued that a posterior growth phase controlling posterior body development is broadly conserved in the Bilateria (de Rosa et al., 2005; Martin and Kimelman, 2009). The mechanisms by which Wnt signaling controls posterior body formation in arthropods and other organisms will be an important future area of study.

In summary, roles for β -catenin and Wnt signaling in protostomes include head-to-tail axis maintenance and regeneration, animal-vegetal polarization of early embryos, asymmetric cell divisions, cell migrations, axon outgrowth, synapse formation, and posterior growth and development. In all of these cases, Wnts and/or β -catenin are active in the posterior or vegetal orientation. Therefore, β -catenin and Wnt signaling can influence the earliest animal-vegetal polarization events in protostomes as well as later A-P polarized events. These observations share similarities in the orientation and polarizing role for Wnt signaling or β -catenin activity to observations in deuterostomes described above. The diversity of developmental stages and physical processes under the influence of Wnt signaling and/or β -catenin activity make it difficult to identify a single ancestral and conserved process controlled by this pathway. One possibility is that polarized pathway activity along the primary axis represents the ancestral condition and that as animals diversified, multiple processes independently evolved to come under the control of polarized Wnt signaling or β -catenin activity. Examination of pre-bilaterians (described below) can help to identify the candidate role(s) of Wnt signaling and β -catenin activity that represents the ancestral state for the Bilateria.

Wnt Signaling Controls Axial Identity in Pre-Bilaterians

Basal metazoans such as cnidarians and sponges diverged from bilaterians over 500 million years ago and lack overt bilateral symmetry. Experiments in these animals can help identify developmental mechanisms present in the ancestors of all bilaterians. For example, the anthozoan sea anemone *Nematostella vectensis* has emerged as a model system for studying deeply conserved processes in animal evolution (Putnam et al., 2007). What roles do Wnt signaling and β -catenin activity have in body plan development in these pre-bilaterians?

β-Catenin and Wnt Signaling Control Animal-Vegetal Axis Polarity in Cnidarians

Cnidarians have a primary axis, the oral-aboral axis, with overt radial symmetry about it. This anatomy allows analysis of axis development in animals serving as outgroups to the bilaterians. Because cnidarians lack many of the features of the bilaterian primary axis (for example, a recognizable brain), it has been difficult to ascertain the relationship between the cnidarian primary axis and the bilaterian primary axis. Comparisons of gene expression patterns, however, suggest that the cnidarian oral-aboral axis might be similar to the bilaterian anteroposterior axis. The oral end of the cnidarian axis emerges from the animal pole and the aboral end emerges from the vegetal pole.

Nuclear β -catenin is abundant near the site of gastrulation in the animal pole in cnidarians (Wikramanayake et al., 2003; Momose et al., 2008). In cnidarians, gastrulation occurs and endoderm is formed in the animal pole, whereas ectoderm is derived from the vegetal pole (Martindale, 2005). Inhibition of β -catenin in *Nematostella* with dominant-negative constructs causes defects in processes occurring in the animal pole (gastrulation and endoderm formation) (Wikramanayake et al., 2003). Conversely, overactivation of β -catenin by lithium chloride treatment in *Nematostella* results in an apparent expansion of animal pole-derived endoderm (Wikramanayake et al., 2003). Similar observations have been made in the hydrozoan jellyfish *Clytia hemisphaerica*. Morpholino knockdown of *Clytia Wnt3* leads to loss of oral tissue identity and an expansion of aboral tissue (Figure 2H) (Momose et al., 2008). Overexpression of *Wnt3* in *Clytia* causes an expansion of animal/oral tissue identity at the expense of vegetal/aboral identity (Momose et al., 2008). Together, these results suggest that Wnts/ β -catenin control animal-vegetal polarization in cnidarian embryogenesis. These data are reminiscent of the vegetal pole localization of β -catenin in sea urchins (basal deuterostome) and nemertean worms (lophotrochozoan) described above, but with opposite orientation. It is possible that animal-vegetal polarized β -catenin represents the ancestral state for bilaterians, and that its orientation with respect to the animal-vegetal axis has inverted in either bilaterians or cnidarians (Martindale and Hejnal, 2009).

Primary Axis Polarity in Cnidarians

Does Wnt signaling also pattern the cnidarian primary axis after early development? Remarkably, multiple *Nematostella* Wnt genes are expressed in domains along the oral-aboral axis throughout embryonic and larval development, with a bias toward the oral end (Figure 1) (Kusserow et al., 2005). Similarly, in *Clytia*, five Wnt genes are expressed in nested axial domains emanating from the oral pole throughout embryonic and larval development (Momose et al., 2008). By contrast, a Wnt inhibitor is expressed at the opposite end (aborally) of *Nematostella* larva (Lee et al., 2006). These data indicate that, as seen in deuterostomes and protostomes, Wnt signaling occurs in a polarized manner on the primary axis.

Further evidence for a continued requirement for Wnt signaling in postembryonic cnidarian development comes from studies on adult freshwater *Hydra*, which are capable of regenerating an entire body from tissue fragments. Expression of a *Hydra* Wnt inhibitor is observed within the body column (Guder

et al., 2006), and expression of *Hydra* Wnt genes and the highest levels of nuclear β -catenin are found at the oral terminus (Figure 1) (Hobmayer et al., 2000; Broun et al., 2005; Lengfeld et al., 2009). Overactivation of Wnt signaling (with GSK-3 β inhibition) results in a striking phenotype involving ectopic tentacles throughout the body column, suggesting that spatial restriction of β -catenin activity is necessary to limit the region containing cell types or structures associated with the oral pole (Figure 3B) (Broun et al., 2005). Therefore, Wnt signaling might control fate along the primary axis in adult cnidarians.

In summary, an early role for β -catenin in animal-vegetal axis polarization and a later role for Wnt signaling in primary axis polarization are both observed in cnidarians. β -catenin is active in the animal pole and Wnts are predominantly expressed at the oral end that develops from the animal pole. This common orientation of early and later roles for β -catenin activity is reminiscent of the vegetal (sea urchin, ascidian, nemertean) and posterior roles for β -catenin/Wnt signaling (*Xenopus*, zebrafish, mouse, planarian, nematode, arthropods) observed in multiple bilaterians. On the basis of *Wnt* gene expression, the cnidarian oral end might correspond to the bilaterian posterior. On the basis of transcription factor expression along the main axis, however, the oral end of different cnidarians has been proposed to correspond to either the bilaterian anterior or posterior (Yanze et al., 2001; Finnerty et al., 2004). Whether there is an orientation of Wnt signaling with respect to other features of the primary axis (e.g., Hox expression) that is opposite to that in bilaterians therefore remains an important area of investigation (Martindale and Hejnal, 2009). Regardless, these data suggest that a role for β -catenin/Wnt signaling in polarization of the primary axis might predate the emergence of the Bilateria.

Polarized Wnt Expression in Sponge Larva

The irregular form of adult sponges might at first indicate that they have no axis at all. However, the embryos of these early diverging animals are free swimming and have some hallmarks of axial polarity. After gastrulation, larva of the haplosclerid demosponge *Amphimedon queenslandica* form a pigmentation spot at the pole away from the direction of movement. Although there is currently no gene function data available, an *Amphimedon* Wnt is expressed at the pigmented pole (Figure 1) (Adamska et al., 2007), consistent with the possibility of a role of Wnt signaling in primary axis development in the earliest multicellular animals.

Conclusions

The processes involved in the formation and patterning of the animal main axis have fascinated biologists for much of the past century. An increasing body of work, as summarized here, indicates that most animals utilize canonical Wnt signaling to control features of the primary axis (Figure 3A). Wnt pathway perturbation causes dramatic axial consequences throughout the animal kingdom (Figure 3B). Remarkably, the spatial orientation of Wnt signaling (active in the posterior and often involving anterior Wnt inhibition) is found in nearly all animals examined, suggesting that this orientation is representative of the ancestral condition for the Bilateria. β -catenin activity is also often polarized along the animal-vegetal axis of animals, before gastrulation

has occurred and the anteroposterior axis has developed (Martindale, 2005). After initial anteroposterior axis formation, Wnt signaling also often has a role in posterior growth (Martin and Kimelman, 2009). In support of the notion of an ancestral state involving Wnt signaling in the regulation of polarized features of the primary axis, pre-bilaterian cnidarians possess axial polarity of Wnt expression, in which Wnts are expressed at the oral pole and Wnt inhibitors are expressed aborally. The hypothesized central role of Wnt signaling in promoting posterior rather than anterior aspects of animal tissue provides a candidate explanation for a large array of data and predicts that additional posterior-promoting roles for Wnts will continue to be uncovered.

What is the significance of the fact that a great diversity of processes—ranging from neurectoderm patterning to regeneration polarity—are under control of Wnt signaling along the primary axis? Many of these varied processes undoubtedly have distinct evolutionary origins yet appear to share similarity in usage of posterior Wnt signaling. One possibility is that a prominent conserved aspect of Wnt signaling in development is to convey position along the primary axis rather than to control any particular process. Presented data indicate that Wnt signaling might have been involved in the polarization of the primary axis in the very earliest animals. It is therefore possible that there existed an ancestral state of posterior Wnt signaling that came to regulate many processes, such as neurectoderm development, regeneration, posterior growth, and/or head-tail identity, as animals diversified. Derived usage of the polarizing role of Wnt signaling may have evolved in some animals, for example to control asymmetric cell divisions in *C. elegans* and *Platynereis* or to establish segment polarity in arthropods.

Other interpretations of the data presented here are possible, although such interpretations are generally less parsimonious than the model that canonical Wnt signaling has a conserved role in regulating positional patterning of the primary body axis. For example, it is possible that Wnt signaling has been recruited variably and independently to axial patterning throughout evolution. However, this hypothesis fails to explain why so many studied animals employ Wnt signaling in A-P axis development in particular and with the same polarity (Wnt activation posterior and Wnt inhibition anterior). Another possible interpretation for some of the data would be that Wnt signaling/ β -catenin controls a particular activity (such as growth or gastrulation) that commonly occurs in the posterior or vegetal pole. However, given the large morphological diversity of animals that utilize Wnt signaling to control aspects of the primary axis, it seems that no particular regional structure or process could account for all of the data. The continued exploration of roles for Wnt signaling in axis polarity in diverse organisms will be important for assessing whether specific roles in different animals reflect conservation of polarity control through common ancestry.

Together, a wealth of data demonstrates that the use of canonical Wnt signaling in control of posterior polarized tissue identity is remarkably widespread, similar in its ubiquity to the axial expression of *Hox* genes or the utilization of BMP signaling to pattern the D-V axis. These observations indicate that A-P patterning by posterior Wnt activation and anterior Wnt inhibition is a core determinate of polarity along the metazoan primary axis.

ACKNOWLEDGMENTS

We acknowledge support by NIH R01GM080639 and ACS RSG-07-180-01-DDC, an American Cancer Society postdoctoral fellowship to C.P.P., and Rita Allen, Searle, Smith, and Keck Foundation support to P.W.R. P.W.R. is an HHMI Early Career Scientist. The authors thank T. DiCesare for illustration assistance, and E. Andersen, I. Cheeseman, C. Extavour, R. Harland, C. Lowe, H. Sive, and members of the Reddien laboratory for helpful comments and criticism.

REFERENCES

- Adamska, M., Degnan, S.M., Green, K.M., Adamski, M., Craigie, A., Larroux, C., and Degnan, B.M. (2007). Wnt and TGF- β expression in the sponge *Amphimedon queenslandica* and the origin of metazoan embryonic patterning. *PLoS ONE* 2, e1031.
- Adell, T., Saló, E., Boutros, M., and Bartscherer, K. (2009). *Smed-Evi/Wntless* is required for β -catenin-dependent and -independent processes during planarian regeneration. *Development* 136, 905–910.
- Adoutte, A., Balavoine, G., Lartillot, N., Lospinet, 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.
- Aulehla, A., Wiegraebe, W., Baubet, V., Wahl, M.B., Deng, C., Taketo, M., Lewandoski, M., and Pourquié, O. (2008). A β -catenin gradient links the clock and wavefront systems in mouse embryo segmentation. *Nat. Cell Biol.* 10, 186–193.
- Bolognesi, R., Beermann, A., Farzana, L., Wittkopp, N., Lutz, R., Balavoine, G., Brown, S.J., and Schröder, R. (2008a). *Tribolium* Wnts: evidence for a larger repertoire in insects with overlapping expression patterns that suggest multiple redundant functions in embryogenesis. *Dev. Genes Evol.* 218, 193–202.
- Bolognesi, R., Farzana, L., Fischer, T.D., and Brown, S.J. (2008b). Multiple Wnt genes are required for segmentation in the short-germ embryo of *tribolium castaneum*. *Curr. Biol.* 18, 1624–1629.
- 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.
- Christian, J.L., and Moon, R.T. (1993). Interactions between *Xwnt-8* and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. *Genes Dev.* 7, 13–28.
- Coudreuse, D.Y., Roël, G., Betist, M.C., Destrée, O., and Korswagen, H.C. (2006). Wnt gradient formation requires retromer function in Wnt-producing cells. *Science* 312, 921–924.
- de Rosa, R., Prud'homme, B., and Balavoine, G. (2005). Caudal and even-skipped in the annelid *Platynereis dumerilii* and the ancestry of posterior growth. *Evol. Dev.* 7, 574–587.
- De Robertis, E.M. (2006). Spemann's organizer and self-regulation in amphibian embryos. *Nat. Rev. Mol. Cell Biol.* 7, 296–302.
- De Robertis, E.M. (2008). Evo-devo: variations on ancestral themes. *Cell* 132, 185–195.
- De Robertis, E.M., and Sasai, Y. (1996). A common plan for dorsoventral patterning in Bilateria. *Nature* 380, 37–40.
- Dubrulle, J., and Pourquié, O. (2004). Coupling segmentation to axis formation. *Development* 131, 5783–5793.
- Dunty, W.C., Jr., Biris, K.K., Chalamalasetty, R.B., Taketo, M.M., Lewandoski, M., and Yamaguchi, T.P. (2008). Wnt3a/ β -catenin signaling controls posterior body development by coordinating mesoderm formation and segmentation. *Development* 135, 85–94.
- 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 posterioriza-

tion in vivo. *Development* 128, 3571–3583.

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.

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.

Fredieu, J.R., Cui, Y., Maier, D., Danilchik, M.V., and Christian, J.L. (1997). *Xwnt-8* and lithium can act upon either dorsal mesodermal or neurectodermal cells to cause a loss of forebrain in *Xenopus* embryos. *Dev. Biol.* 186, 100–114.

Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C., and Niehrs, C. (1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391, 357–362.

Goldstein, B., Takeshita, H., Mizumoto, K., and Sawa, H. (2006). Wnt signals can function as positional cues in establishing cell polarity. *Dev. Cell* 10, 391–396.

Guder, C., Pinho, S., Nacac, T.G., Schmidt, H.A., Hobmayer, B., Niehrs, C., and Holstein, T.W. (2006). An ancient Wnt-Dickkopf antagonism in *Hydra*. *Development* 133, 901–911.

Gurley, K.A., Rink, J.C., and Sánchez Alvarado, A. (2008). β -catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science* 319, 323–327.

Haegel, H., Larue, L., Ohsugi, M., Fedorov, L., Herrenknecht, K., and Kemler, R. (1995). Lack of β -catenin affects mouse development at gastrulation. *Development* 121, 3529–3537.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, 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). β -catenin is required for the establishment of vegetal embryonic fates in the nemertean, *Cerebratulus lacteus*. *Dev. Biol.* 317, 368–379.

Herman, M.A., and Horvitz, H.R. (1994). The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions. *Development* 120, 1035–1047.

Herman, M.A., Vassilieva, L.L., Horvitz, H.R., Shaw, J.E., and Herman, R.K. (1995). The *C. elegans* gene *lin-44*, which controls the polarity of certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. *Cell* 83, 101–110.

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. (2002). Heads or tails? Amphioxus and the evolution of anterior-posterior patterning in deuterostomes. *Dev. Biol.* 247, 209–228.

Holland, L.Z., Panfilio, K.A., Chastain, R., Schubert, M., and Holland, N.D. (2005). Nuclear β -catenin promotes non-neural ectoderm and posterior cell fates in amphioxus embryos. *Dev. Dyn.* 233, 1430–1443.

Huang, S., Shetty, P., Robertson, S.M., and Lin, R. (2007). Binary cell fate specification during *C. elegans* embryogenesis driven by reiterated reciprocal asymmetry of TCF POP-1 and its coactivator β -catenin SYS-1. *Development* 134, 2685–2695.

Huelsken, J., Vogel, R., Brinkmann, V., Erdmann, B., Birchmeier, C., and Birchmeier, W. (2000). Requirement for β -catenin in anterior-posterior axis formation in mice. *J. Cell Biol.* 148, 567–578.

Iglesias, M., Gomez-Skarmeta, J.L., Saló, E., and Adell, T. (2008). Silencing of *Smed- β -catenin1* generates radial-like hypercephalized planarians. *Development* 135, 1215–1221.

Imai, K., Takada, N., Satoh, N., and Satou, Y. (2000). β -catenin mediates the specification of endoderm cells in ascidian embryos. *Development* 127,

3009–3020.

Ishikawa, T.O., Tamai, Y., Li, Q., Oshima, M., and Taketo, M.M. (2003). Requirement for tumor suppressor Apc in the morphogenesis of anterior and ventral mouse embryo. *Dev. Biol.* 253, 230–246.

Kao, K.R., and Elinson, R.P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* 127, 64–77.

Kelly, G.M., Greenstein, P., Erezylmaz, D.F., and Moon, R.T. (1995). Zebrafish *wnt8* and *wnt8b* share a common activity but are involved in distinct developmental pathways. *Development* 121, 1787–1799.

Khokha, M.K., Yeh, J., Grammer, T.C., and Harland, R.M. (2005). Depletion of three BMP antagonists from Spemann's organizer leads to a catastrophic loss of dorsal structures. *Dev. Cell* 8, 401–411.

Kiecker, C., and Niehrs, C. (2001). A morphogen gradient of Wnt/ β -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.

Klassen, M.P., and Shen, K. (2007). Wnt signaling positions neuromuscular connectivity by inhibiting synapse formation in *C. elegans*. *Cell* 130, 704–716.

Kusserow, A., Pang, K., Sturm, C., Hrouda, M., Lentfer, J., Schmidt, H.A., Technau, U., von Haeseler, A., Hobmayer, B., Martindale, M.Q., and Holstein, T.W. (2005). Unexpected complexity of the Wnt gene family in a sea anemone. *Nature* 433, 156–160.

Larabell, C.A., Torres, M., Rowing, B.A., Yost, C., Miller, J.R., Wu, M., Kimelman, D., and Moon, R.T. (1997). Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. *J. Cell Biol.* 136, 1123–1136.

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.

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.

Leyns, L., Bouwmeester, T., Kim, S.H., Piccolo, S., and De Robertis, E.M. (1997). Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 88, 747–756.

Li, Y., Rankin, S.A., Sinner, D., Kenny, A.P., Krieg, P.A., and Zorn, A.M. (2008). Sfrp5 coordinates foregut specification and morphogenesis by antagonizing both canonical and noncanonical Wnt11 signaling. *Genes Dev.* 22, 3050–3063.

Lin, R., Thompson, S., and Priess, J.R. (1995). *pop-1* encodes an HMG box protein required for the specification of a mesoderm precursor in early *C. elegans* embryos. *Cell* 83, 599–609.

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., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* 20, 781–810.

Logan, C.Y., Miller, J.R., Ferkowicz, M.J., and McClay, D.R. (1999). Nuclear β -catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* 126, 345–357.

Martin, B.L., and Kimelman, D. (2009). Wnt signaling and the evolution of embryonic posterior development. *Curr. Biol.* 19, R215–R219.

- Martindale, M.Q. (2005). The evolution of metazoan axial properties. *Nat. Rev. Genet.* 6, 917–927.
- Martindale, M.Q., and Hejnal, 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.
- 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.
- McGrew, L.L., Lai, C.J., and Moon, R.T. (1995). Specification of the antero-posterior neural axis through synergistic interaction of the Wnt signaling cascade with noggin and follistatin. *Dev. Biol.* 172, 337–342.
- McGrew, L.L., Hoppler, S., and Moon, R.T. (1997). Wnt and FGF pathways cooperatively pattern anteroposterior neural ectoderm in *Xenopus*. *Mech. Dev.* 69, 105–114.
- McGrew, L.L., Takemaru, K., Bates, R., and Moon, R.T. (1999). Direct regulation of the *Xenopus* engrailed-2 promoter by the Wnt signaling pathway, and a molecular screen for Wnt-responsive genes, confirm a role for Wnt signaling during neural patterning in *Xenopus*. *Mech. Dev.* 87, 21–32.
- McLin, V.A., Rankin, S.A., and Zorn, A.M. (2007). Repression of Wnt/ β -catenin signaling in the anterior endoderm is essential for liver and pancreas development. *Development* 134, 2207–2217.
- Merrill, B.J., Pasolli, H.A., Polak, L., Rendl, M., Garcia-Garcia, M.J., Anderson, K.V., and Fuchs, E. (2004). Tcf3: a transcriptional regulator of axis induction in the early embryo. *Development* 131, 263–274.
- Miyawaki, K., Yamamoto, M., Saito, K., Saito, S., Kobayashi, N., and Matsuda, S. (2003). Nuclear localization of beta-catenin in vegetal pole cells during early embryogenesis of the starfish *Asterina pectinifera*. *Dev. Growth Differ.* 45, 121–128.
- 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.
- Mohamed, O.A., Clarke, H.J., and Dufort, D. (2004). β -catenin signaling marks the prospective site of primitive streak formation in the mouse embryo. *Dev. Dyn.* 231, 416–424.
- Molina, M.D., Saló, E., and Cebrià, 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., Derelle, R., and Houliston, E. (2008). A maternally localised Wnt ligand required for axial patterning in the cnidarian *Clytia hemisphaerica*. *Development* 135, 2105–2113.
- Moussian, B., and Roth, S. (2005). Dorsoventral axis formation in the *Drosophila* embryo—shaping and transducing a morphogen gradient. *Curr. Biol.* 15, R887–R899.
- Mukhopadhyay, M., Shtrom, S., Rodriguez-Esteban, C., Chen, L., Tsukui, T., Gomer, L., Dorward, D.W., Glinka, A., Grinberg, A., Huang, S.P., et al. (2001). *Dickkopf1* is required for embryonic head induction and limb morphogenesis in the mouse. *Dev. Cell* 1, 423–434.
- Nakamura, K., Kim, S., Ishidate, T., Bei, Y., Pang, K., Shirayama, M., Trzepak, C., Brownell, D.R., and Mello, C.C. (2005). Wnt signaling drives WRM-1/ β -catenin asymmetries in early *C. elegans* embryos. *Genes Dev.* 19, 1749–1754.
- Niehrs, C. (2004). Regionally specific induction by the Spemann-Mangold organizer. *Nat. Rev. Genet.* 5, 425–434.
- Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287, 795–801.
- 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/ β -catenin have complementary roles in anterior/posterior patterning embryos of the basal chordate amphioxus. *Dev. Biol.* 332, 223–233.
- Orii, H., and Watanabe, K. (2007). Bone morphogenetic protein is required for dorso-ventral patterning in the planarian *Dugesia japonica*. *Dev. Growth Differ.* 49, 345–349.
- Pan, C.L., Howell, J.E., Clark, S.G., Hilliard, M., Cordes, S., Bargmann, C.I., and Garriga, G. (2006). Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in *Caenorhabditis elegans*. *Dev. Cell* 10, 367–377.
- Petersen, C.P., and Reddien, P.W. (2008). *Smed- β -catenin-1* is required for anteroposterior blastema polarity in planarian regeneration. *Science* 319, 327–330.
- Petersen, C.P., and Reddien, P.W. (2009). A wound-induced Wnt expression program controls planarian regeneration polarity. *Proc. Natl. Acad. Sci. USA* 106, 17061–17066.
- Pöpperl, H., Schmidt, C., Wilson, V., Hume, C.R., Dodd, J., Krumlauf, R., and Beddington, R.S. (1997). Misexpression of *Cwnt8C* in the mouse induces an ectopic embryonic axis and causes a truncation of the anterior neuroectoderm. *Development* 124, 2997–3005.
- 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.
- Reddien, P.W., and Sánchez Alvarado, A. (2004). Fundamentals of planarian regeneration. *Annu. Rev. Cell Dev. Biol.* 20, 725–757.
- Reddien, P.W., Bermange, A.L., Kicza, A.M., and Sánchez Alvarado, A. (2007). BMP signaling regulates the dorsal planarian midline and is needed for asymmetric regeneration. *Development* 134, 4043–4051.
- Rhinn, M., Lun, K., Luz, M., Werner, M., and Brand, M. (2005). Positioning of the midbrain-hindbrain boundary organizer through global posteriorization of the neuroectoderm mediated by *Wnt8* signaling. *Development* 132, 1261–1272.
- Rocheleau, C.E., Downs, W.D., Lin, R., Wittmann, C., Bei, Y., Cha, Y.H., Ali, M., Priess, J.R., and Mello, C.C. (1997). Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* 90, 707–716.
- Schneider, S., Steinbeisser, H., Warga, R.M., and Hausen, P. (1996). Beta-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* 57, 191–198.
- Schneider, S.Q., and Bowerman, B. (2007). β -Catenin asymmetries after all animal/vegetal-oriented cell divisions in *Platynereis dumerilii* embryos mediate binary cell-fate specification. *Dev. Cell* 13, 73–86.
- Schohl, A., and Fagotto, F. (2002). β -catenin, MAPK and Smad signaling during early *Xenopus* development. *Development* 129, 37–52.
- Schubert, M., Holland, L.Z., Stokes, M.D., and Holland, N.D. (2001). Three amphioxus Wnt genes (*AmphiWnt3*, *AmphiWnt5*, and *AmphiWnt6*) associated with the tail bud: the evolution of somitogenesis in chordates. *Dev. Biol.* 240, 262–273.
- Shimizu, T., Bae, Y.K., Muraoka, O., and Hibi, M. (2005). Interaction of Wnt and caudal-related genes in zebrafish posterior body formation. *Dev. Biol.* 279, 125–141.
- Silhankova, M., and Korswagen, H.C. (2007). Migration of neuronal cells along the anterior-posterior body axis of *C. elegans*: Wnts are in control. *Curr. Opin. Genet. Dev.* 17, 320–325.
- Slack, J.M., Holland, P.W., and Graham, C.F. (1993). The zootype and the phylotypic stage. *Nature* 361, 490–492.
- Takada, S., Stark, K.L., Shea, M.J., Vassileva, G., McMahon, J.A., and McMahon, A.P. (1994). *Wnt-3a* regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* 8, 174–189.

- Takeshita, H., and Sawa, H. (2005). Asymmetric cortical and nuclear localizations of WRM-1/ β -catenin during asymmetric cell division in *C. elegans*. *Genes Dev.* 19, 1743–1748.
- Tendeng, C., and Houart, C. (2006). Cloning and embryonic expression of five distinct *sfrp* genes in the zebrafish *Danio rerio*. *Gene Expr. Patterns* 6, 761–771.
- Thorpe, C.J., Schlesinger, A., Carter, J.C., and Bowerman, B. (1997). Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. *Cell* 90, 695–705.
- Thorpe, C.J., Weidinger, G., and Moon, R.T. (2005). Wnt/ β -catenin regulation of the Sp1-related transcription factor *sp5l* promotes tail development in zebrafish. *Development* 132, 1763–1772.
- Wang, S., Krinks, M., Lin, K., Luyten, F.P., and Moos, M., Jr. (1997). Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* 88, 757–766.
- 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 β -catenin in the evolution of axial polarity and germ layer segregation. *Nature* 426, 446–450.
- Wikramanayake, A.H., Huang, L., and Klein, W.H. (1998). β -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., Peterson, R., Chen, J., Huang, L., Bince, J.M., McClay, D.R., and Klein, W.H. (2004). Nuclear β -catenin-dependent Wnt8 signaling in vegetal cells of the early sea urchin embryo regulates gastrulation and differentiation of endoderm and mesodermal cell lineages. *Genesis* 39, 194–205.
- Yanze, N., Spring, J., Schmidli, C., and Schmid, V. (2001). Conservation of Hox/ParaHox-related genes in the early development of a cnidarian. *Dev. Biol.* 236, 89–98.
- 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.
- Zeng, L., Fagotto, F., Zhang, T., Hsu, W., Vasicek, T.J., Perry, W.L., 3rd, Lee, J.J., Tilghman, S.M., Gumbiner, B.M., and Costantini, F. (1997). The mouse *Fused* locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* 90, 181–192.