Wnt Signaling in Vertebrate Axis Specification

Hiroki Hikasa¹ and Sergei Y. Sokol²

¹Division of Cancer Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan

²Mount Sinai School of Medicine, New York, New York 10029

Correspondence: sergei.sokol@mssm.edu

The Wnt pathway is a major embryonic signaling pathway that controls cell proliferation, cell fate, and body-axis determination in vertebrate embryos. Soon after egg fertilization, Wnt pathway components play a role in microtubule-dependent dorsoventral axis specification. Later in embryogenesis, another conserved function of the pathway is to specify the anteroposterior axis. The dual role of Wnt signaling in *Xenopus* and zebrafish embryos is regulated at different developmental stages by distinct sets of Wnt target genes. This review highlights recent progress in the discrimination of different signaling branches and the identification of specific pathway targets during vertebrate axial development.

Wnt pathways play major roles in cell-fate specification, proliferation and differentiation, cell polarity, and morphogenesis (Clevers 2006; van Amerongen and Nusse 2009). Signaling is initiated in the responding cell by the interaction of Wnt ligands with different receptors and coreceptors, including Frizzled, LRP5/6, ROR1/2, RYK, PTK7, and proteoglycans (Angers and Moon 2009; Kikuchi et al. 2009; MacDonald et al. 2009). Receptor activation is accompanied by the phosphorylation of Dishevelled (Yanagawa et al. 1995), which appears to transduce the signal to both the cell membrane and the nucleus (Cliffe et al. 2003; Itoh et al. 2005; Bilic et al. 2007). Another common pathway component is β -catenin, an abundant component of adherens junctions (Nelson and Nusse 2004; Grigoryan et al. 2008). In response to signaling, β -catenin associates with T-cell factors (TCFs) and translocates to the nucleus to

stimulate Wnt target gene expression (Behrens et al. 1996; Huber et al. 1996; Molenaar et al. 1996).

This β -catenin-dependent activation of specific genes is often referred to as the “canonical” pathway. In the absence of Wnt signaling, β -catenin is destroyed by the protein complex that includes Axin, GSK3, and the tumor suppressor APC (Clevers 2006; MacDonald et al. 2009). Wnt proteins, such as Wnt1, Wnt3, and Wnt8, stimulate Frizzled and LRP5/6 receptors to inactivate this β -catenin destruction complex, and, at the same time, trigger the phosphorylation of TCF proteins by homeodomain-interacting protein kinase 2 (HIPK2) (Hikasa et al. 2010; Hikasa and Sokol 2011). Both β -catenin stabilization and the regulation of TCF protein function by phosphorylation appear to represent general strategies that are conserved in multiple systems (Sokol 2011). Thus, the signaling

H. Hikasa and S.Y. Sokol

pathway consists of two branches that together regulate target gene expression (Fig. 1).

Other Wnt proteins, such as Wnt5a or Wnt11, strongly affect the cytoskeletal organization and morphogenesis without stabilizing β -catenin (Torres et al. 1996; Angers and Moon 2009; Wu and Mlodzik 2009). These “noncanonical” ligands do not influence TCF3 phosphorylation (Hikasa and Sokol 2011), but may use distinct receptors such as ROR1/2 and RYK instead of or in addition to Frizzled (Hikasa et al. 2002; Lu et al. 2004; Mikels and Nusse 2006; Nishita et al. 2006, 2010; Schambony and Wedlich 2007; Grumolato et al. 2010; Lin et al. 2010; Gao et al. 2011). In such cases, signaling

mechanisms are likely to include planar cell polarity (PCP) components, such as Vangl2, Flamingo, Prickle, Diversin, Rho GTPases, and c-Jun amino-terminal kinases (JNKs), which do not directly affect β -catenin stability (Fig. 1) (Sokol 2000; Schwarz-Romond et al. 2002; Schambony and Wedlich 2007; Komiya and Habas 2008; Axelrod 2009; Itoh et al. 2009; Tada and Kai 2009; Sato et al. 2010; Gao et al. 2011). This simplistic dichotomy of the Wnt pathway does not preclude some Wnt ligands from using both β -catenin-dependent and -independent routes in a context-specific manner.

Despite the existence of many pathway branches, only the β -catenin-dependent branch

Figure 1. Conserved Wnt pathway branches and components. In the absence of Wnt signals, glycogen synthase kinase 3 (GSK3) binds Axin and APC to form the β -catenin destruction complex. Some Wnt proteins, such as Wnt8 and Wnt3a, stimulate Frizzled and LRP5/6 receptors to inhibit GSK3 activity and stabilize β -catenin (β -cat). Stabilized β -cat forms a complex with T-cell factors (e.g., TCF1/LEF1) to activate target genes. Moreover, GSK3 inhibition leads to target gene derepression by promoting TCF3 phosphorylation by homeodomain-interacting protein kinase 2 (HIPK2) through an unknown mechanism, for which β -catenin is required as a scaffold. This phosphorylation results in TCF3 removal from target promoters and gene activation. Other Wnt proteins, such as Wnt5a and Wnt11, use distinct receptors such as ROR2 and RYK, in addition to Frizzled, to control the the cytoskeletal organization through core planar cell polarity (PCP) proteins, small GTPases (Rho/Rac/Cdc42), and c-Jun amino-terminal kinase (JNK).

has been implicated in body-axis specification. Recent experiments in lower vertebrates have identified additional pathway components and targets and provided new insights into the underlying mechanisms.

ROLE OF CYTOPLASMIC DETERMINANTS AND WNT SIGNALING IN DORSOVENTRAL AXIS SPECIFICATION

The specification of the dorsoventral axis coincides with the formation of an essential embryonic signaling center known as the Spemann organizer in amphibians, the shield in zebrafish, and the node in the mouse (Harland and Gerhart 1997; Schier and Talbot 2005; Marlow 2010). During egg fertilization, sperm entry triggers microtubule-dependent rearrangement of the cortical cytoplasm from the vegetal pole toward the future dorsal side, a process known as the cortical rotation (Houliston and Elinson 1992). Experiments with cytoplasm microinjections or removal revealed vegetally localized dorsalizing activity that moves to the dorsal region before the first cleavage in *Xenopus* or zebrafish eggs (Fujisue et al. 1993; Holowacz and Elinson 1993; Mizuno et al. 1999; Ober and Schulte-Merker 1999). Treatment of fertilized *Xenopus* or zebrafish eggs with microtubule-depolymerizing agents, such as UV irradiation or nocodazole, causes embryo ventralization (Chung and Malacinski 1980; Scharf and Gerhart 1980; Jesuthasan and Stahle 1997). These observations indicated that the movement of the dorsalizing activity is essential for the establishment of the primary dorsoventral axis in both *Xenopus* and zebrafish.

One of the important outcomes of the cortical rotation is the accumulation of β -catenin in dorsal nuclei of early blastulae, whereas β -catenin remains largely cytoplasmic and cortical in ventral cells (Schneider et al. 1996; Schohl and Fagotto 2002). This difference in β -catenin localization can be detected as early as the 2- to 4-cell stage (Larabell et al. 1997). Supporting the critical role of this localization for dorsal development, several maternal effect mutants in zebrafish such as *hecate*, *ichabod*, and *tokkaebi* result in the reduction of β -catenin dorsal ac-

Wnt Signaling in Vertebrate Axis Specification

cumulation and defects in dorsal development (Kelly et al. 2000; Nojima et al. 2004; Lyman Gingerich et al. 2005; Bellipanni et al. 2006). The *ichabod* mutation has been mapped to the proximity of the gene encoding β -catenin-2, a second zebrafish β -catenin that is essential for maternal Wnt signaling (Bellipanni et al. 2006). Consistent with the ventralized phenotype of the *ichabod* embryos, maternal β -catenin-2 RNA is down-regulated. *Hecate* is another zebrafish mutation that is manifested by deficient dorsal development and enhanced intracellular calcium release; however, the mutated gene has not yet been identified (Lyman Gingerich et al. 2005). Positional cloning revealed that the *tokkaebi* gene encodes syntabulin, a microtubule-binding protein connecting kinesin I to syntaxin-containing cargo vesicles (Nojima et al. 2010). This finding further implicates microtubule-dependent trafficking in dorsoventral axis specification. Reminiscent of the key function of β -catenin in *Xenopus* and zebrafish, mouse embryos lacking β -catenin reveal abnormal visceral endoderm patterning before gastrulation and do not form the primitive streak (Haegel et al. 1995; Huelsken et al. 2000).

An essential role for Wnt/ β -catenin signaling in dorsoventral axis determination has been initially suggested in gain-of-function experiments, in which Wnt and Dishevelled messenger RNAs (mRNAs) triggered ectopic organizer formation (McMahon and Moon 1989; Smith and Harland 1991; Sokol et al. 1991, 1995; Rothbacher et al. 1995). The requirement for β -catenin has been established by antisense oligonucleotide-mediated depletion of β -catenin in *Xenopus* and zebrafish embryos (Heasman et al. 1994; Bellipanni et al. 2006). β -catenin activates several major targets in *Xenopus* embryos that are responsible for Spemann organizer formation. Activation of many organizer-specific genes depends on functional TCF sites in their promoters (Brannon et al. 1997; Laurent et al. 1997; McKendry et al. 1997; Fan et al. 1998; Ryu et al. 2001; Leung et al. 2003). These early β -catenin targets include *Nodal* group genes that are essential for dorsal mesoderm formation in frogs, zebrafish, and mammals (Conlon et al. 1994; Feldman et al. 1998; Osada

H. Hikasa and S.Y. Sokol

and Wright 1999; Takahashi et al. 2000; Yang et al. 2002; Hilton et al. 2003; Zhang et al. 2003). Besides *Nodals*, other direct targets of β -catenin are *Siamois* and *Twin* in *Xenopus* (Lemaire et al. 1995; Brannon et al. 1997; Laurent et al. 1997; Fan et al. 1998), and *Bozozok/Nieuwkoid* in zebrafish (Ryu et al. 2001), which are required for organizer formation (Fig. 2; Table 1) (Fan and Sokol 1997; Kessler 1997; Koos and Ho 1998; Yamanaka et al. 1998; Fekany et al. 1999). Taken together, available data establish a key role for Wnt signaling and its early targets in early dorsoventral axis specification (Moon and Kimelman 1998; Schroeder et al. 1999; Sokol 1999; Tao et al. 2005).

Although lack of effect of a dominant-negative form of Dishevelled (Dvl) on axis specification does not eliminate Dvl participation; it suggests that β -catenin is stabilized by a downstream Wnt pathway component that is translocated dorsally during the cortical rotation (Sokol 1996; Harland and Gerhart 1997). One of the key factors is GSK3-binding protein or Frat, which inhibits GSK3 and is essential for

dorsal development in *Xenopus* embryos (Yost et al. 1998). However, the triple knockout of the existing Frat homologs in the mouse does not produce a morphological phenotype, suggesting that this mechanism is not conserved in mammals (van Amerongen et al. 2005). More recently, antisense oligonucleotide-mediated depletion of maternal Wnt5a, Wnt11, and Fz7 indicated their roles in dorsoventral axis specification (Sumanas et al. 2000; Tao et al. 2005; Cha et al. 2008). These observations suggest a different model, in which Wnt5a and Wnt11 influence β -catenin levels in the oocyte, raising the question how β -catenin becomes localized to the dorsal side of the embryo. Because maternal Wnt11 RNA is vegetally localized (Ku and Melton 1993), one possibility is that Wnt11 is delivered to its future site of action by the cortical rotation (Tao et al. 2005). So far, dorsoventral patterning defects have not been described in embryos with genetically reduced Wnt5a or Wnt11 function (Rauch et al. 1997; Yamaguchi et al. 1999; Heisenberg et al. 2000; Kilian et al. 2003; Majumdar et al. 2003; Ulrich

Figure 2. Axis specification by early and late Wnt signaling involves distinct targets. After cortical rotation (black dotted arrow), early β -catenin accumulation in the dorsal equatorial region activates gene targets to generate the Spemann organizer. β -Catenin up-regulates several Wnt and bone morphogenetic protein (BMP) antagonists, including *Dkk1*, *Cerberus*, *Shisa*, *Noggin*, and *Chordin*. The pathway is inhibited by Wnt antagonists in the anterior tissues, but the zygotic activation of Wnt8 causes ventral and posterior accumulation of β -catenin during gastrulation. The target genes of this late Wnt signaling, including *Cdx*, *Vent*, *Meis*, *Gbx*, and *Msx*, are critical for the specification of ventroposterior mesodermal fates and lead to tail formation.

Table 1. Putative Wnt targets during anteroposterior axis specification

Genes activated by Wnt3a	Genes inhibited by Dickkopf-1	References
<i>Axin1/2</i>	<i>Axin1/2</i>	Zeng et al. 1997; Jho et al. 2002
<i>Kremen2</i>	<i>Kremen2</i>	Mao et al. 2002
<i>Cdx2, 4</i>	<i>Cdx2, 4</i>	Isaacs et al. 1998
<i>Gbx2</i>	<i>Gbx2</i>	von Bubnoff et al. 1996
<i>HoxA1/D1</i>	<i>HoxA1/D1</i>	Kolm and Sive 1995
<i>Esr9/10</i>	<i>Esr9/10</i>	Li et al. 2003
<i>Fz10</i>	<i>Fz10</i>	Wheeler and Hoppler 1999; Garcia-Morales et al. 2009
<i>Foxi1</i>		Suri et al. 2005; Mir et al. 2007
<i>Meis3</i>	<i>Meis-like</i>	Elkouby et al. 2010
<i>Msx1/2</i>	<i>Msx1/2</i>	Maeda et al. 1997; Marazzi et al. 1997; Mathers et al. 1997; Suzuki et al. 1997; Willert et al. 2002
<i>Nz11</i>	<i>Nz11</i>	Andreazzoli et al. 2001; Nakamura et al. 2008
<i>Nrh1</i>		Bromley et al. 2004; Sasai et al. 2004
<i>Riddle2</i>		Shibata et al. 2008
<i>Spr2</i>		Weidinger et al. 2005
<i>Vent/PV1</i>	<i>Vent/PV1</i>	Onichtchouk et al. 1996
<i>XARP</i>	<i>XARP</i>	Itoh et al. 2000
<i>Xmc</i>		Frazzetto et al. 2002
<i>Xpo</i>	<i>Xpo</i>	Sato and Sargent 1991; Amaya et al. 1993; Hoppler et al. 1996

Only some of these genes have been shown to contain TCF/LEF sites in their promoters.

et al. 2003; Fossat et al. 2011). Potentially, these observations could be explained by functional redundancy or by the presence of maternally encoded proteins. On the other hand, overexpression of Wnt5a or Wnt11 does not lead to β -catenin stabilization (Torres et al. 1996; Mikels and Nusse 2006). Owing to these contradictory observations, the final conclusion regarding the involvement of these Wnt proteins in β -catenin

Wnt Signaling in Vertebrate Axis Specification

stabilization and body-axis formation awaits additional experimental insights. Despite the variability in the existing models of vertebrate axis specification, there is a common outcome: the establishment of a signaling center that dorsalizes all three germ layers and mediates anteroposterior patterning.

ZYGOTIC WNT SIGNALING DURING ANTEROPOSTERIOR AXIS SPECIFICATION

Early studies revealed that the effect of Wnt proteins on embryogenesis is stage specific. When injected as mRNA, Wnt8 triggers secondary axis formation (Smith and Harland 1991; Sokol et al. 1991). However, when supplied as a plasmid, Wnt8 expression is initiated only at midblastula transition and the delayed stimulation leads to posteriorization, characterized by lack of head structures (Christian and Moon 1993). These observations correlated well with the time-sensitive effect of lithium chloride, a GSK3 inhibitor (Klein and Melton 1996), on anteroposterior patterning (Yamaguchi and Shinagawa 1989). Of note, Dkk1 RNA does not interfere with the early dorsal signaling, although being fully capable of inhibiting ectopic Wnt expression (Glinka et al. 1998; Brott and Sokol 2002), consistent with the idea that Wnt ligands are not accessible to Dkk-dependent inhibition at this early stage. These observations indicate that Wnt signaling plays different roles in axis specification at different developmental stages.

Several Wnt ligands, including Wnt3a, Wnt5a, Wnt8, and Wnt11, are expressed in the ventral or posterior region of the embryo (Christian et al. 1991a; Krauss et al. 1992; Moon et al. 1993; Kelly et al. 1995; Hong et al. 2008). On the other hand, multiple Wnt antagonists, such as Frzb/Sfrp3, Crescent, Shisa, and Dkk1, are expressed in the head region (Leyns et al. 1997; Wang et al. 1997; Glinka et al. 1998; Shibata et al. 2005; Yamamoto et al. 2005). This complementary expression pattern indicates a function for the Wnt pathway in anteroposterior axis specification. The involvement of Wnt signaling in anteroposterior patterning was first revealed in studies with overexpressed Wnt pathway modulators. Twofold titration of *Xenopus*

H. Hikasa and S.Y. Sokol

Dishevelled RNA caused graded appearance of region-specific positional markers, indicating a morphogenlike dose response with the highest point at the posterior of the embryo (Itoh and Sokol 1997). Wnt pathway antagonists, such as GSK3, Dkk1, and Shisa, cause anteriorization, in agreement with the proposed posteriorizing activity of Wnt ligands (Itoh et al. 1995; Glinka et al. 1998; Yamamoto et al. 2005). Further supporting this hypothesis, TCF-dependent reporter activity and the nuclear localization of endogenous β -catenin are elevated in the anterior-to-posterior gradient (Kiecker and Niehrs 2001; Dorsky et al. 2002).

The critical role of Wnt signaling in anteroposterior axis specification has been corroborated by depletion experiments for pathway components (Heasman et al. 2000; Erter et al. 2001; Lekven et al. 2001; Shimizu et al. 2005; Bellipanni et al. 2006; Hikasa et al. 2010) and the analysis of the corresponding zebrafish mutations. *Headless* (*hdl*) was identified as a point mutation in a *tcf3* gene homolog, which represses Wnt target genes in zebrafish embryos (Kim et al. 2000). Loss of forebrain and eyes and the modest expansion of ventral mesoderm in *hdl* embryos are consistent with up-regulated Wnt signaling. When both *Headless* and a closely related protein *Tcf3b* are down-regulated, this posteriorization is more severe (Dorsky et al. 2003). Similarly, *mastermind* zebrafish embryos with a mutation in *Axin1* are posteriorized with telencephalon and eyes transformed to diencephalon (Heisenberg et al. 2001). Thus, interference with Wnt pathway antagonists mimics Wnt protein overexpression and results in posteriorization. Phenotypes of mice lacking the genes for Dkk1, APC, TCF3, Wnt3, ICAT, and β -catenin also support the role of the Wnt pathway in posterior development (Takada et al. 1994; Liu et al. 1999b; Mukhopadhyay et al. 2001; Ishikawa et al. 2003; Merrill et al. 2004; Satoh et al. 2004). Combined with more recent studies of Cnidaria and Planaria (Holstein 2008; Martin and Kimmel 2009; Petersen and Reddien 2009), these findings establish a conserved key role for Wnt signaling in anteroposterior axis specification (Fig. 2).

PUTATIVE TRANSCRIPTIONAL TARGETS OF THE WNT PATHWAY IN AXIS SPECIFICATION

Molecular screens have identified transcriptional Wnt targets in early embryos and embryonic stem cells (Willert et al. 2002; Li et al. 2004; Hufton et al. 2006). A major obstacle to identifying Wnt gene targets is the stage and context dependence of cellular responses to Wnt ligands. Therefore, a special effort is needed to elucidate the Wnt targets that are relevant to anteroposterior axis specification.

Hybridization of RNA probes prepared from the control and Wnt3a-stimulated ectoderm cells with *Xenopus* copy DNA (cDNA) microarrays (Affymetrix) has identified a number of candidate Wnt targets (Table 1) (H Hikasa and SY Sokol, unpubl.). Among these are Wnt pathway components and modulators, such as Fz9, Fz10, Kremen 2, and the Axin family, highlighting the need for feedback regulation at this developmental stage. A second class contains many of the previously identified Wnt targets that are expressed ventrally or posteriorly. These genes are *Gbx2* (von Bubnoff et al. 1996), the *Cdx* group (Epstein et al. 1997; Haremak et al. 2003; Shimizu et al. 2005; Keenan et al. 2006; Pilon et al. 2006), the *Vent/Vox/Ved/Xom* group (Gawantka et al. 1995; Ladher et al. 1996; Onichtchouk et al. 1996; Schmidt et al. 1996; Imai et al. 2001; Ramel and Lekven 2004; Thorpe and Moon 2004), and the *Meis* group (*Meis3*) (Choe et al. 2009; Elkouby et al. 2010). The remaining genes include some known bone morphogenetic protein (BMP)- and fibroblast growth factor (FGF)-inducible genes, reiterating the importance of the cross talk between these pathways, and novel putative Wnt targets (Table 1). Whereas some of these genes may be activated indirectly, *Meis3*, *Gbx2*, *Cdx4*, and *Vent1/2* are known to contain functional TCF-binding sites in their promoters that are required for Wnt responsiveness (Haremak et al. 2003; Li et al. 2009; Elkouby et al. 2010; Hikasa et al. 2010). Supporting these findings, a complementary analysis revealed a similar list of genes that are inhibited in ventral mesoderm by Dkk1 (Table 1) (Hufton et al. 2006).

The *Cdx* genes, a group of four conserved vertebrate homologs of *Drosophila caudal*, have been widely implicated in posterior development (Northrop and Kimelman 1994; Isaacs et al. 1998; Pillemer et al. 1998; van den Akker et al. 2002; Shimizu et al. 2005, 2006; Faas and Isaacs 2009; Young and Deschamps 2009; Young et al. 2009; Beck and Stringer 2010). Loss of the *Cdx4* gene function in the zebrafish mutant *kugelrig* shows its essential role in posterior development (Davidson et al. 2003). The promoter of the *Xenopus* and mammalian *Cdx4* gene is Wnt inducible and contains multiple TCF/LEF binding sites (Harekaki et al. 2003; Shimizu et al. 2005; Pilon et al. 2006). Up-regulation of *Cdx* genes causes the anterior shift of posterior markers in *Xenopus* and zebrafish (Isaacs et al. 1998; Shimizu et al. 2005; Faas and Isaacs 2009). Similar to *Cdx* genes, *Vent* family proteins (*Vent/Vox/Ved/Xom*) in *Xenopus* and zebrafish are homeodomain-containing transcriptional repressors that promote ventroposterior fates by suppressing organizer genes such as *bozozok* (in zebrafish), *chordin*, and *goosecoid* (Schmidt et al. 1996; Melby et al. 1999, 2000; Imai et al. 2001; Ramel and Lekven 2004; Sander et al. 2007). Inactivation of *Vent* genes results in a severe loss of ventroposterior structures and enhanced dorsoanterior fate, similar to the Wnt8 knockdown (Ramel and Lekven 2004; Sander et al. 2007). Together, these findings show that the Wnt pathway controls posterior development through multiple gene targets.

Putative Wnt target genes that are involved in anteroposterior patterning differ from the early β -catenin targets that mark the Spemann organizer region. These organizer-specific genes include *Xenopus Siamois* (Lemaire et al. 1995; Brannon et al. 1997; Fan et al. 1998) and *Twin* (Laurent et al. 1997), zebrafish *Bozozok/dharma/nieuwkoid* (Koos and Ho 1998; Yamanaka et al. 1998; Fekany et al. 1999), *Xnr3* (Smith et al. 1995), *Xnr5/6* (Takahashi et al. 2000; Yang et al. 2002) and several Wnt antagonists: *Frzb/Sfrp3* (Leyns et al. 1997), *Dkk1* (Glinka et al. 1998), *Crescent* (Shibata et al. 2000), *Cerberus* (Bouwmeester et al. 1996), *Shisa* (Yamamoto et al. 2005), as well as other genes listed in Table 2. The difference between the early and late targets for

Wnt Signaling in Vertebrate Axis Specification

Table 2. Putative Wnt targets during dorsoventral axis specification

Genes activated by maternal β -catenin	References
<i>Siamois</i>	Lemaire et al. 1995; Brannon et al. 1997; Fan et al. 1998
<i>Twin</i>	Laurent et al. 1997
<i>Xnr3</i>	Smith et al. 1995
<i>Goosecoid</i>	Blumberg et al. 1991; Cho et al. 1991
<i>Cerberus</i>	Bouwmeester et al. 1996
<i>Chordin</i>	Sasai et al. 1994
<i>Crescent</i>	Shibata et al. 2000; Shibata et al. 2001
<i>Noggin</i>	Smith and Harland 1992
<i>Xlim-1</i>	Taira et al. 1994
<i>Xnr5/6</i>	Takahashi et al. 2000; Yang et al. 2002
<i>Xnot1</i>	von Dassow et al. 1993
<i>Shisa</i>	Yamamoto et al. 2005
<i>Dkk1</i>	Glinka et al. 1998
<i>Frzb</i>	Leyns et al. 1997
<i>Frizzled 8</i>	Deardorff et al. 1998; Itoh et al. 1998a
<i>Bozozok</i>	Koos and Ho 1998; Yamanaka et al. 1998; Fekany et al. 1999 (zebrafish-specific, also known as <i>Dharma</i> and <i>Nieuwkoid</i>)

Known antagonists of Wnt and BMP signaling are shown in bold.

the Wnt pathway corresponds to the switch between stage-specific Wnt signaling mechanisms that are critical for vertebrate axis specification. For additional information about Wnt targets in different experimental systems, the reader is referred to Roel Nusse's Wnt Window (http://www.stanford.edu/group/nusse/lab/cgi-bin/wnt/target_genes).

REGULATION OF TCF PROTEIN FUNCTION DURING AXIS SPECIFICATION

How does Wnt signaling lead to target gene activation during anteroposterior patterning? A critical event seems to be TCF3 phosphorylation by HIPK2 allowing one to derepress the target genes (Hikasa et al. 2010; Sokol 2011), although

H. Hikasa and S.Y. Sokol

in some cases TCF3 is likely to function in a Wnt-independent manner (Cole et al. 2008; Gribble et al. 2009). Homeodomain-interacting protein kinases (HIPKs) have been shown to function in a context-dependent manner in response to Wnt signaling (Wei et al. 2007; Lee et al. 2009; Louie et al. 2009; Kim et al. 2010). Loss-of-function studies reveal that HIPK2 is required in vivo for Wnt8-dependent ventroposterior development and antagonizes the function of TCF3 in axis specification (Hikasa et al. 2010). Besides HIPK2, TCF3 phosphorylation requires β -catenin. TCF3 with a mutation that disrupts β -catenin binding is not phosphorylated (Hikasa et al. 2010). Thus, β -catenin appears to act by providing a scaffold for TCF3 phosphorylation by HIPK2. This is a novel function of β -catenin that differs from its commonly proposed role as transcriptional co-activator (Behrens et al. 1996; van de Wetering et al. 1997; Cavallo et al. 1998; Daniels and Weis 2005). Although TCF3 phosphorylation has been shown to require LRP6 activity and GSK3 inhibition (Hikasa and Sokol 2011), the mechanistic role of GSK3 in HIPK2 activation remains a mystery.

Nemolike kinase (Nlk) has been previously reported to phosphorylate TCF proteins (Ishitani et al. 1999; Meneghini et al. 1999) and is a positive regulator of Wnt8 signaling in anteroposterior patterning in zebrafish embryos (Thorpe and Moon 2004). In mammalian cells, Nlk and HIPK2 have been shown to regulate the stability of Myb oncoprotein in response to Wnt1 (Kaneishi et al. 2004), indicating that these kinases might also function together during axis specification. The pathway leading to TCF regulation in vertebrates is reminiscent of Wnt signaling in *C. elegans*, because LIT-1, a homolog of Nlk, and WRM-1, a β -catenin paralog, phosphorylates POP-1/TCF to enhance its nuclear export, leading to transcriptional derepression (Rocheleau et al. 1997, 1999; Meneghini et al. 1999; Lo et al. 2004). Because POP-1 plays a dual role in gene target regulation, the reader is referred to other reviews for additional information (Phillips and Kimble 2009; Sokol 2011).

Chromatin precipitation analysis has shown that phosphorylated TCF3 is removed from

target promoters, resulting in gene activation (Hikasa et al. 2010). Subsequent experiments showed that *Vent2* gene activation is accompanied by the removal of TCF3 from the promoter and its replacement by TCF1, an activator form of TCF that is resistant to HIPK2-mediated phosphorylation (Hikasa and Sokol 2011). This finding strongly supports the view that the four vertebrate homologs of TCF, expressed in a tissue-specific manner (Molenaar et al. 1998; Dorsky et al. 1999, 2003; Konig et al. 2000; Roel et al. 2003; Veien et al. 2005), play diverse roles in Wnt signaling and axis specification (Hamilton et al. 2001; Houston et al. 2002; Roel et al. 2002; Liu et al. 2005; Standley et al. 2006). Although both TCF3 and TCF1 can bind β -catenin and Groucho corepressors (Roose et al. 1998; Brantjes et al. 2001), the two proteins appear to function very differently. TCF3 serves exclusively as a transcriptional repressor, whereas TCF1 is a transcriptional activator (Kim et al. 2000; Gradl et al. 2002; Houston et al. 2002; Merrill et al. 2004; Liu et al. 2005; Nguyen et al. 2006; Gribble et al. 2009; Hikasa and Sokol 2011). Additional studies are needed to explain the difference in the activity of TCF1 and TCF3, which is likely caused by a distinct set of interacting partners.

PATHWAY SWITCH MECHANISMS

Whereas the early dorsal accumulation of β -catenin is essential for the activation of organizer genes, it is later followed by the stabilization of β -catenin ventrally (Kiecker and Niehrs 2001; Schohl and Fagotto 2002; Hikasa et al. 2010). This reversed distribution of β -catenin correlates with the ventroposterior expression of Wnt8 (Christian et al. 1991a,b; Kelly et al. 1995; Lekven et al. 2001) and dorsoanterior expression of several Wnt antagonists in both *Xenopus* and zebrafish (Fig. 2). Thus, a likely explanation for this change is that dorsal β -catenin stimulates the transcription of pathway antagonists, such as *Dkk1*, *Shisa*, *FrzB*, and, likely, *Axin* family proteins (Leyns et al. 1997; Glinka et al. 1998; Yamamoto et al. 2005). The negative feedback from Wnt antagonists decreases β -catenin in the dorsoanterior region, whereas

ventroposterior activity of Wnt8 up-regulates β -catenin ventrally. In agreement with this model, the increased level of β -catenin in ventroposterior tissues relies mostly on Wnt8 activity, rather than dorsally derived signals (Hikasa et al. 2010).

Although the molecular cause for the switch from early dorsal Wnt signaling to late ventroposterior Wnt signaling has not yet been identified, the gene targets for each round of signaling appear to be very different. A number of questions remain to be answered. (1) What prevents late Wnt targets from being activated early at the dorsal side? (2) Why are the early targets silent ventrally at gastrulation when β -catenin is stabilized in response to Wnt8 signals at later stages? A possible explanation of this context dependence is related to epigenetic changes in chromatin that underlie the developmental control of transcription (Li 2002; Kouzarides 2007; Ng and Gurdon 2008; Akkers et al. 2009; Xu et al. 2010). A plasmid reporter for the *Siamois* gene failed to respond to a constitutive TCF activator after gastrulation (Darken and Wilson 2001), suggesting that the stage-dependent regulation of target genes depends on some downstream components of the pathway. Moreover, β -catenin can modulate histone methylation of poised genes, indicating local epigenetic changes in chromatin (Blythe et al. 2010). Thus, ventroposterior Wnt targets, such as *Vent* or *Cdx*, may be poised for activation at a later time, predicting the existence of epigenetic differences in the chromatin state that are critical for axis specification.

Another explanation is that functionally diverse vertebrate TCF proteins are responsible for the early to late transition in Wnt target specificity. Besides the distinct roles of TCF proteins as discussed above, the switch from early to late Wnt signaling can be linked to the phosphorylation of TCF by Wnt8/HIPK2, attenuating target DNA binding. This takes place only after the beginning of gastrulation, likely depending on a factor that becomes available after the onset of zygotic transcription. Notably, Wnt8/HIPK2 signal can trigger the phosphorylation of TCF3, TCF4, and Lef1 (but not TCF1), owing to the conservation of the HIPK2 phosphorylation

sites. Thus, context-dependent activation of early and late Wnt target genes may be explained by the opposing action of tissue-specific TCF proteins and their distinct response to Wnt8-mediated phosphorylation (Sokol 2011). Whether this explanation is correct remains to be experimentally tested.

CROSS TALK WITH OTHER PATHWAYS

Organizer-derived posteriorizing signals reach neuroectoderm during gastrulation to specify the anteroposterior axis (Nieuwkoop 1952; Doniach et al. 1992; Holowacz and Sokol 1999). Besides Wnt signaling, this process requires additional pathways, such as BMP and FGF signaling. The region- and stage-specific integration of distinct signals contributes to the differential selection of pathway targets that is crucial for the establishment of embryonic axes. The best known examples are the cross talk between the Wnt pathway and the BMP pathway in the ventroposterior region (Itasaki and Hoppler 2010) or FGF/IGF (insulinlike growth factor) receptor tyrosine kinase signaling dorsally and posteriorly (Schohl and Fagotto 2002; Pera et al. 2003; Kudoh et al. 2004; Marchal et al. 2009).

Both BMP and Wnt signaling are involved in setting up ventroposterior gene expression in vertebrate embryos (Hoppler and Moon 1998; De Robertis and Kuroda 2004; Schier and Talbot 2005). The synergistic function of Wnt8 and BMP4 in ventral mesoderm patterning has been first shown in *Xenopus* embryos (Hoppler and Moon 1998) and confirmed by genetic studies in zebrafish. Reduced *Vent* expression in zebrafish embryos depleted of β -catenin or containing a mutation in the *Wnt8* gene (Ramel and Lekven 2004; Bellipanni et al. 2006) indicates a requirement for the Wnt pathway in *Vent* activation. The *Wnt8* and *Swr* (*Bmp2*) double mutants display a progressive reduction of *Vent* gene expression and a concomitant expansion of the dorsal domain (Ramel and Lekven 2004; Ramel et al. 2005). The synergy between BMP and Wnt signaling has been documented in many other developmental processes, including bone, kidney, limb, and tooth development (Perantoni 2003; Tucker and Sharpe 2004;

H. Hikasa and S.Y. Sokol

Hartmann 2006; Yokoyama 2008; Itasaki and Hoppler 2010).

In contrast to the BMP pathway that is active in the ventral region of the blastula embryo, FGF signaling seems to be essential for dorsal posterior patterning (Amaya et al. 1991; Cox and Hemmati-Brivanlou 1995; Lamb and Harland 1995; Holowacz and Sokol 1999; Schohl and Fagotto 2002; Kudoh et al. 2004; Marchal et al. 2009; Martin and Kimelman 2009). Many Wnt-inducible genes, including *Cdx4*, *Xpo*, *Meis*, and *Marginal coil* (*Xmc*), have been previously identified as FGF-responsive genes (Table 1) (Amaya et al. 1993; Frazzetto et al. 2002; Amar and Frank 2004; Chung et al. 2004; Keenan et al. 2006). In studies using dominant-negative FGF receptor constructs and specific morpholino oligonucleotides, FGF signaling has been reported to be essential for the Wnt pathway to activate its targets during anteroposterior axis development (McGrew et al. 1997; Maegawa et al. 2006; Burks et al. 2009). The cross talk between the Wnt and the FGF pathway has been preserved in many other models, including brain patterning, limb formation, tail regeneration, and lung and kidney morphogenesis (Martinez et al. 1999; Schedl and Hastie 2000;

Kawakami et al. 2001; Lupo et al. 2002; Villanueva et al. 2002; Lin and Slack 2008).

The mechanisms underlying these examples of pathway cross talk are being revealed. In some cases two pathways operate sequentially, with one pathway inducing the other. For example, FGF8a signaling transcriptionally activates Wnt8 to promote neural crest development (Fig. 3A) (Hong et al. 2008). Alternatively, two pathways may function in parallel, when a target promoter contains transcription factor binding sites that are specific for each pathway (Fig. 3B). Both Wnt and BMP signals promote ventral fate and directly activate the expression of *Vent* genes. The *Vent2* promoter contains a unique Wnt-responsive TCF-binding site and the Smad and OAZ sites needed for the BMP response (Candia et al. 1997; Hata et al. 2000; Karaulanov et al. 2004; Hikasa et al. 2010). Mutagenesis of individual promoter elements indicates that the Wnt and the BMP pathways separately control the *Vent2* gene (Hikasa et al. 2010).

Similarly, a combination of Wnt and FGF signals leads to a robust expression of *Cdx4* in *Xenopus* and zebrafish (Shimizu et al. 2005; Keenan et al. 2006). The regulatory region of the *Cdx4* gene contains multiple TCF and Ets

Figure 3. Cross talk between Wnt, BMP, and FGF pathways during axis specification. (A) Sequential interaction of two pathways. FGF8a induces the expression of Wnt8 transcripts to promote neural crest formation in *Xenopus* neurulae. (B) Parallel interaction of two pathways at a target promoter (*Vent2*). *Vent2* is up-regulated by BMP proteins acting through Smad1 interacting with the Smad binding site (SBS), whereas Wnt proteins act through TCF and the TCF-binding site (TBS). Both signals are responsible for the maximal activation of *Vent2*. (C) The cytoplasmic integration of two pathways. GSK3 phosphorylates the activated form of Smad1, marking it for degradation. Wnt proteins signal to inhibit or sequester GSK3, thereby promoting BMP target gene activation.

DNA-binding elements that are located in close proximity (Haremak et al. 2003). This structure of the *Cdx4* regulatory region explains the observation that *Cdx4* is a direct target of both Wnt and FGF pathways, because Ets factors are known to regulate gene expression downstream from FGF (Nentwich et al. 2009; Znosko et al. 2010). This transcriptional cross talk does not preclude a more close interaction, in which two transcription factors interact physically, resulting in a synergistic response (Fig. 3B). The physical interaction of LEF1 with Smad3 and Smad4 may help explain the cross talk between Wnt and TGF β signaling, relevant to the specification of the primary dorsoventral axis by early Wnt signaling (Sokol and Melton 1992; Sokol 1993; Labbe et al. 2000; Nishita et al. 2000).

A direct cytoplasmic mechanism has been proposed for Wnt-BMP and FGF-BMP pathway cross talk. Smad1 proteins are phosphorylated by MAPK in response to FGF and IGF, targeting Smad1 for degradation by the proteasome (Massague 2003; Pera et al. 2003; Sapkota et al. 2007). Similarly, GSK3 phosphorylates Smad1 and this phosphorylation can be inhibited by Wnt signaling in tissue culture cells (Fig. 3C) (Fuentelba et al. 2007; Sapkota et al. 2007). These phosphorylation events may provide specific interfaces for pathway cross talk. Nevertheless, this mode of regulation may not be the main mechanism for Wnt8-mediated anteroposterior axis determination, because no significant change in the amount of active Smad1 (phosphorylated at the carboxyl terminus) is observed in *Xenopus* embryos with altered Wnt8 activity (Hikasa et al. 2010). On the contrary, the requirement for the TCF-binding site in *Vent2* reporter activation and the regulation of TCF by Wnt signaling reiterates the importance of TCF regulation for Wnt signaling (Hikasa et al. 2010).

Besides FGF and BMP signaling, there are examples of Wnt pathway cross talk with other signaling pathways, especially those linked to proteasome-mediated protein degradation. The β -transducin repeat-containing protein (β TrCP) is an E3 ubiquitin ligase that represents a nodal point for down-regulation of β -catenin, Gli, and NF- κ B levels (Liu et al. 1999a; Maniatis 1999). As Wnt signaling has been shown to up-regulate

β TrCP activity in a negative-feedback loop (Spiegelman et al. 2000), the effect on the Hh and NF- κ B signaling may be expected. Also, the Wnt pathway negatively regulates the activity of GSK3 (Cook et al. 1996; Itoh et al. 1998b; Taelman et al. 2010), an enzyme that has been implicated in the regulation (e.g., degradation) of a large number of proteins, in addition to β -catenin (Zhou et al. 2004; Xu et al. 2009). These findings suggest the interaction of the Wnt pathway with many signaling pathways, which is likely to be better understood in the course of future studies.

CONCLUDING REMARKS

Recent studies support the view that Wnt signaling plays a dual role in vertebrate axis specification. Initially, the maternally encoded components of the pathway help to establish the dorsoventral axis, whereas at a later stage the zygotic Wnt pathway is involved in anteroposterior axis specification. These two roles are mediated by two distinct sets of specific targets, yet the underlying mechanisms for target selection are unclear. Further work is needed to understand the role of maternally produced Wnt ligands in early axis determination in the context of the abundant evidence for the involvement of microtubule-dependent trafficking in the dorsal accumulation of β -catenin. Although HIPK2-mediated phosphorylation of TCF3 is a major route for anteroposterior target activation, this phosphorylation is undetectable before gastrulation, suggesting an alternative mechanism for early target derepression (Hikasa et al. 2010). Other possibilities should also be explored, given that β -catenin can use other co-factors, besides TCFs, for transcriptional control (Olson et al. 2006; Takao et al. 2007; Abu-Rmaleh et al. 2010). Future research will be driven by the need to better understand pathway target selection and the contribution of Wnt5 and Wnt11 signaling to body-axis specification.

ACKNOWLEDGMENTS

We thank Stefan Hoppler, Keiji Itoh, and Jenya Grinblat for comments on the manuscript. We

H. Hikasa and S.Y. Sokol

apologize to those investigators, whose work has not been cited here because of limited space. The work in the Sokol laboratory is supported by National Institutes of Health grants.

REFERENCES

- Aamar E, Frank D. 2004. *Xenopus* Meis3 protein forms a hindbrain-inducing center by activating FGF/MAP kinase and PCP pathways. *Development* **131**: 153–163.
- Abu-Remaileh M, Gerson A, Farago M, Nathan G, Alkalay I, Zins Rouso S, Gur M, Fainsod A, Bergman Y. 2010. Oct-3/4 regulates stem cell identity and cell fate decisions by modulating Wnt/ β -catenin signalling. *EMBO J* **29**: 3236–3248.
- Akkers RC, van Heeringen SJ, Jacobi UG, Janssen-Megens EM, Francoijs KJ, Stunnenberg HG, Veenstra GJ. 2009. A hierarchy of H3K4me3 and H3K27me3 acquisition in spatial gene regulation in *Xenopus* embryos. *Dev Cell* **17**: 425–434.
- Amaya E, Musci TJ, Kirschner MW. 1991. Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell* **66**: 257–270.
- Amaya E, Stein PA, Musci TJ, Kirschner MW. 1993. FGF signalling in the early specification of mesoderm in *Xenopus*. *Development* **118**: 477–487.
- Andreazzoli M, Broccoli V, Dawid IB. 2001. Cloning and expression of *noz1*, a zebrafish zinc finger gene related to *Drosophila* *nocA*. *Mech Dev* **104**: 117–120.
- Angers S, Moon RT. 2009. Proximal events in Wnt signal transduction. *Nat Rev Mol Cell Biol* **10**: 468–477.
- Axelrod JD. 2009. Progress and challenges in understanding planar cell polarity signaling. *Semin Cell Dev Biol* **20**: 964–971.
- Beck F, Stringer EJ. 2010. The role of Cdx genes in the gut and in axial development. *Biochem Soc Trans* **38**: 353–357.
- Behrens J, von Kries JB, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. 1996. Functional interaction of β -catenin with the transcription factor LEF-1. *Nature* **382**: 638–642.
- Bellipanni G, Varga M, Maegawa S, Imai Y, Kelly C, Myers AP, Chu F, Talbot WS, Weinberg ES. 2006. Essential and opposing roles of zebrafish β -catenins in the formation of dorsal axial structures and neurectoderm. *Development* **133**: 1299–1309.
- Bilic J, Huang YL, Davidson G, Zimmermann T, Cruciat CM, Bienz M, Niehrs C. 2007. Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science* **316**: 1619–1622.
- Blumberg B, Wright CV, De Robertis EM, Cho KW. 1991. Organizer-specific homeobox genes in *Xenopus laevis* embryos. *Science* **253**: 194–196.
- Blythe SA, Cha SW, Tadjuidje E, Heasman J, Klein PS. 2010. β -Catenin primes organizer gene expression by recruiting a histone H3 arginine 8 methyltransferase, Prmt2. *Dev Cell* **19**: 220–231.
- Bouwmeester T, Kim S, Sasai Y, Lu B, De Robertis EM. 1996. Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382**: 595–601.
- Brannon M, Gomperts M, Sumoy L, Moon RT, Kimelman D. 1997. A β -catenin/XTcf-3 complex binds to the siamois promoter to regulate dorsal axis specification in *Xenopus*. *Genes Dev* **11**: 2359–2370.
- Brantjes H, Roose J, van De Wetering M, Clevers H. 2001. All Tcf HMG box transcription factors interact with Groucho-related co-repressors. *Nucleic Acids Res* **29**: 1410–1419.
- Bromley E, Knapp D, Wardle FC, Sun BI, Collins-Racie L, LaVallie E, Smith JC, Sive HL. 2004. Identification and characterisation of the posteriorly-expressed *Xenopus* neurotrophin receptor homolog genes fullback and full-back-like. *Gene Expr Patterns* **5**: 135–140.
- Brott BK, Sokol SY. 2002. Regulation of Wnt/LRP signaling by distinct domains of Dickkopf proteins. *Mol Cell Biol* **22**: 6100–6110.
- Burks PJ, Isaacs HV, Pownall ME. 2009. FGF signalling modulates transcriptional repression by *Xenopus* groucho-related-4. *Biol Cell* **101**: 301–308.
- Candia AE, Watabe T, Hawley SH, Onichtchouk D, Zhang Y, Derynck R, Niehrs C, Cho KW. 1997. Cellular interpretation of multiple TGF- β signals: Intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. *Development* **124**: 4467–4480.
- Cavallo RA, Cox RT, Moline MM, Roose J, Polevoy GA, Clevers H, Peifer M, Bejsovec A. 1998. *Drosophila* Tcf and Groucho interact to repress Wingless signalling activity. *Nature* **395**: 604–608.
- Cha SW, Tadjuidje E, Tao Q, Wylie C, Heasman J. 2008. Wnt5a and Wnt11 interact in a maternal Dkk1-regulated fashion to activate both canonical and non-canonical signaling in *Xenopus* axis formation. *Development* **135**: 3719–3729.
- Cho KW, Blumberg B, Steinbeisser H, De Robertis EM. 1991. Molecular nature of Spemann's organizer: The role of the *Xenopus* homeobox gene goosecoid. *Cell* **67**: 1111–1120.
- Choe SK, Lu P, Nakamura M, Lee J, Sagerstrom CG. 2009. Meis cofactors control HDAC and CBP accessibility at Hox-regulated promoters during zebrafish embryogenesis. *Dev Cell* **17**: 561–567.
- Christian JL, Moon RT. 1993. Interactions between Xwnt-8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. *Genes Dev* **7**: 13–28.
- Christian JL, Gavin BJ, McMahon AP, Moon RT. 1991a. Isolation of cDNAs partially encoding four *Xenopus* Wnt-1/int-1-related proteins and characterization of their transient expression during embryonic development. *Dev Biol* **143**: 230–234.
- Christian JL, McMahon JA, McMahon AP, Moon RT. 1991b. Xwnt-8, a *Xenopus* Wnt-1/int-1-related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesodermal patterning during embryogenesis. *Development* **111**: 1045–1055.
- Chung HM, Malacinski GM. 1980. Establishment of the dorsal/ventral polarity of the amphibian embryo: Use

- of ultraviolet irradiation and egg rotation as probes. *Dev Biol* **80**: 120–133.
- Chung HA, Hyodo-Miura J, Kitayama A, Terasaka C, Nagamune T, Ueno N. 2004. Screening of FGF target genes in *Xenopus* by microarray: Temporal dissection of the signalling pathway using a chemical inhibitor. *Genes Cells* **9**: 749–761.
- Clevers H. 2006. Wnt/ β -catenin signaling in development and disease. *Cell* **127**: 469–480.
- Cliffe A, Hamada F, Bienz M. 2003. A role of Dishevelled in relocating Axin to the plasma membrane during wingless signaling. *Curr Biol* **13**: 960–966.
- Cole MF, Johnstone SE, Newman JJ, Kagey MH, Young RA. 2008. Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells. *Genes Dev* **22**: 746–755.
- Conlon FL, Lyons KM, Takaesu N, Barth KS, Kispert A, Herrmann B, Robertson EJ. 1994. A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* **120**: 1919–1928.
- Cook D, Fry MJ, Hughes K, Sumathipala R, Woodgett JR, Dale TC. 1996. Wingless inactivates glycogen synthase kinase-3 via an intracellular signalling pathway which involves a protein kinase C. *EMBO J* **15**: 4526–4536.
- Cox WG, Hemmati-Brivanlou A. 1995. Caudalization of neural fate by tissue recombination and bFGF. *Development* **121**: 4349–4358.
- Daniels DL, Weis WI. 2005. β -Catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nat Struct Mol Biol* **12**: 364–371.
- Darken RS, Wilson PA. 2001. Axis induction by wnt signaling: Target promoter responsiveness regulates competence. *Dev Biol* **234**: 42–54.
- Davidson AJ, Ernst P, Wang Y, Dekens MP, Kingsley PD, Palis J, Korsmeyer SJ, Daley GQ, Zon LI. 2003. *cdx4* mutants fail to specify blood progenitors and can be rescued by multiple *hox* genes. *Nature* **425**: 300–306.
- Deardorff MA, Tan C, Conrad LJ, Klein PS. 1998. Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. *Development* **125**: 2687–2700.
- De Robertis EM, Kuroda H. 2004. Dorsal-ventral patterning and neural induction in *Xenopus* embryos. *Annu Rev Cell Dev Biol* **20**: 285–308.
- Doniach T, Phillips CR, Gerhart JC. 1992. Planar induction of anteroposterior pattern in the developing central nervous system of *Xenopus laevis*. *Science* **257**: 542–545.
- Dorsky RI, Snyder A, Cretekos CJ, Grunwald DJ, Geisler R, Haffter P, Moon RT, Raible DW. 1999. Maternal and embryonic expression of zebrafish *lef1*. *Mech Dev* **86**: 147–150.
- Dorsky RI, Sheldahl LC, Moon RT. 2002. A transgenic *Left1*/ β -catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. *Dev Biol* **241**: 229–237.
- Dorsky RI, Itoh M, Moon RT, Chitnis A. 2003. Two *tcf3* genes cooperate to pattern the zebrafish brain. *Development* **130**: 1937–1947.
- Elkouby YM, Elias S, Casey ES, Blythe SA, Tsabar N, Klein PS, Root H, Liu KJ, Frank D. 2010. Mesodermal Wnt signaling organizes the neural plate via *Meis3*. *Development* **137**: 1531–1541.
- Epstein M, Pillemer G, Yelin R, Yisraeli JK, Fainsod A. 1997. Patterning of the embryo along the anterior-posterior axis: The role of the caudal genes. *Development* **124**: 3805–3814.
- Erter CE, Wilm TP, Basler N, Wright CV, Solnica-Krezel L. 2001. Wnt8 is required in lateral mesendodermal precursors for neural posteriorization in vivo. *Development* **128**: 3571–3583.
- Faas L, Isaacs HV. 2009. Overlapping functions of *Cdx1*, *Cdx2*, and *Cdx4* in the development of the amphibian *Xenopus tropicalis*. *Dev Dyn* **238**: 835–852.
- Fan MJ, Sokol SY. 1997. A role for Siamois in Spemann organizer formation. *Development* **124**: 2581–2589.
- Fan MJ, Gruning W, Walz G, Sokol SY. 1998. Wnt signaling and transcriptional control of Siamois in *Xenopus* embryos. *Proc Natl Acad Sci* **95**: 5626–5631.
- Fekany K, Yamanaka Y, Leung T, Sirotkin HI, Topczewski J, Gates MA, Hibi M, Renucci A, Stemple D, Radbill A, et al. 1999. The zebrafish *bozozok* locus encodes Dharma, a homeodomain protein essential for induction of gastrula organizer and dorsoanterior embryonic structures. *Development* **126**: 1427–1438.
- Feldman B, Gates MA, Egan ES, Dougan ST, Rennebeck G, Sirotkin HI, Schier AF, Talbot WS. 1998. Zebrafish organizer development and germ-layer formation require nodal-related signals. *Nature* **395**: 181–185.
- Fossat N, Jones V, Khoo PL, Bogani D, Hardy A, Steiner K, Mukhopadhyay M, Westphal H, Nolan PM, Arkell R, et al. 2011. Stringent requirement of a proper level of canonical WNT signalling activity for head formation in mouse embryo. *Development* **138**: 667–676.
- Frazzetto G, Klingbeil P, Bouwmeester T. 2002. *Xenopus* marginal coil (*Xmc*), a novel FGF inducible cytosolic coiled-coil protein regulating gastrulation movements. *Mech Dev* **113**: 3–14.
- Fuentealba LC, Eivers E, Ikeda A, Hurtado C, Kuroda H, Pera EM, De Robertis EM. 2007. Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal. *Cell* **131**: 980–993.
- Fujisue M, Kobayakawa Y, Yamana K. 1993. Occurrence of dorsal axis-inducing activity around the vegetal pole of an uncleaved *Xenopus* egg and displacement to the equatorial region by cortical rotation. *Development* **118**: 163–170.
- Gao B, Song H, Bishop K, Elliot G, Garrett L, English MA, Andre P, Robinson J, Sood R, Minami Y, et al. 2011. Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. *Dev Cell* **20**: 163–176.
- Garcia-Morales C, Liu CH, Abu-Elmagd M, Hajihosseini MK, Wheeler GN. 2009. Frizzled-10 promotes sensory neuron development in *Xenopus* embryos. *Dev Biol* **335**: 143–155.
- Gawantka V, Delius H, Hirschfeld K, Blumenstock C, Niehrs C. 1995. Antagonizing the Spemann organizer: Role of the homeobox gene *Xvent-1*. *EMBO J* **14**: 6268–6279.
- Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. 1998. Dickkopf-1 is a member of a new family

H. Hikasa and S.Y. Sokol

- of secreted proteins and functions in head induction. *Nature* **391**: 357–362.
- Grädl D, König A, Wedlich D. 2002. Functional diversity of *Xenopus* lymphoid enhancer factor/T-cell factor transcription factors relies on combinations of activating and repressing elements. *J Biol Chem* **277**: 14159–14171.
- Gribble SL, Kim HS, Bonner J, Wang X, Dorsky RI. 2009. Tcf3 inhibits spinal cord neurogenesis by regulating sox4a expression. *Development* **136**: 781–789.
- Grigoryan T, Wend P, Klaus A, Birchmeier W. 2008. Deciphering the function of canonical Wnt signals in development and disease: Conditional loss- and gain-of-function mutations of β -catenin in mice. *Genes Dev* **22**: 2308–2341.
- Grumolato L, Liu G, Mong P, Mudbhary R, Biswas R, Arroyave R, Vijayakumar S, Economides AN, Aaronson SA. 2010. Canonical and noncanonical Wnts use a common mechanism to activate completely unrelated coreceptors. *Genes Dev* **24**: 2517–2530.
- Haegel H, Larue L, Ohsugi M, Fedorov L, Herrenknecht K, Kemler R. 1995. Lack of β -catenin affects mouse development at gastrulation. *Development* **121**: 3529–3537.
- Hamilton FS, Wheeler GN, Hoppler S. 2001. Difference in XTcf-3 dependency accounts for change in response to β -catenin-mediated Wnt signalling in *Xenopus* blastula. *Development* **128**: 2063–2073.
- Haremak T, Tanaka Y, Hongo I, Yuge M, Okamoto H. 2003. Integration of multiple signal transducing pathways on Fgf response elements of the *Xenopus* caudal homologue Xcad3. *Development* **130**: 4907–4917.
- Harland R, Gerhart J. 1997. Formation and function of Spemann's organizer. *Annu Rev Cell Dev Biol* **13**: 611–667.
- Hartmann C. 2006. A Wnt canon orchestrating osteoblastogenesis. *Trends Cell Biol* **16**: 151–158.
- Hata A, Seoane J, Lagna G, Montalvo E, Hemmati-Brivanlou A, Massague J. 2000. OAZ uses distinct DNA- and protein-binding zinc fingers in separate BMP-Smad and Olf signaling pathways. *Cell* **100**: 229–240.
- Heasman J, Crawford A, Goldstone K, Garner-Hamrick P, Gumbiner B, McCrear P, Kintner C, Noro CY, Wylie C. 1994. Overexpression of cadherins and underexpression of β -catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell* **79**: 791–803.
- Heasman J, Kofron M, Wylie C. 2000. β -Catenin signaling activity dissected in the early *Xenopus* embryo: A novel antisense approach. *Dev Biol* **222**: 124–134.
- Heisenberg CP, Tada M, Rauch GJ, Saude L, Concha ML, Geisler R, Stemple DL, Smith JC, Wilson SW. 2000. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**: 76–81.
- Heisenberg CP, Houart C, Take-Uchi M, Rauch GJ, Young N, Coutinho P, Masai I, Caneparo L, Concha ML, Geisler R, et al. 2001. A mutation in the Gsk3-binding domain of zebrafish Masterblind/Axin1 leads to a fate transformation of telencephalon and eyes to diencephalon. *Genes Dev* **15**: 1427–1434.
- Hikasa H, Sokol SY. 2011. Phosphorylation of TCF proteins by homeodomain-interacting protein kinase 2. *J Biol Chem* **286**: 12093–12100.
- Hikasa H, Shibata M, Hiratani I, Taira M. 2002. The *Xenopus* receptor tyrosine kinase Xror2 modulates morphogenetic movements of the axial mesoderm and neuroectoderm via Wnt signaling. *Development* **129**: 5227–5239.
- Hikasa H, Ezan J, Itoh K, Li X, Klymkowsky MW, Sokol SY. 2010. Regulation of TCF3 by Wnt-dependent phosphorylation during vertebrate axis specification. *Dev Cell* **19**: 521–532.
- Hilton E, Rex M, Old R. 2003. VegT activation of the early zygotic gene Xnr5 requires lifting of Tcf-mediated repression in the *Xenopus* blastula. *Mech Dev* **120**: 1127–1138.
- Holowacz T, Elinson RP. 1993. Cortical cytoplasm, which induces dorsal axis formation in *Xenopus*, is inactivated by UV irradiation of the oocyte. *Development* **119**: 277–285.
- Holowacz T, Sokol S. 1999. FGF is required for posterior neural patterning but not for neural induction. *Dev Biol* **205**: 296–308.
- Holstein TW. 2008. Wnt signaling in cnidarians. *Methods Mol Biol* **469**: 47–54.
- Hong CS, Park BY, Saint-Jeannet JP. 2008. Fgf8a induces neural crest indirectly through the activation of Wnt8 in the paraxial mesoderm. *Development* **135**: 3903–3910.
- Hoppler S, Moon RT. 1998. BMP-2/-4 and Wnt-8 cooperatively pattern the *Xenopus* mesoderm. *Mech Dev* **71**: 119–129.
- Hoppler S, Brown JD, Moon RT. 1996. Expression of a dominant-negative Wnt blocks induction of MyoD in *Xenopus* embryos. *Genes Dev* **10**: 2805–2817.
- Houliston E, Elinson RP. 1992. Microtubules and cytoplasmic reorganization in the frog egg. *Curr Top Dev Biol* **26**: 53–70.
- Houston DW, Kofron M, Resnik E, Langland R, Destree O, Wylie C, Heasman J. 2002. Repression of organizer genes in dorsal and ventral *Xenopus* cells mediated by maternal XTcf3. *Development* **129**: 4015–4025.
- Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R. 1996. Nuclear localization of β -catenin by interaction with transcription factor LEF-1. *Mech Dev* **59**: 3–10.
- Huelsken J, Vogel R, Brinkmann V, Erdmann B, Birchmeier C, Birchmeier W. 2000. Requirement for β -catenin in anterior-posterior axis formation in mice. *J Cell Biol* **148**: 567–578.
- Huften AL, Vinayagam A, Suhai S, Baker JC. 2006. Genomic analysis of *Xenopus* organizer function. *BMC Dev Biol* **6**: 27.
- Imai Y, Gates MA, Melby AE, Kimelman D, Schier AF, Talbot WS. 2001. The homeobox genes *vox* and *vent* are redundant repressors of dorsal fates in zebrafish. *Development* **128**: 2407–2420.
- Isaacs HV, Pownall ME, Slack JM. 1998. Regulation of Hox gene expression and posterior development by the *Xenopus* caudal homologue Xcad3. *EMBO J* **17**: 3413–3427.
- Ishikawa TO, Tamai Y, Li Q, Oshima M, Taketo MM. 2003. Requirement for tumor suppressor Apc in the morphogenesis of anterior and ventral mouse embryo. *Dev Biol* **253**: 230–246.
- Ishitani T, Ninomiya-Tsuji J, Nagai S, Nishita M, Meneghini M, Barker N, Waterman M, Bowerman B, Clevers H,

- Shibuya H, et al. 1999. The TAK1-NLK-MAPK-related pathway antagonizes signalling between β -catenin and transcription factor TCF. *Nature* **399**: 798–802.
- Itasaki N, Hoppler S. 2010. Crosstalk between Wnt and bone morphogenic protein signaling: A turbulent relationship. *Dev Dyn* **239**: 16–33.
- Itoh K, Sokol SY. 1997. Graded amounts of *Xenopus* dishevelled specify discrete anteroposterior cell fates in prospective ectoderm. *Mech Dev* **61**: 113–125.
- Itoh K, Tang TL, Neel BG, Sokol SY. 1995. Specific modulation of ectodermal cell fates in *Xenopus* embryos by glycogen synthase kinase 3 and β -catenin. *Development* **121**: 3979–3988.
- Itoh K, Jacob J, Sokol YS. 1998a. A role for *Xenopus* Frizzled 8 in dorsal development. *Mech Dev* **74**: 145–157.
- Itoh K, Krupnik VE, Sokol SY. 1998b. Axis determination in *Xenopus* involves biochemical interactions of axin, glycogen synthase kinase 3 and β -catenin. *Curr Biol* **8**: 591–594.
- Itoh K, Antipova A, Ratcliffe MJ, Sokol S. 2000. Interaction of dishevelled and *Xenopus* axin-related protein is required for wnt signal transduction. *Mol Cell Biol* **20**: 2228–2238.
- Itoh K, Brott BK, Bae GU, Ratcliffe MJ, Sokol SY. 2005. Nuclear localization is required for Dishevelled function in Wnt/ β -catenin signaling. *J Biol* **4**: 3.
- Itoh K, Jenny A, Mlodzik M, Sokol SY. 2009. Centrosomal localization of Diversin and its relevance to Wnt signaling. *J Cell Sci* **122**: 3791–3798.
- Jesuthasan S, Stahle U. 1997. Dynamic microtubules and specification of the zebrafish embryonic axis. *Curr Biol* **7**: 31–42.
- Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. 2002. Wnt/ β -catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol Cell Biol* **22**: 1172–1183.
- Kanei-Ishii C, Ninomiya-Tsuji J, Tanikawa J, Nomura T, Ishitani T, Kishida S, Kokura K, Kurahashi T, Ichikawa-Iwata E, Kim Y, et al. 2004. Wnt-1 signal induces phosphorylation and degradation of c-Myc protein via TAK1, HIPK2, and NLK. *Genes Dev* **18**: 816–829.
- Karaulanov E, Knochel W, Niehrs C. 2004. Transcriptional regulation of BMP4 synexpression in transgenic *Xenopus*. *EMBO J* **23**: 844–856.
- Kawakami Y, Capdevila J, Buscher D, Itoh T, Rodriguez Esteban C, Izpisua Belmonte JC. 2001. WNT signals control FGF-dependent limb initiation and AER induction in the chick embryo. *Cell* **104**: 891–900.
- Keenan ID, Sharrard RM, Isaacs HV. 2006. FGF signal transduction and the regulation of Cdx gene expression. *Dev Biol* **299**: 478–488.
- Kelly GM, Greenstein B, Erezylmaz DE, Moon RT. 1995. Zebrafish wnt8 and wnt8b share a common activity but are involved in distinct developmental pathways. *Development* **121**: 1787–1799.
- Kelly C, Chin AJ, Leatherman JL, Kozlowski DJ, Weinberg ES. 2000. Maternally controlled β -catenin-mediated signaling is required for organizer formation in the zebrafish. *Development* **127**: 3899–3911.
- Kessler DS. 1997. Siamois is required for formation of Spemann's organizer. *Proc Natl Acad Sci* **94**: 13017–13022.
- Kiecker C, Niehrs C. 2001. A morphogen gradient of Wnt/ β -catenin signalling regulates anteroposterior neural patterning in *Xenopus*. *Development* **128**: 4189–4201.
- Kikuchi A, Yamamoto H, Sato A. 2009. Selective activation mechanisms of Wnt signaling pathways. *Trends Cell Biol* **19**: 119–129.
- Kilian B, Mansukoski H, Barbosa FC, Ulrich F, Tada M, Heisenberg CP. 2003. The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation. *Mech Dev* **120**: 467–476.
- Kim CH, Oda T, Itoh M, Jiang D, Artinger KB, Chandrasekharappa SC, Driever W, Chitnis AB. 2000. Repressor activity of Headless/Tcf3 is essential for vertebrate head formation. *Nature* **407**: 913–916.
- Kim EA, Kim JE, Sung KS, Choi DW, Lee BJ, Choi CY. 2010. Homeodomain-interacting protein kinase 2 (HIPK2) targets β -catenin for phosphorylation and proteasomal degradation. *Biochem Biophys Res Commun* **394**: 966–971.
- Klein PS, Melton DA. 1996. A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci* **93**: 8455–8459.
- Kolm PJ, Sive HL. 1995. Regulation of the *Xenopus* labial homeodomain genes, HoxA1 and HoxD1: Activation by retinoids and peptide growth factors. *Dev Biol* **167**: 34–49.
- Komiya Y, Habas R. 2008. Wnt signal transduction pathways. *Organogenesis* **4**: 68–75.
- Konig A, Gradl D, Kuhl M, Wedlich D. 2000. The HMG-box transcription factor XTcf-4 demarcates the forebrain-midbrain boundary. *Mech Dev* **93**: 211–214.
- Koos DS, Ho RK. 1998. The *nieuwkoid* gene characterizes and mediates a Nieuwkoop-center-like activity in the zebrafish. *Curr Biol* **8**: 1199–1206.
- Kouzarides T. 2007. Chromatin modifications and their function. *Cell* **128**: 693–705.
- Krauss S, Korzh V, Fjose A, Johansen T. 1992. Expression of four zebrafish wnt-related genes during embryogenesis. *Development* **116**: 249–259.
- Ku M, Melton DA. 1993. Xwnt-11: A maternally expressed *Xenopus* wnt gene. *Development* **119**: 1161–1173.
- Kudoh T, Concha ML, Houart C, Dawid IB, Wilson SW. 2004. Combinatorial Fgf and Bmp signalling patterns the gastrula ectoderm into prospective neural and epidermal domains. *Development* **131**: 3581–3592.
- Labbe E, Letamendia A, Attisano L. 2000. Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor- β and wnt pathways. *Proc Natl Acad Sci* **97**: 8358–8363.
- Ladher R, Mohun TJ, Smith JC, Snape AM. 1996. Xom: A *Xenopus* homeobox gene that mediates the early effects of BMP-4. *Development* **122**: 2385–2394.
- Lamb TM, Harland RM. 1995. Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. *Development* **121**: 3627–3636.
- Larabell CA, Torres M, Rowing BA, Yost C, Miller JR, Wu M, Kimelman D, Moon RT. 1997. Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in β -catenin that are modulated by the Wnt signaling pathway. *J Cell Biol* **136**: 1123–1136.
- Laurent MN, Blitz IL, Hashimoto C, Rothbacher U, Cho KW. 1997. The *Xenopus* homeobox gene *twin* mediates

H. Hikasa and S.Y. Sokol

- Wnt induction of goosecoid in establishment of Spemann's organizer. *Development* **124**: 4905–4916.
- Lee W, Swarup S, Chen J, Ishitani T, Verheyen EM. 2009. Homeodomain-interacting protein kinases (Hipks) promote Wnt/Wg signaling through stabilization of β -catenin/Arm and stimulation of target gene expression. *Development* **136**: 241–251.
- Lekven AC, Thorpe CJ, Waxman JS, Moon RT. 2001. Zebrafish wnt8 encodes two wnt8 proteins on a bicistronic transcript and is required for mesoderm and neurectoderm patterning. *Dev Cell* **1**: 103–114.
- Lemaire P, Garrett N, Gurdon JB. 1995. Expression cloning of Siamois, a *Xenopus* homeobox gene expressed in dorsal-vegetal cells of blastulae and able to induce a complete secondary axis. *Cell* **81**: 85–94.
- Leung T, Soll I, Arnold SJ, Kemler R, Driever W. 2003. Direct binding of Lef1 to sites in the boz promoter may mediate pre-midblastula-transition activation of boz expression. *Dev Dyn* **228**: 424–432.
- Leys L, Bouwmeester T, Kim SH, Piccolo S, De Robertis EM. 1997. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* **88**: 747–756.
- Li E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* **3**: 662–673.
- Li Y, Fenger U, Niehrs C, Pollet N. 2003. Cyclic expression of *esr9* gene in *Xenopus* presomitic mesoderm. *Differentiation* **71**: 83–89.
- Li H, Malbon CC, Wang HY. 2004. Gene profiling of Frizzled-1 and Frizzled-2 signaling: Expression of G-protein-coupled receptor chimeras in mouse F9 teratocarcinoma embryonal cells. *Mol Pharmacol* **65**: 45–55.
- Li B, Kuriyama S, Moreno M, Mayor R. 2009. The posteriorizing gene *Gbx2* is a direct target of Wnt signalling and the earliest factor in neural crest induction. *Development* **136**: 3267–3278.
- Lin G, Slack JM. 2008. Requirement for Wnt and FGF signaling in *Xenopus* tadpole tail regeneration. *Dev Biol* **316**: 323–335.
- Lin S, Baye LM, Westfall TA, Slusarski DC. 2010. Wnt5b-Ryk pathway provides directional signals to regulate gastrulation movement. *J Cell Biol* **190**: 263–278.
- Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X. 1999a. β -Trcp couples β -catenin phosphorylation-degradation and regulates *Xenopus* axis formation. *Proc Natl Acad Sci* **96**: 6273–6278.
- Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. 1999b. Requirement for Wnt3 in vertebrate axis formation. *Nat Genet* **22**: 361–365.
- Liu F, van den Broek O, Destree O, Hoppler S. 2005. Distinct roles for *Xenopus* Tcf/Lef genes in mediating specific responses to Wnt/ β -catenin signalling in mesoderm development. *Development* **132**: 5375–5385.
- Lo MC, Gay F, Odom R, Shi Y, Lin R. 2004. Phosphorylation by the β -catenin/MAPK complex promotes 14–3–3-mediated nuclear export of TCF/POP-1 in signal-responsive cells in *C. elegans*. *Cell* **117**: 95–106.
- Louie SH, Yang XY, Conrad WH, Muster J, Angers S, Moon RT, Cheyette BN. 2009. Modulation of the β -catenin signaling pathway by the dishevelled-associated protein Hipk1. *PLoS ONE* **4**: e4310.
- Lu W, Yamamoto V, Ortega B, Baltimore D. 2004. Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. *Cell* **119**: 97–108.
- Lupo G, Harris WA, Barsacchi G, Vignali R. 2002. Induction and patterning of the telencephalon in *Xenopus laevis*. *Development* **129**: 5421–5436.
- Lyman Gingerich J, Westfall TA, Slusarski DC, Pelegri F. 2005. Hecate, a zebrafish maternal effect gene, affects dorsal organizer induction and intracellular calcium transient frequency. *Dev Biol* **286**: 427–439.
- MacDonald BT, Tamai K, He X. 2009. Wnt/ β -catenin signaling: Components, mechanisms, and diseases. *Dev Cell* **17**: 9–26.
- Maeda R, Kobayashi A, Sekine R, Lin JJ, Kung H, Maeno M. 1997. Xmsx-1 modifies mesodermal tissue pattern along dorsoventral axis in *Xenopus laevis* embryo. *Development* **124**: 2553–2560.
- Maegawa S, Varga M, Weinberg ES. 2006. FGF signaling is required for β -catenin-mediated induction of the zebrafish organizer. *Development* **133**: 3265–3276.
- Majumdar A, Vainio S, Kispert A, McMahon J, McMahon AP. 2003. Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. *Development* **130**: 3175–3185.
- Maniatis T. 1999. A ubiquitin ligase complex essential for the NF- κ B, Wnt/Wingless, and Hedgehog signaling pathways. *Genes Dev* **13**: 505–510.
- Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, Delius H, Hoppe D, Stanek P, Walter C, et al. 2002. Kremen proteins are Dickkopf receptors that regulate Wnt/ β -catenin signalling. *Nature* **417**: 664–667.
- Marazzi G, Wang Y, Sassoon D. 1997. Msx2 is a transcriptional regulator in the BMP4-mediated programmed cell death pathway. *Dev Biol* **186**: 127–138.
- Marchal L, Luxardi G, Thome V, Kodjabachian L. 2009. BMP inhibition initiates neural induction via FGF signaling and *Zic* genes. *Proc Natl Acad Sci* **106**: 17437–17442.
- Marlow FL. 2010. *Maternal control of development in vertebrates: My mother made me do it!* Morgan & Claypool Life Sciences, San Rafael, CA.
- Martin BL, Kimelman D. 2009. Wnt signaling and the evolution of embryonic posterior development. *Curr Biol* **19**: R215–R219.
- Martinez S, Crossley PH, Cobos I, Rubenstein JL, Martin GR. 1999. FGF8 induces formation of an ectopic isthmus organizer and isthmocerebellar development via a repressive effect on *Otx2* expression. *Development* **126**: 1189–1200.
- Massague J. 2003. Integration of Smad and MAPK pathways: A link and a linker revisited. *Genes Dev* **17**: 2993–2997.
- Mathers PH, Grinberg A, Mahon KA, Jamrich M. 1997. The Rx homeobox gene is essential for vertebrate eye development. *Nature* **387**: 603–607.
- McGrew LL, Hoppler S, Moon RT. 1997. Wnt and FGF pathways cooperatively pattern anteroposterior neural ectoderm in *Xenopus*. *Mech Dev* **69**: 105–114.
- McKendry R, Hsu SC, Harland RM, Grosschedl R. 1997. LEF-1/TCF proteins mediate wnt-inducible trans-

- cription from the *Xenopus* nodal-related 3 promoter. *Dev Biol* **192**: 420–431.
- McMahon AP, Moon RT. 1989. Ectopic expression of the proto-oncogene *int-1* in *Xenopus* embryos leads to duplication of the embryonic axis. *Cell* **58**: 1075–1084.
- Melby AE, Clements WK, Kimelman D. 1999. Regulation of dorsal gene expression in *Xenopus* by the ventralizing homeodomain gene *Vox*. *Dev Biol* **211**: 293–305.
- Melby AE, Beach C, Mullins M, Kimelman D. 2000. Patterning the early zebrafish by the opposing actions of *bozozok* and *vox*/vent. *Dev Biol* **224**: 275–285.
- Meneghini MD, Ishitani T, Carter JC, Hisamoto N, Nino-miya-Tsuiji J, Thorpe CJ, Hamill DR, Matsumoto K, Bowerman B. 1999. MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* **399**: 793–797.
- Merrill BJ, Pasolli HA, Polak L, Rendl M, Garcia-Garcia MJ, Anderson KV, Fuchs E. 2004. Tcf3: A transcriptional regulator of axis induction in the early embryo. *Development* **131**: 263–274.
- Mikels AJ, Nusse R. 2006. Purified Wnt5a protein activates or inhibits β -catenin-TCF signaling depending on receptor context. *PLoS Biol* **4**: e115.
- Mir A, Kofron M, Zorn AM, Bajzer M, Haque M, Heasman J, Wylie CC. 2007. FoxI1e activates ectoderm formation and controls cell position in the *Xenopus* blastula. *Development* **134**: 779–788.
- Mizuno T, Yamahara E, Kuroiwa A, Takeda H. 1999. Removal of vegetal yolk causes dorsal deficiencies and impairs dorsal-inducing ability of the yolk cell in zebrafish. *Mech Dev* **81**: 51–63.
- Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destree O, Clevers H. 1996. XTcf-3 transcription factor mediates β -catenin-induced axis formation in *Xenopus* embryos. *Cell* **86**: 391–399.
- Molenaar M, Roose J, Peterson J, Venanzi S, Clevers H, Destree O. 1998. Differential expression of the HMG box transcription factors XTcf-3 and XLe-1 during early *Xenopus* development. *Mech Dev* **75**: 151–154.
- Moon RT, Kimelman D. 1998. From cortical rotation to organizer gene expression: Toward a molecular explanation of axis specification in *Xenopus*. *Bioessays* **20**: 536–545.
- Moon RT, Campbell RM, Christian JL, McGrew LL, Shih J, Fraser S. 1993. Xwnt-5A: A maternal Wnt that affects morphogenetic movements after overexpression in embryos of *Xenopus laevis*. *Development* **119**: 97–111.
- Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L, Dorward DW, Glinka A, Grinberg A, Huang SP, et al. 2001. Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev Cell* **1**: 423–434.
- Nakamura M, Choe SK, Runko AP, Gardner PD, Sagerstrom CG. 2008. Nlz1/Znf703 acts as a repressor of transcription. *BMC Dev Biol* **8**: 108.
- Nelson WJ, Nusse R. 2004. Convergence of Wnt, β -catenin, and cadherin pathways. *Science* **303**: 1483–1487.
- Nentwich O, Dingwell KS, Nordheim A, Smith JC. 2009. Downstream of FGF during mesoderm formation in *Xenopus*: The roles of Elk-1 and Egr-1. *Dev Biol* **336**: 313–326.
- Ng RK, Gurdon JB. 2008. Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. *Nat Cell Biol* **10**: 102–109.
- Nguyen H, Rendl M, Fuchs E. 2006. Tcf3 governs stem cell features and represses cell fate determination in skin. *Cell* **127**: 171–183.
- Nieuwkoop PD. 1952. Activation and organization of the central nervous system in amphibians. *J Exp Zool* **120**: 83–108.
- Nishita M, Hashimoto MK, Ogata S, Laurent MN, Ueno N, Shibuya H, Cho KW. 2000. Interaction between Wnt and TGF- β signalling pathways during formation of Spemann's organizer. *Nature* **403**: 781–785.
- Nishita M, Yoo SK, Nomachi A, Kani S, Sougawa N, Ohta Y, Takada S, Kikuchi A, Minami Y. 2006. Filopodia formation mediated by receptor tyrosine kinase Ror2 is required for Wnt5a-induced cell migration. *J Cell Biol* **175**: 555–562.
- Nishita M, Itsukushima S, Nomachi A, Endo M, Wang Z, Inaba D, Qiao S, Takada S, Kikuchi A, Minami Y. 2010. Ror2/Frizzled complex mediates Wnt5a-induced AP-1 activation by regulating Dishevelled polymerization. *Mol Cell Biol* **30**: 3610–3619.
- Nojima H, Shimizu T, Kim CH, Yabe T, Bae YK, Muraoka O, Hirata T, Chitnis A, Hirano T, Hibi M. 2004. Genetic evidence for involvement of maternally derived Wnt canonical signaling in dorsal determination in zebrafish. *Mech Dev* **121**: 371–386.
- Nojima H, Rothamel S, Shimizu T, Kim CH, Yonemura S, Marlow FL, Hibi M. 2010. Syntabulin, a motor protein linker, controls dorsal determination. *Development* **137**: 923–933.
- Northrop JL, Kimelman D. 1994. Dorsal-ventral differences in Xcad-3 expression in response to FGF-mediated induction in *Xenopus*. *Dev Biol* **161**: 490–503.
- Ober EA, Schulte-Merker S. 1999. Signals from the yolk cell induce mesoderm, neuroectoderm, the trunk organizer, and the notochord in zebrafish. *Dev Biol* **215**: 167–181.
- Olson LE, Tollkuhn J, Scafoglio C, Kronen A, Zhang J, Ohgi KA, Wu W, Taketo MM, Kemler R, Grosschedl R, et al. 2006. Homeodomain-mediated β -catenin-dependent switching events dictate cell-lineage determination. *Cell* **125**: 593–605.
- Onichtchouk D, Gawanke V, Dosch R, Delius H, Hirschfeld K, Blumenstock C, Niehrs C. 1996. The Xvent-2 homeobox gene is part of the BMP-4 signalling pathway controlling dorsoventral patterning of *Xenopus* mesoderm. *Development* **122**: 3045–3053.
- Osada SI, Wright CV. 1999. *Xenopus* nodal-related signaling is essential for mesendodermal patterning during early embryogenesis. *Development* **126**: 3229–3240.
- Pera EM, Ikeda A, Eivers E, De Robertis EM. 2003. Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. *Genes Dev* **17**: 3023–3028.
- Perantoni AO. 2003. Renal development: Perspectives on a Wnt-dependent process. *Semin Cell Dev Biol* **14**: 201–208.

H. Hikasa and S.Y. Sokol

- Petersen CP, Reddien PW. 2009. Wnt signaling and the polarity of the primary body axis. *Cell* **139**: 1056–1068.
- Phillips BT, Kimble J. 2009. A new look at TCF and β -catenin through the lens of a divergent *C. elegans* Wnt pathway. *Dev Cell* **17**: 27–34.
- Pillemer G, Yelin R, Epstein M, Gont L, Frumkin Y, Yisraeli JK, Steinbeisser H, Fainsod A. 1998. The Xcad-2 gene can provide a ventral signal independent of BMP-4. *Mech Dev* **74**: 133–143.
- Pilon N, Oh K, Sylvestre JR, Bouchard N, Savory J, Lohnes D. 2006. Cdx4 is a direct target of the canonical Wnt pathway. *Dev Biol* **289**: 55–63.
- Ramel MC, Lekven AC. 2004. Repression of the vertebrate organizer by Wnt8 is mediated by Vent and Vox. *Development* **131**: 3991–4000.
- Ramel MC, Buckles GR, Baker KD, Lekven AC. 2005. WNT8 and BMP2B co-regulate non-axial mesoderm patterning during zebrafish gastrulation. *Dev Biol* **287**: 237–248.
- Rauch GJ, Hammerschmidt M, Blader P, Schauerte HE, Strahle U, Ingham PW, McMahon AP, Haftter P. 1997. Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harb Symp Quant Biol* **62**: 227–234.
- Rocheleau CE, Downs WD, Lin R, Wittmann C, Bei Y, Cha YH, Ali M, Priess JR, Mello CC. 1997. Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* **90**: 707–716.
- Rocheleau CE, Yasuda J, Shin TH, Lin R, Sawa H, Okano H, Priess JR, Davis RJ, Mello CC. 1999. WRM-1 activates the LIT-1 protein kinase to transduce anterior/posterior polarity signals in *C. elegans*. *Cell* **97**: 717–726.
- Roel G, Hamilton FS, Gent Y, Bain AA, Destree O, Hoppler S. 2002. Lef-1 and Tcf-3 transcription factors mediate tissue-specific Wnt signaling during *Xenopus* development. *Curr Biol* **12**: 1941–1945.
- Roel G, van den Broek O, Spieker N, Peterson-Maduro J, Destree O. 2003. Tcf-1 expression during *Xenopus* development. *Gene Expr Patterns* **3**: 123–126.
- Roose J, Molenaar M, Peterson J, Hurenkamp J, Brantjes H, Moerer P, van de Wetering M, Destree O, Clevers H. 1998. The *Xenopus* Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature* **395**: 608–612.
- Rothbacher U, Laurent MN, Blitz IL, Watabe T, Marsh JL, Cho KWY. 1995. Functional conservation of the Wnt signaling pathway revealed by ectopic expression of *Drosophila* dishevelled in *Xenopus*. *Dev Biol* **170**: 717–721.
- Ryu SL, Fujii R, Yamanaka Y, Shimizu T, Yabe T, Hirata T, Hibi M, Hirano T. 2001. Regulation of dharma/bozozok by the Wnt pathway. *Dev Biol* **231**: 397–409.
- Sander V, Reversade B, De Robertis EM. 2007. The opposing homeobox genes Gooseoid and Vent1/2 self-regulate *Xenopus* patterning. *EMBO J* **26**: 2955–2965.
- Sapkota G, Alarcon C, Spagnoli FM, Brivanlou AH, Massague J. 2007. Balancing BMP signaling through integrated inputs into the Smad1 linker. *Mol Cell* **25**: 441–454.
- Sasai Y, Lu B, Steinbeisser H, Geissert D, Gont LK, De Robertis EM. 1994. *Xenopus* chordin: A novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell* **79**: 779–790.
- Sasai N, Nakazawa Y, Haraguchi T, Sasai Y. 2004. The neurotrophin-receptor-related protein NRH1 is essential for convergent extension movements. *Nat Cell Biol* **6**: 741–748.
- Sato SM, Sargent TD. 1991. Localized and inducible expression of *Xenopus-posterior* (Xpo), a novel gene active in early frog embryos, encoding a protein with a “CCHC” finger domain. *Development* **112**: 747–754.
- Sato A, Yamamoto H, Sakane H, Koyama H, Kikuchi A. 2010. Wnt5a regulates distinct signalling pathways by binding to Frizzled2. *EMBO J* **29**: 41–54.
- Satoh K, Kasai M, Ishida T, Tago K, Ohwada S, Hasegawa Y, Senda T, Takada S, Nada S, Nakamura T, et al. 2004. Anteriorization of neural fate by inhibitor of β -catenin and T cell factor (ICAT), a negative regulator of Wnt signaling. *Proc Natl Acad Sci* **101**: 8017–8021.
- Schambony A, Wedlich D. 2007. Wnt-5A/Ror2 regulate expression of XPAPC through an alternative noncanonical signaling pathway. *Dev Cell* **12**: 779–792.
- Scharf SR, Gerhart JC. 1980. Determination of the dorsal-ventral axis in eggs of *Xenopus laevis*: Complete rescue of uv-impaired eggs by oblique orientation before first cleavage. *Dev Biol* **79**: 181–198.
- Schedl A, Hastie ND. 2000. Cross-talk in kidney development. *Curr Opin Genet Dev* **10**: 543–549.
- Schier AF, Talbot WS. 2005. Molecular genetics of axis formation in zebrafish. *Annu Rev Genet* **39**: 561–613.
- Schmidt JE, von Dassow G, Kimelman D. 1996. Regulation of dorsal-ventral patterning: The ventralizing effects of the novel *Xenopus* homeobox gene Vox. *Development* **122**: 1711–1721.
- Schneider S, Steinbeisser H, Warga RM, Hausen P. 1996. β -Catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech Dev* **57**: 191–198.
- Schohl A, Fagotto E. 2002. β -Catenin, MAPK and Smad signaling during early *Xenopus* development. *Development* **129**: 37–52.
- Schroeder KE, Condic ML, Eisenberg LM, Yost HJ. 1999. Spatially regulated translation in embryos: Asymmetric expression of maternal Wnt-11 along the dorsal-ventral axis in *Xenopus*. *Dev Biol* **214**: 288–297.
- Schwarz-Romond T, Asbrand C, Bakkers J, Kuhl M, Schaeffer HJ, Huelsken J, Behrens J, Hammerschmidt M, Birchmeier W. 2002. The ankyrin repeat protein Diversin recruits Casein kinase I ϵ to the β -catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling. *Genes Dev* **16**: 2073–2084.
- Shibata M, Ono H, Hikasa H, Shinga J, Taira M. 2000. *Xenopus* crescent encoding a Frizzled-like domain is expressed in the Spemann organizer and pronephros. *Mech Dev* **96**: 243–246.
- Shibata M, Itoh M, Ohmori SY, Shinga J, Taira M. 2001. Systematic screening and expression analysis of the head organizer genes in *Xenopus* embryos. *Dev Biol* **239**: 241–256.
- Shibata M, Itoh M, Hikasa H, Taira S, Taira M. 2005. Role of crescent in convergent extension movements by modulating Wnt signaling in early *Xenopus* embryogenesis. *Mech Dev* **122**: 1322–1339.

- Shibata T, Takahashi Y, Tasaki J, Saito Y, Izutsu Y, Maeno M. 2008. A role of D domain-related proteins in differentiation and migration of embryonic cells in *Xenopus laevis*. *Mech Dev* **125**: 284–298.
- Shimizu T, Bae YK, Muraoka O, Hibi M. 2005. Interaction of Wnt and caudal-related genes in zebrafish posterior body formation. *Dev Biol* **279**: 125–141.
- Shimizu T, Bae YK, Hibi M. 2006. Cdx-Hox code controls competence for responding to Fgfs and retinoic acid in zebrafish neural tissue. *Development* **133**: 4709–4719.
- Smith WC, Harland RM. 1991. Injected Xwnt-8 RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing center. *Cell* **67**: 753–765.
- Smith WC, Harland RM. 1992. Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell* **70**: 829–840.
- Smith WC, McKendry R, Ribisi S Jr, Harland RM. 1995. A nodal-related gene defines a physical and functional domain within the Spemann organizer. *Cell* **82**: 37–46.
- Sokol SY. 1993. Mesoderm formation in *Xenopus* ectodermal explants overexpressing Xwnt8: Evidence for a cooperating signal reaching the animal pole by gastrulation. *Development* **118**: 1335–1342.
- Sokol SY. 1996. Analysis of *Dishevelled* signalling pathways during *Xenopus* development. *Curr Biol* **6**: 1456–1467.
- Sokol SY. 1999. Wnt signaling and dorso-ventral axis specification in vertebrates. *Curr Opin Genet Dev* **9**: 405–410.
- Sokol SY. 2000. A role for Wnts in morpho-genesis and tissue polarity. *Nat Cell Biol* **2**: E124–E125.
- Sokol SY. 2011. Wnt signaling through T-cell factor phosphorylation. *Cell Res* **21**: 1002–1012.
- Sokol SY, Melton DA. 1992. Interaction of Wnt and activin in dorsal mesoderm induction in *Xenopus*. *Dev Biol* **154**: 348–355.
- Sokol S, Christian JL, Moon RT, Melton DA. 1991. Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. *Cell* **67**: 741–752.
- Sokol SY, Klingensmith J, Perrimon N, Itoh K. 1995. Dorsalizing and neuralizing properties of Xdsh, a maternally expressed *Xenopus* homolog of dishevelled. *Development* **121**: 3487.
- Spiegelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai Z, Fuchs SY. 2000. Wnt/ β -catenin signaling induces the expression and activity of β TrCP ubiquitin ligase receptor. *Mol Cell* **5**: 877–882.
- Standley HJ, Destree O, Kofron M, Wylie C, Heasman J. 2006. Maternal XTcf1 and XTcf4 have distinct roles in regulating Wnt target genes. *Dev Biol* **289**: 318–328.
- Sumanas S, Stregge P, Heasman J, Ekker SC. 2000. The putative wnt receptor *Xenopus* frizzled-7 functions upstream of β -catenin in vertebrate dorsoventral mesoderm patterning. *Development* **127**: 1981–1990.
- Suri C, Haremak T, Weinstein DC. 2005. Xema, a foxi-class gene expressed in the gastrula stage *Xenopus* ectoderm, is required for the suppression of mesendoderm. *Development* **132**: 2733–2742.
- Suzuki A, Ueno N, Hemmati-Brivanlou A. 1997. *Xenopus* msx1 mediates epidermal induction and neural inhibition by BMP4. *Development* **124**: 3037–3044.
- Tada M, Kai M. 2009. Noncanonical Wnt/PCP signaling during vertebrate gastrulation. *Zebrafish* **6**: 29–40.
- Taelman VF, Dobrowolski R, Plouhinec JL, Fuentealba LC, Vorwald PP, Gumper I, Sabatini DD, De Robertis EM. 2010. Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. *Cell* **143**: 1136–1148.
- Taira M, Otani H, Saint-Jeannet JP, Dawid IB. 1994. Role of the LIM class homeodomain protein Xlim-1 in neural and muscle induction by the Spemann organizer in *Xenopus*. *Nature* **372**: 677–679.
- Takada S, Stark KL, Shea MJ, Vassileva G, McMahon JA, McMahon AP. 1994. Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev* **8**: 174–189.
- Takahashi S, Yokota C, Takano K, Tanegashima K, Onuma Y, Goto J, Asashima M. 2000. Two novel nodal-related genes initiate early inductive events in *Xenopus* Nieuwkoop center. *Development* **127**: 5319–5329.
- Takao Y, Yokota T, Koide H. 2007. β -Catenin up-regulates Nanog expression through interaction with Oct-3/4 in embryonic stem cells. *Biochem Biophys Res Commun* **353**: 699–705.
- Tao Q, Yokota C, Puck H, Kofron M, Birsoy B, Yan D, Asashima M, Wylie CC, Lin X, Heasman J. 2005. Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* **120**: 857–871.
- Thorpe CJ, Moon RT. 2004. Nemo-like kinase is an essential co-activator of Wnt signaling during early zebrafish development. *Development* **131**: 2899–2909.
- Torres MA, Yang-Snyder JA, Purcell SM, DeMarais AA, McGrew LL, Moon RT. 1996. Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early *Xenopus* development. *J Cell Biol* **133**: 1123–1137.
- Tucker A, Sharpe P. 2004. The cutting-edge of mammalian development; how the embryo makes teeth. *Nat Rev Genet* **5**: 499–508.
- Ulrich F, Concha ML, Heid PJ, Voss E, Witzel S, Roehl H, Tada M, Wilson SW, Adams RJ, Soll DR, et al. 2003. Slb/Wnt11 controls hypoblast cell migration and morphogenesis at the onset of zebrafish gastrulation. *Development* **130**: 5375–5384.
- van Amerongen R, Nusse R. 2009. Towards an integrated view of Wnt signaling in development. *Development* **136**: 3205–3214.
- van Amerongen R, Nawijn M, Franca-Koh J, Zevenhoven J, van der Gulden H, Jonkers J, Berns A. 2005. Frat is dispensable for canonical Wnt signaling in mammals. *Genes Dev* **19**: 425–430.
- van den Akker E, Forlani S, Chawengsaksophak K, de Graaff W, Beck F, Meyer BI, Deschamps J. 2002. Cdx1 and Cdx2 have overlapping functions in anteroposterior patterning and posterior axis elongation. *Development* **129**: 2181–2193.
- van de Wetering M, Cavallo R, Dooijes D, van Beest M, van Es J, Loureiro J, Ypma A, Hursh D, Jones T, Bejsovec A, et al. 1997. Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCE. *Cell* **88**: 789–799.

H. Hikasa and S.Y. Sokol

- Veien ES, Grierson MJ, Saund RS, Dorsky RI. 2005. Expression pattern of zebrafish *tcf7* suggests unexplored domains of Wnt/ β -catenin activity. *Dev Dyn* **233**: 233–239.
- Villanueva S, Glavic A, Ruiz P, Mayor R. 2002. Posteriorization by FGF, Wnt, and retinoic acid is required for neural crest induction. *Dev Biol* **241**: 289–301.
- von Bubnoff A, Schmidt JE, Kimelman D. 1996. The *Xenopus laevis* homeobox gene *Xgbx-2* is an early marker of anteroposterior patterning in the ectoderm. *Mech Dev* **54**: 149–160.
- von Dassow G, Schmidt JE, Kimelman D. 1993. Induction of the *Xenopus* organizer: Expression and regulation of *Xnot*, a novel FGF and activin-regulated homeo box gene. *Genes Dev* **7**: 355–366.
- Wang S, Krinks M, Lin K, Luyten FP, Moos M Jr. 1997. Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* **88**: 757–766.
- Wei G, Ku S, Ma GK, Saito S, Tang AA, Zhang J, Mao JH, Appella E, Balmain A, Huang EJ. 2007. HIPK2 represses β -catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis. *Proc Natl Acad Sci* **104**: 13040–13045.
- Weidinger G, Thorpe CJ, Wuennenberg-Stapleton K, Ngai J, Moon RT. 2005. The Sp1-related transcription factors sp5 and sp5-like act downstream of Wnt/ β -catenin signaling in mesoderm and neuroectoderm patterning. *Curr Biol* **15**: 489–500.
- Wheeler GN, Hoppler S. 1999. Two novel *Xenopus* frizzled genes expressed in developing heart and brain. *Mech Dev* **86**: 203–207.
- Willert J, Epping M, Pollack JR, Brown PO, Nusse R. 2002. A transcriptional response to Wnt protein in human embryonic carcinoma cells. *BMC Dev Biol* **2**: 8.
- Wu J, Mlodzik M. 2009. A quest for the mechanism regulating global planar cell polarity of tissues. *Trends Cell Biol* **19**: 295–305.
- Xu C, Kim NG, Gumbiner BM. 2009. Regulation of protein stability by GSK3 mediated phosphorylation. *Cell Cycle* **8**: 4032–4039.
- Xu PF, Zhu KY, Jin Y, Chen Y, Sun XJ, Deng M, Chen SJ, Chen Z, Liu TX. 2010. Setdb2 restricts dorsal organizer territory and regulates left-right asymmetry through suppressing *fgf8* activity. *Proc Natl Acad Sci* **107**: 2521–2526.
- Yamaguchi Y, Shinagawa A. 1989. Marked alteration at mid-blastula transition in the effect of lithium on formation of the larval body pattern of *Xenopus laevis*. *Dev Growth Differ* **31**: 531–541.
- Yamaguchi TP, Bradley A, McMahon AP, Jones S. 1999. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* **126**: 1211–1223.
- Yamamoto A, Nagano T, Takehara S, Hibi M, Aizawa S. 2005. Shisa promotes head formation through the inhibition of receptor protein maturation for the caudalizing factors, Wnt and FGF. *Cell* **120**: 223–235.
- Yamanaka Y, Mizuno T, Sasai Y, Kishi M, Takeda H, Kim CH, Hibi M, Hirano T. 1998. A novel homeobox gene, *dharma*, can induce the organizer in a non-cell-autonomous manner. *Genes Dev* **12**: 2345–2353.
- Yanagawa S, van Leeuwen F, Wodarz A, Klingensmith J, Nusse R. 1995. The dishevelled protein is modified by wingless signaling in *Drosophila*. *Genes Dev* **9**: 1087–1097.
- Yang J, Tan C, Darken RS, Wilson PA, Klein PS. 2002. β -Catenin/Tcf-regulated transcription prior to the mid-blastula transition. *Development* **129**: 5743–5752.
- Yokoyama H. 2008. Initiation of limb regeneration: The critical steps for regenerative capacity. *Dev Growth Differ* **50**: 13–22.
- Yost C, Farr GH III, Pierce SB, Ferkey DM, Chen MM, Kimelman D. 1998. GBP, an inhibitor of GSK-3, is implicated in *Xenopus* development and oncogenesis. *Cell* **93**: 1031–1041.
- Young T, Deschamps J. 2009. Hox, Cdx, and anteroposterior patterning in the mouse embryo. *Curr Top Dev Biol* **88**: 235–255.
- Young T, Rowland JE, van de Ven C, Bialecka M, Novoa A, Carapuco M, van Nes J, de Graaff W, Duluc I, Freund JN, et al. 2009. Cdx and Hox genes differentially regulate posterior axial growth in mammalian embryos. *Dev Cell* **17**: 516–526.
- Zeng L, Fagotto F, Zhang T, Hsu W, Vasicek TJ, Perry WL III, Lee JJ, Tilghman SM, Gumbiner BM, 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.
- Zhang C, Basta T, Jensen ED, Klymkowsky MW. 2003. The β -catenin/VegT-regulated early zygotic gene *Xnr5* is a direct target of SOX3 regulation. *Development* **130**: 5609–5624.
- Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC. 2004. Dual regulation of Snail by GSK-3 β -mediated phosphorylation in control of epithelial-mesenchymal transition. *Nat Cell Biol* **6**: 931–940.
- Znosko WA, Yu S, Thomas K, Molina GA, Li C, Tsang W, Dawid IB, Moon AM, Tsang M. 2010. Overlapping functions of Pea3 ETS transcription factors in FGF signaling during zebrafish development. *Dev Biol* **342**: 11–25.

Cold Spring Harbor Perspectives in Biology

Wnt Signaling in Vertebrate Axis Specification

Hiroki Hikasa and Sergei Y. Sokol

Cold Spring Harb Perspect Biol 2013; doi: 10.1101/cshperspect.a007955 originally published online August 22, 2012

Subject Collection [Wnt Signaling](#)

Wnt Signaling in Vertebrate Axis Specification

Hiroki Hikasa and Sergei Y. Sokol

Secreted and Transmembrane Wnt Inhibitors and Activators

Cristina-Maria Cruciat and Christof Niehrs

Wnt Signaling in Normal and Malignant Hematopoiesis

William Lento, Kendra Congdon, Carlijn Voermans, et al.

Frizzled and LRP5/6 Receptors for Wnt/ β -Catenin Signaling

Bryan T. MacDonald and Xi He

TCF/LEFs and Wnt Signaling in the Nucleus

Ken M. Cadigan and Marian L. Waterman

Alternative Wnt Pathways and Receptors

Renée van Amerongen

β -Catenin-Dependent Wnt Signaling in *C. elegans*: Teaching an Old Dog a New Trick

Belinda M. Jackson and David M. Eisenmann

The Evolution of the Wnt Pathway

Thomas W. Holstein

The β -Catenin Destruction Complex

Jennifer L. Stamos and William I. Weis

Wnt Signaling in Skin Development, Homeostasis, and Disease

Xinhong Lim and Roel Nusse

Wnt Signaling in Bone Development and Disease: Making Stronger Bone with Wnts

Jean B. Regard, Zhendong Zhong, Bart O. Williams, et al.

Targeting Wnt Pathways in Disease

Zachary F. Zimmerman, Randall T. Moon and Andy J. Chien

Wnt Signaling in Mammary Glands: Plastic Cell Fates and Combinatorial Signaling

Caroline M. Alexander, Shruti Goel, Saja A. Fakhruldeen, et al.

Wnt Signaling and Injury Repair

Jemima L. Whyte, Andrew A. Smith and Jill A. Helms

Wnt Signaling and Forebrain Development

Susan J. Harrison-Uy and Samuel J. Pleasure

Wnt Signaling in Neuromuscular Junction Development

Kate Koles and Vivian Budnik

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**

Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?

Your wish is our command.