Molecular Genetics of Axis Formation in Zebrafish

Alexander F. Schier[1] and William S. Talbot[2]

[1]Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, Department of Cell Biology, New York University School of Medicine, New York, NY 10016-6497; email: schier@saturn.med.nyu.edu; present address: Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138

[2]Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305; email: talbot@cmgm.stanford.edu

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Abstract

The basic vertebrate body plan of the zebrafish embryo is established in the first 10 hours of development. This period is characterized by the formation of the anterior-posterior and dorsal-ventral axes, the development of the three germ layers, the specification of organ progenitors, and the complex morphogenetic movements of cells. During the past 10 years a combination of genetic, embryological, and molecular analyses has provided detailed insights into the mechanisms underlying this process. Maternal determinants control the expression of transcription factors and the location of signaling centers that pattern the blastula and gastrula. Bmp, Nodal, FGF, canonical Wnt, and retinoic acid signals generate positional information that leads to the restricted expression of transcription factors that control cell type specification. Noncanonical Wnt signaling is required for the morphogenetic movements during gastrulation. We review how the coordinated interplay of these molecules determines the fate and movement of embryonic cells.

Contents

INTRODUCTION

Over the past 25 years, the zebrafish has become a powerful model system for investigation of vertebrate development, physiology, and disease mechanisms. Recognizing important attributes such as high fecundity, a three-month generation time, and accessibility of the embryo, Streisinger introduced the zebrafish as a model system, developed methods for constructing haploid and gynogenetic diploid fish, and identified the first few zebrafish mutants (308). Exploiting the optical transparency of the embryo, Kimmel established essential embryological tools, including time-lapse imaging, lineage-tracing, and cellular transplantation, which are now widely used in analyses of wild-type and mutant embryos (reviewed in 154). In the mid-1990s, the Nüsslein-Volhard and Driever groups conducted two large-scale genetic screens that identified genes with essential functions in a wide array of biological processes, ranging

from early embryonic patterning to organogenesis (68, 104). The 1990s also witnessed the advent of key resources for the molecular analysis of zebrafish mutations, including genetic maps, radiation hybrid maps, and large-insert genomic libraries (91, 130, 164, 244). These areas have all progressed rapidly, and the zebrafish field continues to be invigorated by the identification of new mutants in screens targeted for specific phenotypes and by the development of new tools and resources (e.g., 26, 194, 349). Examples of other important advances include retroviral insertional mutagenesis, in vivo analysis of gene expression with GFP (green fluorescent protein) transgenes, the use of morpholino oligonucleotides and target-selected mutagenesis approaches for reverse genetic studies, and a concerted effort to obtain the genome sequence (88, 190, 223, 340). Because of these experimental advantages, the zebrafish system has yielded important insights into many areas of vertebrate biology; especially noteworthy among these is the genetic control of embryonic axis formation, the subject of this review.

OVERVIEW OF ZEBRAFISH DEVELOPMENT

Only 10 h post fertilization (hpf), the zebrafish embryo has clearly recognizable anterior-posterior and dorsal-ventral axes (**Figure 1**). Moreover, the embryo is exquisitely patterned so that the precursors for different regions and cell types of the embryo can be recognized using molecular markers. To generate this basic body plan, the embryo undergoes rapid developmental and morphogenetic changes (reviewed in 155). Upon fertilization, cytoplasmic streaming generates a large blastodisc on top of the yolk. During the following 3 h of development, rapid, synchronous cleavage divisions occur within the blastodisc to generate a blastula embryo consisting of ~1000 cells, initially arranged in a pile (blastoderm) atop the yolk. During cleavage, the volume of the embryo remains essentially constant, so that the divisions produce a larger number of smaller cells. The cells in the blastoderm form the embryo proper, whereas the yolk is an extraembryonic structure. Cell cycles lengthen and become asynchronous during the mid-blastula transition (MBT). The MBT begins at the 512-cell stage (2.75 hpf), when cell division has increased the DNA:cytoplasm ratio to a critical threshold (58, 136). The MBT also marks the time when zygotic transcription begins (although a few genes may be transcribed prior to the MBT), so that the zygotic genome begins to govern embryonic development. Also around the time of the MBT, cells at the blastoderm margin collapse into the yolk and form the yolk syncytial layer, a thin, multinucleate structure at the interface of the blastoderm and the yolk (157).

At about 4 hpf, cellular rearrangements begin to reshape the blastoderm into a characteristic vertebrate body plan (reviewed in 298) (**Figure 2**). In the process of epiboly, cells intercalate radially, thereby thinning the blastoderm and spreading over the yolk. By the end of gastrulation, epiboly movements have spread the blastomeres so that the blastoderm covers the entire yolk cell; the extent of yolk cell coverage (measured as "percent epiboly") provides a convenient way to determine an embryo's developmental stage. Three other movements contribute to the formation of the axis. Beginning at 5 hpf, cells at the margin internalize and form the so-called hypoblast, the precursors of the mesoderm and endoderm (this usage of the term hypoblast is different from that in mouse and chick, where it denotes extraembryonic tissue). By 6 hpf, convergence and extension movements have begun, resulting in the dorsal accumulation of cells moving from lateral and ventral regions of the blastoderm (convergence). Concomitantly, converging cells intercalate with dorsal blastomeres, spreading them along the animal-vegetal axis, leading to a lengthening of the anterior-posterior axis (extension). Convergence of cells toward the dorsal side of the embryo marks the first clearly apparent break in radial symmetry and forms the

Anterior-posterior axis: the line from head to tail

Endoderm: the inner germ layer, which gives rise to the gastrointestinal tract and associated structures

Gastrulation: the process by which blastoderm cells are specified and move to generate an embryo with three germ layers and anterior-posterior and dorsal-ventral polarity

Mesoderm: the middle germ layer, which gives rise to bone, muscle, connective tissue, urogenital and circulatory system

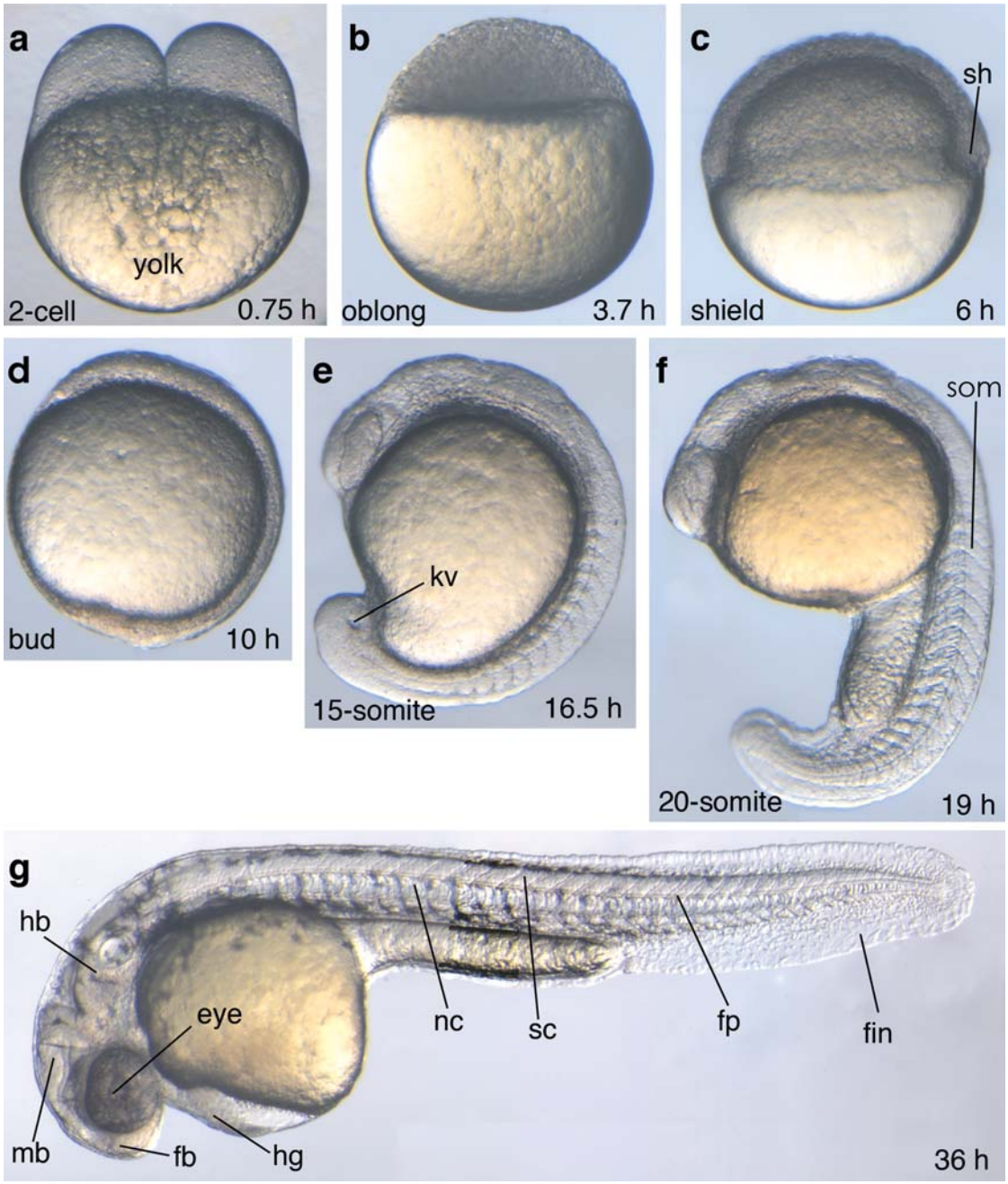

Figure 1

Zebrafish embryogenesis. Living zebrafish embryos are shown at the indicated developmental stages. Approximate developmental ages in hours postfertilization (h) are shown. Embryos are oriented: (*a*,*b*) animal pole to top; (*c*) animal pole to top, dorsal to the right; (*d–f*) anterior to the top, dorsal to the right; (*g*) anterior to the left, dorsal to the top. Abbreviations: sh, embryonic shield; kv, Kupffer's vesicle; som, somite; hg, hatching gland; fb, forebrain; mb, midbrain; hb, hindbrain; nc, notochord; sc, spinal cord; fp, floor plate. For further details see Reference 155.

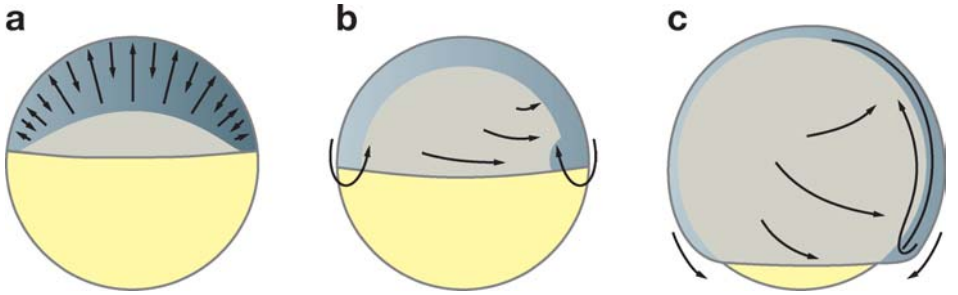

Figure 2

Gastrulation movements. (*a*) Dome stage. Cells intercalate radially, contributing to epiboly. (*b*) Shield stage. Cells at the margin internalize and migrate toward the animal pole. Cells converge dorsally, with lateral mesodermal cells starting convergence at later stages than cells closer to the shield (282). (*c*) 90% epiboly stage. Epiboly, internalization, convergence and extension continue. Modified from Reference 138.

shield, a thickening at the dorsal blastoderm margin that is the teleost equivalent of the amphibian Spemann-Mangold organizer (266, 286).

FATE MAPS AND ORGANIZING CENTERS

A fate map demarcating the position of precursors for different tissues and organs is apparent at the onset of gastrulation (6 hpf), although different progenitor territories are not sharply demarcated and progenitors are intermingled (161) (**Figure 3**). Because embryological manipulations and mutations in the genes described below alter this fate map, it is important to take a closer look at the arrangement of tissue progenitors. The precursors of the different germ layers are arranged along the animal-vegetal axis, with ectoderm located animally, mesoderm more marginally, and endoderm, intermingled with mesoderm, at the margin itself. Precursors for different mesodermal cell types are arranged along the so-called dorsal-ventral (DV) axis, with dorsal corresponding to the site of the shield. Cells located most dorsally give rise to the axial mesoderm of notochord and prechordal plate. More laterally located cells give rise to trunk somites and heart. Blood and pronephros are derived from marginal blastomeres more distant from the shield, the so-called ventral region. Most of the posterior mesoderm (tail somites) also derives from this ventral territory. Different endodermal progenitors are also located in different dorsal-ventral positions, with pharynx located most dorsally, and stomach, intestine, and liver located more laterally and ventrally (i.e., more distant from the shield) (334). Nonneural ectoderm (epidermis) derives from the animal-ventral territory. Forebrain and midbrain progenitors are found animally and dorsally, whereas hindbrain and spinal cord precursors are located closer to the margin and more laterally and ventrally, respectively (345). Hence, precursors for different anterior-posterior regions in the nervous system do not simply align with the animal-vegetal axis. Similarly, precursors of anterior somites are located more dorsally than posterior somite progenitors. Moreover, prechordal plate precursors are located more vegetally than notochord precursors (101). Because of complex gastrulation movements, there is no completely generalizable connection between dorsal-ventral or animal-vegetal location at early gastrula stages and later anterior-posterior position. This is most clearly exemplified by prechordal plate and forebrain forming the most anterior region of the head but lying at opposite positions of the animal-vegetal axis at the onset of gastrulation. Similarly, posterior notochord and posterior somites together form the tail

Dorsal-ventral axis: the line from back to belly

Ectoderm: the outer germ layer, which gives rise to epidermis, nervous system and sense organs

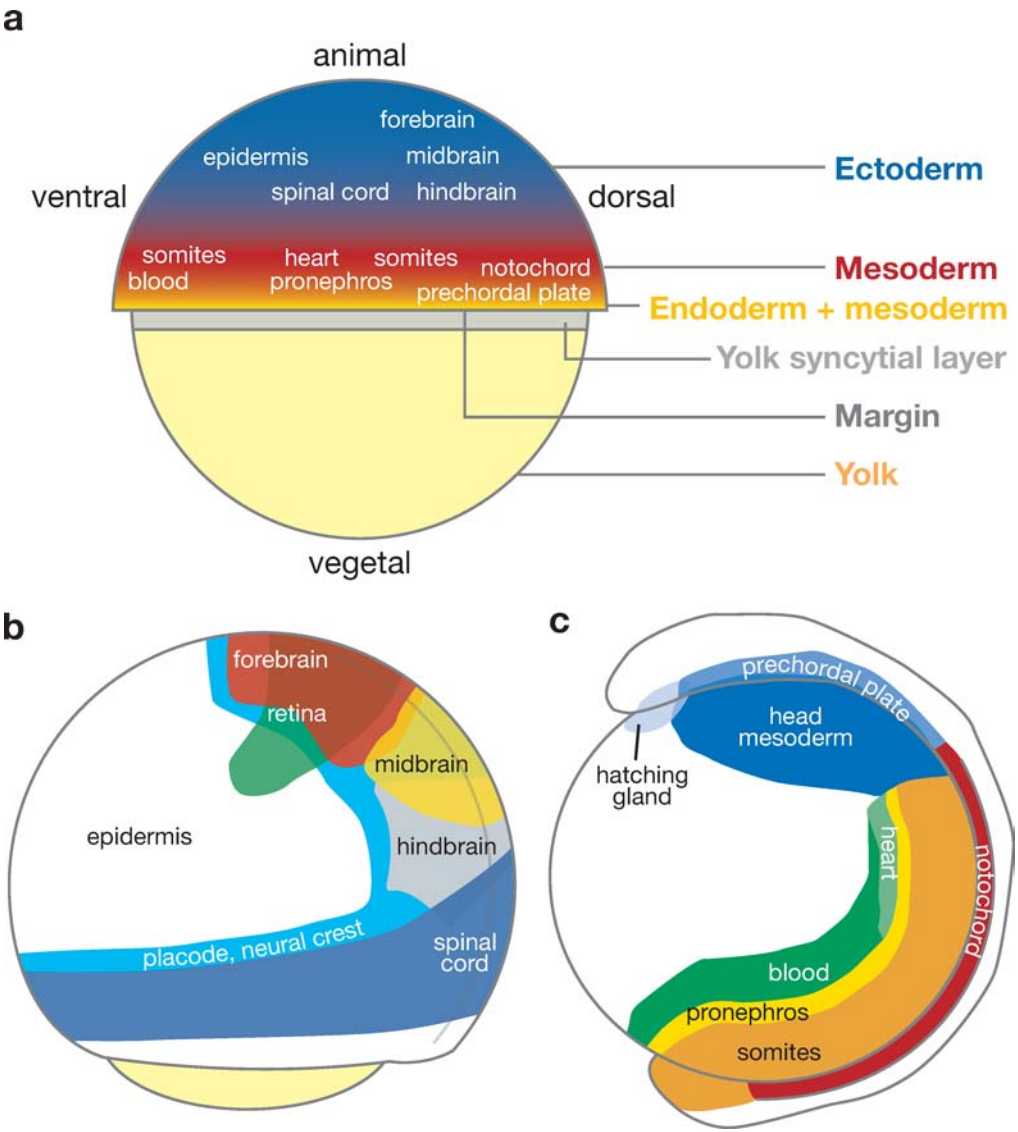

Figure 3

Zebrafish fate maps. (*a*) Fate map at 50% epiboly stage, the onset of gastrulation. Lateral view, dorsal to the right, animal pole to the top. Germ layers are arranged along the animal-vegetal axis. Different mesodermal and ectodermal fates are arranged along the dorsal-ventral axis. For details see References 66, 101, 145, 161, 345. For distribution of endodermal fates see Reference 334. No precise boundaries are depicted because cell fates are often intermingled. Modified from Reference 267. (*b*) Fate map of ectoderm at 90% epiboly. Lateral view, dorsal to the right, animal pole and anterior to the top. Modified from Reference 345; position of spinal cord territory is inferred from Reference 172. (*c*) Model fate map of mesoderm at early somite stage. Lateral view, dorsal to the right, animal pole and anterior to the top. Note that no precise fate map has been established at this stage. Therefore, regions shown here are approximations derived in part from the expression patterns of marker genes (ZFIN.org). The posterior region of the tail bud will continue to extend and give rise to different mesodermal and ectodermal fates. Modified from Reference 138.

mesoderm, but are derived from opposite ends of the DV axis.

Dye labeling experiments at early cleavage stages indicate that the planes of the first cell divisions do not predict the future dorsal-ventral axis (1, 120, 160). In addition, these experiments revealed that there is extensive cell mixing during epiboly such that a cell's position during early cleavage stages does not determine the fates of its descendants, although cells at more vegetal positions tend to contribute more marginal progenitors at the onset of gastrulation. The first lineage restrictions to emerge separate embryonic blastomeres from the extraembryonic blastomeres of the yolk syncytial layer and the enveloping layer, which forms a flattened epithelium that covers the blastoderm. Single embryonic blastomeres at the 1000- to 2000-cell stage can still give rise to several tissue types, and most individual blastomeres are not restricted to particular fates until the early gastrula stage (158). Progenitors of different germ layers begin to occupy definable and distinct positions after the 1000-cell stage, when, for example, ectodermal and mesendodermal progenitors are largely separated, with the exception that some muscle progenitors are intermingled with hindbrain and spinal cord progenitors (161). Although individual blastomeres adopt particular fates that are predictable based on their positions at the early gastrula stage, transplantation experiments show that most individual cells are not committed to particular fates until the mid- to late-gastrula stages (126).

As described in detail below, embryological manipulations have identified regions in the embryo that are required or sufficient to induce specific fates in neighboring cells (reviewed in 267) (**Figure 4**). The dorsal margin is the source of factors that can induce dorsal, anterior and lateral cell types and repress ventral and posterior fates (266, 286). The yolk syncytial layer is the source of mesoderm and endoderm inducers (44, 213), and the ventral margin can induce posterior structures (4, 346).

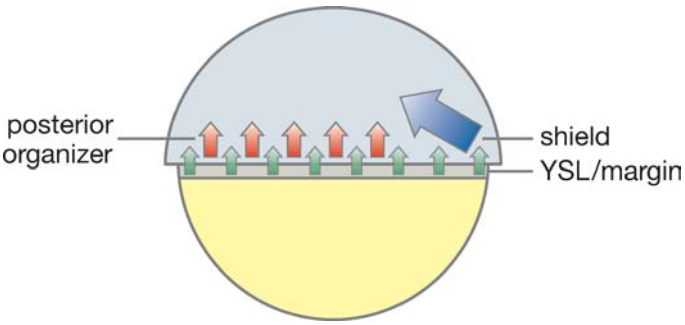

Figure 4

Zebrafish organizing centers. Lateral view, dorsal to the right, animal pole to the top. Yolk syncytial layer (YSL) can induce mesendodermal fates upon transplantation (*green arrows*). Posterior organizer is located at the ventral and lateral margin and can induce tail, posterior trunk, and hindbrain tissue upon transplantation (*red arrows*). Shield corresponds to Spemann-Mangold organizer and can induce dorsal and anterior structures upon transplantation (*blue arrow*).

DORSAL-VENTRAL PATTERNING: MATERNAL FACTORS

The mature zebrafish oocyte is radially symmetric about the animal-vegetal axis, and no dorsal-ventral asymmetry is evident prior to fertilization. During fertilization, the sperm enters the egg through a specialized structure, the micropyle, at the animal pole (344). Thus it seems that the sperm entry point itself cannot be the cue that breaks symmetry in zebrafish, in contrast to the situation in amphibians (reviewed in 336), but the possibility remains that an activity of the sperm after fertilization is somehow involved in establishing the dorsal-ventral axis. Although the first five cleavage divisions occur in a stereotyped alternating orthogonal pattern, these cleavage planes do not correlate with the eventual dorsal-ventral axis (1, 120, 160). Nevertheless, embryological experiments show that events important for the formation of dorsal-ventral asymmetry are occurring even before the first cleavage division. Embryos are ventralized by removal of the vegetal region of the yolk before the first cell division, and the frequency of ventralized embryos rapidly diminishes when the operation is performed at later stages (212, 232). Similarly, treatment

with nocodazole, an inhibitor of microtubule polymerization, causes the loss of dorsal axial structures when applied within 10 min after fertilization, but not after the first cell division (133). Drawing on parallels between these results and previous work on dorsal-ventral axis formation in *Xenopus*, it has been proposed that the dorsal side of the zebrafish embryo is established by a dorsal determinant initially located at the vegetal pole that is translocated along microtubules to the future dorsal side before the first cleavage division occurs (133). This is an intriguing model, but certain key predictions remain untested. For example, directed movement from the vegetal pole toward the dorsal side of the early embryo has not been observed. Likewise, it has not been shown that the vegetal pole contains a determinant sufficient to determine dorsal identity or rescue a ventralized embryo in a transplantation experiment. Thus many questions remain about the mechanisms that establish the earliest dorsal-ventral asymmetries in the zebrafish. The analysis of recently identified maternal-effect mutants with ventralized phenotypes will define important players that act at early stages to establish the dorsal-ventral axis (147, 228, 330).

β-catenin

Evidence suggests that maternal β-catenin acts to establish the dorsal-ventral axis in zebrafish. β-catenin protein acts as a transcriptional effector in the canonical Wnt signaling pathway and also has a function in cell adhesion (reviewed in 129, 188). A complex containing APC, axin, and GSK3β and other components targets β-catenin protein for degradation, thereby allowing only a low level of β-catenin to accumulate. Activation of the canonical Wnt signaling pathway inhibits the β-catenin degradation complex, stabilizing β-catenin and allowing it to enter the nucleus, where it activates transcription of canonical Wnt target genes.

In the zebrafish embryo, β-catenin accumulates specifically in nuclei of dorsal margin blastomeres as early as the 128-cell stage (66, 274). This asymmetric nuclear localization of β-catenin is an early marker of the dorsal-ventral axis (**Figure 5**). As in the amphibian embryo, overexpression of β-catenin leads to axis duplication (148). Moreover, β-catenin seems to be required for dorsal axis formation, as overexpression of proteins that inhibit β-catenin's action as a transcriptional activator (cadherin or a dominant negative form of Tcf3 that binds β-catenin but not DNA) reduces dorsal gene expression and produces ventralized embryos (238). In addition, the maternal effect mutations *ichabod* and *tokkaebi*, whose molecular bases are not known, disrupt the nuclear localization of β-catenin and lead to ventralized embryos (147, 228).

Soon after the mid-blastula transition, β-catenin activates the expression of a number of zygotic genes, including *bozozok* (*boz*, also known as *dharma* and *nieuwkoid*), *chordin*, *dickkopf1* (*dkk1*), *squint* (*sqt*) and FGF signals (63, 66, 75, 79, 87, 113, 147, 165, 247, 261, 263, 292, 324, 353). As detailed below, these β-catenin targets act to inhibit the action of ventralizing factors or, in the case of Sqt, induce mesendodermal fates at the dorsal margin.

Recent work suggests that asymmetric localization of Wnt11 triggers the accumulation of β-catenin in dorsal blastomeres in *Xenopus* (314). Zebrafish *wnt11* mutants (*silberblick*) have defects in morphogenetic movements during gastrulation (see below), but formation of the dorsal-ventral axis is normal, even in embryos lacking maternal and zygotic *wnt11* (119). Moreover, *Xenopus* but not zebrafish *wnt11* mRNA is localized to the vegetal pole. There is another *wnt11* gene in the zebrafish genome (90), and further work is needed to determine if this gene functions in the establishment of the dorsal-ventral axis or if the *wnt11* duplicates might have redundant functions in this process.

Although the asymmetric distribution of β-catenin has not been observed during the first few cleavages, one study suggests that dorsal-ventral asymmetry is evident even in the two-cell embryo (83). Activation of the

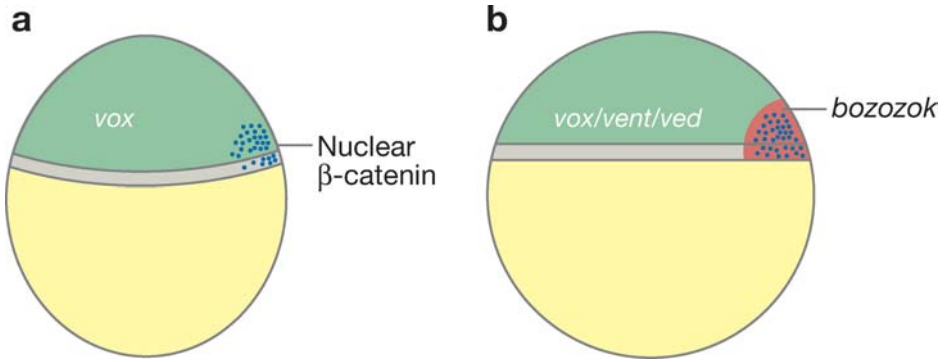

Figure 5

Transcriptional interactions patterning the dorsal-ventral axis. Lateral view, dorsal to the right, animal pole to the top. (*a*) β-catenin is stabilized on the dorsal side during cleavage stages. Soon after mid-blastula transition, *vox* is expressed ubiquitously. (*b*) β-catenin activates *bozozok* (*boz*), which represses *vox*, *vent*,and *ved* expression in dorsal blastomeres.

map kinase p38, assessed with an antibody specific for the doubly phosphorylated form of p38, occurs in the region of the embryo that will eventually become the dorsal side. Despite its early dorsal activation, p38 does not apparently act to specify dorsal fates, and expression of dorsal-specific genes occurs in embryos expressing dominant negative versions of p38. Instead, p38 is required specifically on the dorsal side to control the rate of cell division in dorsal blastomeres, so that there are fewer, larger blastomeres on the dorsal side in embryos expressing dominant negative p38. Activation of p38 does not occur in embryos ventralized by inhibition of microtubules or vegetal yolk depletion, indicating that p38 is regulated by the same factors that establish dorsal-ventral asymmetry and that p38 acts in parallel to the genes that specify dorsal identity (83).

DORSAL-VENTRAL PATTERNING: ZYGOTIC FACTORS

In recent years, the default model for dorsal-ventral patterning has gained widespread acceptance (reviewed in 121). This model, first formulated to explain dorsal-ventral patterning in frog, holds that the Spemann-Mangold organizer induces dorsal fates by inhibiting the action of ventralizing and posteriorizing signals such as Bmp2/4/7 and Wnt8. According to this view, development of dorsal and anterior fates is a "default" state, such that dorsalizing factors act to block the influence of ventralizing signals rather than to actively trigger pathways that specify dorsal fates. Analysis in zebrafish has confirmed certain key predictions of this model, identified genes with essential roles in dorsal-ventral patterning, and advanced the understanding of dorsal-ventral patterning by explaining events that are not wholly accounted for by the simplest version of the default model.

Bmp Signaling

Members of the Bmp family of TGF-β signals induce ventral fates (reviewed in 219, 327). Secreted Bmp ligands bind the extracellular domains of type I and type II Bmp receptors, which are transmembrane proteins with intracellular serine/threonine kinase domains. The closely related Smad family transcription factors Smad1/5/8 are phosphorylated by ligand-bound receptors, allowing these proteins to translocate to the nucleus and regulate target gene expression together with the

nonreceptor-regulated Smad protein Smad4, and other DNA binding cofactors, such as the zinc finger protein Oaz. A large number of inhibitory proteins function to regulate Bmp pathway activity at different levels: for example, Chordin, Noggin, and Follistatin are secreted Bmp antagonists, the transmembrane protein Bambi functions as a decoy receptor, and inhibitory Smads Smad6/7 interfere with Smad1/5/8 phosphorylation (reviewed in 219).

Mutational analysis has demonstrated that a number of Bmp pathway components are essential for formation of ventral cell types in zebrafish (**Table 1**), including the Bmp ligands, Bmp2b and Bmp7, the type I receptor Alk8, the transcriptional effector Smad5, and the protease Tolloid, which cleaves the Bmp antagonist Chordin (22, 49, 60, 124, 162, 209, 226, 272). Although these mutations define components of the same pathway, the mutant phenotypes span a range from weakly dorsalized and viable to strongly dorsalized and lethal in the first day of development (218). Soon after the MBT, *bmp2b* and *bmp7* are widely expressed, but their expression becomes restricted to approximately the ventral half of the embryo by the onset of gastrulation (60, 162, 198, 272) (**Figure 6**). *Swirl/bmp2b* mutants and wild-type embryos overexpressing Chordin or Noggin are strongly dorsalized, with dorsoanterior structures greatly expanded at the expense of ventroposterior structures (162, 218, 219b, 226). In the ectoderm, neural fates including forebrain, midbrain, and hindbrain are expanded to encompass the most ventral regions of the embryo, whereas epidermis, neural crest, and Rohon-Beard sensory neurons are lacking in *swirl/bmp2b* mutant embryos. A similar fate transformation is evident in the margin region of *swirl/bmp2b* mutants, in which anterior (trunk) somites and anterior endoderm are expanded, whereas ventrolateral and posterior fates such as blood, heart, pronephros, pancreas, and tail are reduced or missing. Axial mesoderm is largely unaffected in *swirl/bmp2b* and the other *bmp* pathway mutants, indicating that other factors act to restrict the most dorsal fates to the appropriate territories. Complete loss of *snh/bmp7* function also produces a strongly dorsalized phenotype, indicating that both *bmp2b* and *bmp7* are required for normal dorsal-ventral patterning, despite the fact that the expression of these genes largely overlaps (60, 272). It is possible that the active ventralizing signal in vivo is a Bmp2b-Bmp7 heterodimer (272). Bmp7, however, can induce ventral cell types when overexpressed in *bmp2b* mutants, showing that high levels of Bmp7 are sufficient to specify ventral identity even in the absence of its putative heterodimer partner Bmp2b. Using an inducible dominant negative Bmp receptor, it has been shown that Bmp signaling is required for global dorsal-ventral patterning decisions during early gastrulation, whereas Bmp signals regulate tail development from mid-gastrulation through early somitogenesis (246).

The fly orthologue of the ventralizing Bmps, Decapentaplegic (Dpp), acts as morphogen, and it has been proposed that graded action of Bmp signals directly specifies fates of tissue progenitors across the dorsal-ventral axis in vertebrates (64, 176a, 225, 343). In zebrafish, the evidence for this is best in the ectoderm, where graded inactivation of Bmp signals leads to striking modulations of DV patterning (21, 226). Null mutations in *bmp2b* eliminate epidermis, placodes, neural crest, and Rohon-Beard sensory neurons, whereas forebrain, midbrain, and hindbrain fates are expanded to encompass the most ventral regions of the embryo. When Bmp activity is reduced but not eliminated, as with hypomorphic mutations or overexpression of intermediate concentrations of a Bmp antagonist in wild-type embryos, neural crest and placodal fates are expanded relative to wild type. These seemingly paradoxical results can be explained if the perturbations change the slope of a Bmp gradient. According to this view, a larger region of the DV axis falls within, for example, the neural crest specification threshold when the Bmp gradient is shallower than in wild type. This can account for expansion of

Table 1 Genes essential for zebrafish axis formation and patterning

Mutation	Gene product	Function	Phenotype	Reference
Bmp signaling				
swirl	Bmp2b	Bmp signal	Severely dorsalized	(162)
snailhouse	Bmp7	Bmp signal	Severely dorsalized	(60, 272)
lost-a-fin	Alk8	Type I Bmp receptor	Severely dorsalized	(22, 209)
somitabun	Smad5	Transcription factor	Weakly (zyg.) or strongly (mat.) dorsalized	(124)
morpholino	Twisted Gastrulation	Bmp agonist	Dorsalized	(186, 350)
minifin	Tolloid	Metalloprotease for Chordin	Weakly dorsalized	(49)
chordino	Chordin	Bmp inhibitor	Ventralized	(277)
ogon	Sizzled	Bmp inhibitor	Ventralized	(199, 351)
morpholino	Radar/Gdf6a	Bmp signal	Dorsalized	(293)
dominant negative	Kheper	Zinc finger/homeodomain	Reduced neuroectoderm	(220)
morpholino	ΔNp63	Transcriptional repressor	Reduced ventral ectoderm	(16, 177)
morpholino	ADMP	Divergent Bmp signal	Dorsalized	(180, 341)
Canonical Wnt signaling				
wnt8	Wnt8	Wnt signal	No ventral and posterior structures	(72, 179)
masterblind	Axin	Scaffolding protein	No eyes and telencephalon	(117)
headless	Tcf3	Transcription factor	No forebrain and midbrain	(153)
morpholino	Tlc SFRP	Wnt antagonist	Reduced telencephalon	(127)
ichabod	?	β-catenin localization?	Variably ventralized	(147)
tokkaebi	?	β-catenin stability?	Variably ventralized	(228)
morpholino	Sp5 and Sp5-like	SP1 Zn Finger	Anteriorized and dorsalized	(337)
Nodal signaling				
cyclops	Cyc (Nodal)	Nodal signal	Cyclopia	(115, 252, 265)
squint	Sqt (Nodal)	Nodal signal	Cyclopia, dorsal mesoderm defects	(79)
morpholino	Southpaw (Nodal)	Nodal signal	Loss or randomization of LR asymmetry	(191)
cyclops;squint			No endoderm and head/trunk mesoderm	(79)
one-eyed pinhead	EGF-CFC	Nodal co-receptor	No endoderm and head/trunk mesoderm	(102)
schmalspur	FAST1/FoxH1	Transcription factor	Dorsal mesoderm defects	(243, 295)
bonnie and clyde	Mix homeodomain	Transcription factor	Reduced endoderm	(151)
morpholino	Lefty1 and Lefty2	Antagonist of Nodal signaling	Increased mesoderm and endoderm	(3)
morpholino	Dapper2	Antagonist of Nodal signaling	Increased mesoderm and endoderm	(362)
morpholino	Charon	Antagonist of Nodal signaling	Loss of LR asymmetry	(114)
FGF signaling				
acerebellar	Fgf8	FGF signal	Ventralized with loss of chordin	(87, 253)
morpholino	Fgf24	FGF signal	Loss of posterior structures with loss of fgf8	(67)

(*Continued*)

Table 1 (*Continued*)

Mutation	Gene product	Function	Phenotype	Reference
morpholino	Sef	Antagonist of FGF signaling	Dorsalized	(84, 323)
morpholino	Sprouty2	Antagonist of FGF signaling	Dorsalized	(87)
morpholino	MKP3	Antagonist of FGF signaling	Dorsalized	(324)
Retinoic acid signaling				
neckless	Raldh2	RA synthesis pathway	Anterior spinal cord reduced, myocardial progenitors increased	(24, 144)
giraffe	Cyp26a1	RA degradation	Anterior spinal cord expanded	(70, 172)
Transcription factors				
bozozok	Boz homeodomain	Transcriptional repressor	Variable loss of dorsal mesoderm and forebrain	(75)
vox/vent	Vox, Vent homeodomain	Transcriptional repressor	Severely dorsalized in double mutants	(131)
morpholino	Ved homeodomain	Transcriptional repressor	Severely dorsalized with *vox/vent*	(290)
kugelig	Cdx4 homeodomain	Transcription factor	Reduced tail and blood	(56)
morpholino	Prdm1/Blimp1	Transcriptional repressor	Dorsalized	(342)
dominant negative	Iro3	Transcriptional repressor	Reduced dorsal mesoderm	(171)
spiel ohne grenzen	Pou2/Oct4	Transcription factor	Strongly reduced endoderm in maternal-zygotic mutants	(193, 254)
faust	Gata5 Zinc finger	Transcription factor	Reduced endoderm and heart	(255)
casanaova	HMG domain	Transcription factor	Strongly reduced endoderm	(61, 150)
morpholino	Mezzo homeodomain	Transcription factor	Reduced dorsal mesoderm and endoderm with *bon*	(245)
no tail	Ntl T-box	Transcription factor	Loss of notochord and tail	(106, 278)
floating head	Flh homeodomain	Transcription factor	Loss of notochord	(312)
spadetail	Spt T-box	Transcription factor	Loss of paraxial and lateral mesoderm	(99, 156)
Epiboly				
half-baked	E-cadherin	Cell adhesion	Strongly reduced epiboly	(137)
dominant negative	Eomesodermin T-box	Transcriptional activator	Strongly reduced epiboly	(32)
morpholino	Mtx2 homeodomain	Transcription factor	Disrupted epiboly during gastrulation	(32)
Stat3 pathway				
morpholino	Stat3	Transcription factor	Reduced prechordal plate migration and CE	(354)
morpholino	Liv1	Zinc transporter	Reduced prechordal plate migration and CE	(355)
morpholino	Snail1	Zinc-finger transcription factor	Reduced prechordal plate migration	(355)
Planar cell polarity signaling				
silberblick	Wnt11	Wnt signal	Reduced CE	(119)
pipetail	Wnt5	Wnt signal	Reduced CE	(249)
knypek	Glypican4	Wnt co-receptor?	Reduced CE	(320)
trilobite	Strabismus	Transmembrane protein	Reduced CE	(132)

(*Continued*)

Table 1 (*Continued*)

Mutation	Gene product	Function	Phenotype	Reference
morpholino	Frizzled2	Wnt receptor	Reduced CE	(236, 309)
morpholino	Flamingo1a and 1b	7TM protocadherin	Reduced CE	(82)
morpholino	Prickle1	Regulates Fz/Dsh	Reduced CE	(37)
morpholino	Diversin	Ankyrin repeat protein	Reduced CE	(279)
Others				
morpholino	Gα12/13	G protein subunit	Reduced CE	(184)
morpholino	Quattro	Rho GEF	Abnormal prechordal plate migration and CE	(51)
morpholino	CAP1	Regulates actin distribution	Abnormal prechordal plate migration and CE	(51)
dominant negative	Rok2	Kinase	Reduced CE	(197)
dominant negative	Rac1	Small GTPase	Reduced CE	(17)
inhibitor	Phosphoinosite 3-kinase	Kinase	Abnormal prechordal plate migration and CE	(216)
morpholino	Hyaluronan synthase 2	Polysaccharide synthesis	Reduced CE	(17)
landlocked	Scribble1	LRR/PDZ domain protein	Reduced CE	(329)

Abbreviations: LR, left-right; CE, convergence and extension; TM, transmembrane; for more extensive references see text.

fates specified by intermediate Bmp levels in partial loss-of-function situations, and still explain how these fates are lost when Bmp levels are reduced below the relevant thresholds.

Among the genes acting downstream of Bmp signals to pattern the ectoderm are Δ*Np63* and *kheper*, both of which encode transcriptional repressors (15, 16, 177, 220). The ventrally expressed Δ*Np63* gene is required for development of the epidermis and is directly activated by Bmps. *Kheper*, a zinc finger-homeobox gene expressed in the neural plate, is repressed by Bmp signaling and dorsalizes the ectoderm when overexpressed.

An interesting exception to the neural expansion seen after inactivation of the Bmp pathway is that posterior spinal cord fates are lost rather than expanded in *swirl/bmp2b* mutants. In contrast to other neural progenitors, the tail spinal cord precursors are located on the ventral side of the embryo just above the marginal zone, and it seems that specification of these cells requires ventralizing Bmps, and perhaps other signals such as FGFs (167, 170, 172, 257).

It has also been proposed that graded action of Bmp patterns fates along the dorsal-ventral axis of the mesendoderm (52, 224, 227, 273). Bmps are clearly required for formation of ventrolateral margin fates such as blood, heart, pronephros, and tail somites, but the case for direct action of a Bmp morphogen in patterning different mesodermal fates is weaker than for ectoderm. "Allelic series" experiments have not provided evidence of expansion of intermediate territories as described for the ectoderm above. Thus other signals, including Wnt8 and FGF, are probably involved in patterning these marginal progenitors.

Despite the evidence for DV patterning by a Bmp activity gradient, the postulated gradient has not been directly visualized. Widespread overexpression of synthetic *bmp* mRNA can rescue *bmp* mutants, suggesting that ventral restriction of *bmp* expression is not the only mechanism that operates to form the postulated Bmp activity gradient (226). Instead, it seems that the action of modulators of Bmp signaling ensures the proper levels

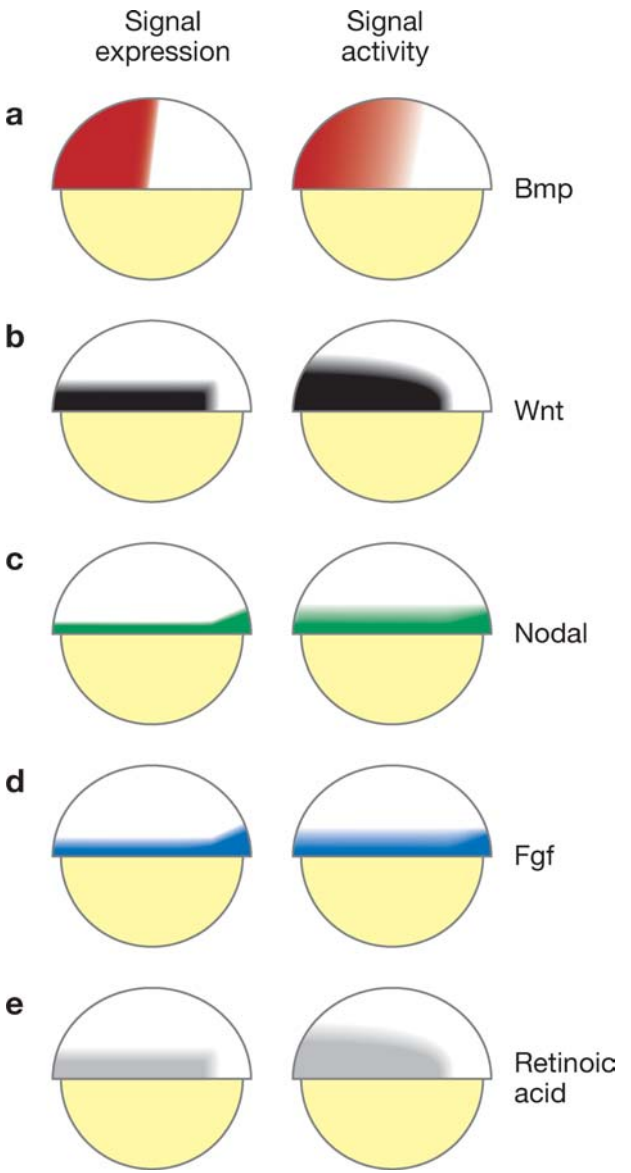

Figure 6

Signals patterning the embryo. Late-blastula stage, lateral view, dorsal to the right, animal pole to the top. Signal expression is based on published reports, but signaling activities are speculative and based on the potential range of signals and the expression pattern and range of antagonists. For example, Bmp signaling activity is inhibited dorsally by antagonists such as Chordin and Noggin. Wnt signaling activity is inhibited by antagonists such as Dickkopf1. Retinoic acid distribution indicates the site of synthesis by RALDH, and activity is inhibited by Cyp26-mediated hydrolysis of retinoic acid dorsally and at the animal pole. Nodal and FGF signals are concentrated on the dorsal side soon after the mid-blastula transition (not shown), but these signals are more uniform across the dorsal-ventral axis by the late-blastula stage that is represented in the figure.

of Bmp signaling activity across the dorsal-ventral axis.

Bmp Signaling is Modulated by Extracellular Factors

Extracellular modifiers of Bmp signals include Chordin, Ogon/Sizzled, Tolloid, and Twisted gastrulation (reviewed in 219, 352) (**Figure 6**). Mutational analysis demonstrates an essential role for Chordin in antagonizing ventralizing Bmps and thereby promoting the development of dorsal fates. *Chordin* mutants have a ventralized phenotype characterized by expansion of blood and tail fin, and a reduction of anterior neural territories (81, 109, 277). Analysis of marker gene expression indicates that DV pattern is disrupted during gastrulation, when ventral territories are expanded at the expense of presumptive neural and paraxial domains. Genetic studies support the biochemical evidence that Chordin acts to inhibit ventralizing Bmp signals: *bmp2b;chordin* double mutants are dorsalized, indicating that *chordin* is not needed for dorsal development if *bmps* are inactivated by mutation (110, 241).

Genetic studies suggest that *ogon* acts in concert with *chordin* to inhibit ventralizing *bmps* (351). Mutants for *ogon* have a ventralized phenotype very similar to *chordin* mutants (109, 208). The *ogon* gene encodes Sizzled, a member of the secreted frizzled related protein family (SFRP) (199, 351). Although SFRPs, which are related to the Wnt receptor Frizzled, were initially recognized as antagonists of Wnt signals, Ogon/Sizzled instead seems to antagonize ventralizing Bmp signals. The *ogon* mutant phenotype can be suppressed by overexpression of Chordin (or Noggin, another secreted Bmp antagonist). Overexpression of Ogon/Sizzled dorsalizes wild-type embryos but has no effect in *chordin* mutants, indicating that the dorsalizing activity of Ogon/Sizzled requires Chordin. The mechanism of Ogon/Sizzled action is not clear, but it seems that Sizzled augments the activity of Chordin, perhaps by inhibiting an inhibitor

of Chordin, by directly making Chordin more active, or by modulating Bmp signals so that they become more susceptible to Chordin inhibition.

Tolloid is a conserved extracellular metalloproteinase that promotes Bmp signaling by cleaving and inactivating Chordin (28, 240). Several homologs of Tolloid, originally identified as an activator of Dpp/Bmp signaling in *Drosophila*, are present in vertebrates (287). Modified Chordins that are resistant to cleavage by Tolloid have more potent dorsalizing activity than wild-type Chordin in overexpression assays, showing that Tolloid activity limits the function of Chordin in the embryo (350). The *tolloid* gene is disrupted in zebrafish *minifin* mutants, which lack ventral tail structures but have normal DV patterning through the end of gastrulation (49). Chordin is cleaved in *tolloid* mutants, suggesting that the lack of an early phenotype in *mfn/tolloid* mutants reflects the action of redundant proteases during gastrulation (350).

Twisted gastrulation (Tsg) is a conserved extracellular protein that binds Bmps and has been implicated as both an agonist and an antagonist of ventralizing Bmp signaling (40, 234, 262, 280). The initial morpholino study in zebrafish reported that *tsg* morphants (embryos injected with antisense morpholino oligonucleotides for *tsg*) have some characteristics of ventralized embryos, supporting a role for Tsg in the antagonism of Bmp signaling (262). In contrast, two studies show that *tsg* morphants are dorsalized and that loss of *tsg* function can partially suppress the ventralized phenotypes of *chordin* and *ogon/sizzled* mutants (186, 350). This provides strong evidence that the predominant function of Tsg in the early zebrafish embryo is to promote Bmp signaling. Overexpressed Chordin accumulates at higher levels in *tsg* morphants than in wild type, suggesting that Tsg promotes Bmp signaling, at least in part, by reducing the level of Chordin (350). Tsg's mechanism of action in not clear, but one model proposes that the action of Tsg depends on the nature of Chordin, that is, whether Chordin is full-length or fragmented by Tolloid cleavage (174). Tsg, however, must have functions independent of Chordin and its fragments, because loss of Tsg function reduces Bmp signaling activity even in the absence of Chordin. Both overexpression and inhibition of *tsg* dorsalize embryos, indicating that too much or too little Tsg activity can inhibit Bmp signals (186, 350). One proposal that accounts for these phenotypes is that Tsg links Bmp proteins to another, as-yet unidentified, cofactor, such that BMP-Tsg-X complex does not form in *tsg* morphants and that inactive BMP-Tsg and Tsg-X complexes form in the presence of excess Tsg (186).

The antidorsalizing morphogenetic protein (ADMP) is a divergent member of the Bmp family that is expressed on the dorsal side of the late blastula and in the axial mesoderm and anterior neuroectoderm during gastrulation (180, 341). Overexpression of *admp* causes ventralization and a reduction of the organizer, whereas injection of morpholino oligonucleotides against *admp* causes a moderate expansion of dorsal mesoderm. The action of *admp* is not well understood, but it may function as part of a negative feedback system to limit the size of the organizer region, perhaps in concert with *bmp2b* and *bmp7*.

Maternal Bmps Activate Expression of Zygotic Bmps

There is evidence from the analysis of *smad5* mutants that maternal Bmp signaling is required for the activation of zygotic *bmp7*. Mutations that eliminate or disrupt the C-terminal domain of Smad5 exhibit a characteristic maternal-zygotic inheritance pattern, which results from a dominant negative function of these mutant Smad5 proteins (124). Homozygotes for a *smad5* null mutation are weakly dorsalized, but *smad5*$^{-/-}$ females produce strongly dorsalized progeny (referred to as maternal *smad5*, or M*smad5*, mutants) (168). The dorsalized phenotype of M*smad5* mutants is apparent before the zygotic *bmp* mutant phenotype, suggesting

that the M*smad5* phenotype reflects more than a simple function as a transcriptional mediator of zygotic *bmp2b* and *bmp*7 (168). The identity of the putative maternal Bmp signal is not clear, but Gdf6a/Radar is one candidate (293). Maternal Radar, however, may not be the signal acting upstream of maternal Smad5, because the *radar* morphant phenotype is different from and weaker than the M*smad5* phenotype (293). *Bmp4* and *bmp*7 are also expressed during oogenesis (168), suggesting that they may act maternally in parallel with *radar*, but there is no evidence that either gene is required maternally for an early patterning function (60, 272).

Wnt Signaling

Signaling through the canonical Wnt pathway is essential for the specification of ventral and posterior fates (reviewed in 129). Wnt signaling through a Frizzled-Lrp receptor complex and a number of cytoplasmic proteins including Dsh, GBP, Axin, Ccd1, APC, and GSK3 stabilizes β-catenin, allowing it to accumulate in the nucleus and activate target gene expression (reviewed in 188). There are several secreted antagonists of Wnt signaling, including SFRPs, Cerberus, and Wnt inhibitory factor (WIF), which act by binding to Wnt proteins, and Dickkopf (Dkk), which binds the LRP subunit of the receptor (reviewed in 143).

Genetic studies in zebrafish show that Wnt8 signals are essential for the establishment of ventral and posterior fates (72, 179). During gastrulation, *wnt8* mRNA and strong activity of a Wnt/β-catenin responsive reporter are evident at the ventrolateral margin (63, 149) (**Figure 6**). Deletion or morpholino-inhibition of both ORFs of the bicistronic *wnt8* gene produces a severe loss of ventroposterior structures, with a concomitant expansion of dorsal fates (179). Simultaneous reduction of Wnt3a and Wnt8 activities results in a stronger expansion of dorsoanterior fates, indicating that these genes have overlapping functions (288). This zygotic role of canonical Wnt signaling in ventral and posterior patterning is opposite to its earlier role in dorsal patterning by maternally provided β-catenin described above.

Wnt signals have a role in repressing dorsal mesodermal fates that is distinct from the action of Bmp signals. In contrast to the *bmp* pathway zygotic mutants, the axial mesodermal territory in *wnt8* mutants is expanded along with the paraxial mesodermal and neural domains. In addition, anterior neural fates are expanded in embryos with reduced *wnt8* function, supporting a role for Wnt8 in posteriorizing the neuroectoderm (72, 179). Furthermore, mutations that inactivate repressors of Wnt signaling lead to an expansion of posterior neural fates at the expense of more anterior territories (62, 117, 153). Embryological and genetic evidence also indicates that the position of the midbrain-hindbrain boundary is established by Wnt8 signals, possibly acting as morphogens, emanating from the blastoderm margin during gastrulation (259, 346, 347).

Among the target genes of Wnt8 and Wnt3a are the homeobox gene *cdx4/kugelig*, which is essential for tail development and the regulation of posterior *hox* genes (56, 94, 288), the T-box gene *tbx6* (311), and the Sp1 class zinc finger gene *Sp5-like* (337). In addition to these functions during gastrulation, experiments with low doses of morpholinos suggest that *wnt8* and *wnt3a* function during segmentation to maintain presomitic mesoderm in the tail bud (319).

The roles of Wnt antagonists have not been extensively studied in zebrafish, but *dkk1*, an early target of maternal β-catenin, is expressed early in the dorsal margin and dorsal yolk syncytial layer and during gastrulation in the developing prechordal plate, where it could function to counteract the ventralizing and posteriorizing effects of canonical Wnt signaling (113, 292).

The SFRP protein Tlc is expressed at the anterior neural border, a region required for induction of anterior neural fates (127, 128). Telencephalic fates are reduced in *tlc* morphant embryos, and it has been proposed

that Tlc acts locally within the neural plate to promote anterior identity by inhibiting Wnt8b signals from the midbrain-hindbrain boundary.

Boz and Vox/Vent Transcriptional Repressors

Inactivation of the redundant homeodomain transcriptional repressors Vox (Vega1) and Vent (Vega2), by deletion, coinjection of morpholino oligonucleotides for both genes, or injection of a *vent* MO into a *vox* point mutant, leads to a severe loss of ventroposterior structures including blood, pronephros, and tail (131, 139, 140, 204). The loss-of-function phenotype is strain dependent, such that AB strain embryos lacking *vox/vent* are essentially wild type (131). Inactivation of a third gene encoding a homeodomain transcriptional repressor, *ved*, along with *vox* and *vent*, is sufficient to strongly dorsalize even AB strain embryos (290). Although embryos lacking *vox/vent* resemble the Bmp pathway mutants, important phenotypic differences are that dorsal mesodermal fates are strongly expanded in embryos lacking *vox/vent*, and anterior neural fates are shifted more toward the margin and less toward the ventral side than in *bmp* pathway mutants such as *swirl/bmp2b* (131). The dorsalized phenotypes of *vox/vent* and *wnt8* mutants are very similar, and there is evidence that *wnt8* activates *vox* and *vent* expression, thereby repressing dorsal genes (248). *chordin* is a key target of Vox and Vent, and these proteins also repress other dorsal genes including *boz*, *goosecoid*, *floating head (flh)*, and *dkk1*.

Mutants for the homeodomain transcriptional repressor Boz have a variable phenotype characterized by cyclopia, reduction of dorsal mesoderm, and, in the most severe cases, reduction of forebrain coupled with an expansion of hindbrain (75, 166, 291, 294, 300). Maternal β-catenin activates *boz* expression in dorsal blastomeres soon after the MBT (263, 353) (**Figure 5**). Beginning shortly thereafter, *boz* expression is confined to the dorsal yolk syncytial layer until *boz* mRNA is no longer detectable at the mid-gastrula stage. Studies with fusion constructs containing the Boz homeodomain and potent transcriptional activator or repressor domains indicate that Boz acts as a transcriptional repressor (290). Although *boz* is predominantly expressed in the yolk syncytial layer, it can act nonautonomously to dorsalize overlying blastomeres, presumably by repressing a ventralizing signal expressed in the yolk syncytial layer (353). Key targets of Boz include *bmp2b*, *wnt8*, and *vox/vent/ved* (76, 96, 131, 182). Thus Boz specifies dorsal fates by repressing the expression of ventralizing factors rather than directly activating dorsal gene expression (**Figure 5**). For example, dorsal mesoderm is expanded in *boz;vox;vent* triple mutants, demonstrating that *boz* is not needed to promote dorsal mesoderm gene expression when the ventralizing repressors are inactivated by mutation (131).

Two additional transcriptional repressors, Prdm/Blimp1 and Iro3, are expressed at the dorsal margin. In contrast to Boz, Prdm1 represses *chordin* expression and antagonizes dorsal fates when overexpressed, and knockdown of Prdm1 function weakly dorsalizes the embryo (342). At later stages, Prdm1 is required for slow muscle development and patterning of cell types at the edge of the neural plate (23, 123). Iro3 appears to act as a repressor of *bmp* transcription (171). These observations indicate that depending on their target genes, dorsally expressed repressors can have opposite roles in DV patterning.

A Model for Dorsal-Ventral Patterning

The detailed analysis of mutants that affect Bmp and Wnt signaling and several transcription factors suggests the following model for DV patterning. Soon after the onset of zygotic transcription, ventralizing genes, including *bmp2b* and *vox*, are widely expressed in the embryo, including in the most dorsal territories (139, 182) (**Figures 5** and **6**).

The maternal pathways inducing the expression of *vox* and *bmp2b* are not known, but Bmp signals likely have a role (168, 293). It seems that *bmp2b* and *vox* are activated in parallel, because zygotic *bmp* and *vox/vent* are not required for each other's expression until the late-gastrula stage (131). In contrast, Wnt8 regulates *vent* expression and mesodermal *vox* expression (248). At the same time, maternal β-catenin protein activates dorsalizing genes, including *boz* among others, specifically in dorsal blastomeres and, soon thereafter, dorsal nuclei in the yolk syncytial layer (353). Hence, the earliest zygotic regulators of DV patterning act downstream of maternal factors to establish a two-state pattern, in which cells express either dorsal and ventral genes or only ventral genes.

After a short lag, presumably reflecting the time needed for Boz protein to accumulate to sufficient levels, Boz represses transcription of *bmp2b*, *vox*, and other ventralizing genes at the dorsal margin (75, 139, 182). This allows for expression of dorsal genes, such as *chordin*, *dkk1*, and *goosecoid*, which would otherwise be repressed by *vox/vent/ved* (131, 290). Thus as the first wave of zygotic genes becomes active, cells have gene expression patterns characteristic of either dorsal cells (e.g., *boz*, *goosecoid*, *chordin*, *dkk1*) or ventrolateral cells (e.g., *bmp2b*, *bmp7*, *vox*, *vent*, *ved*, *wnt8*).

Through the action of Bmp and Wnt8 signals and their antagonists, the simple pattern of mid-blastula stage embryos becomes much more elaborate, with many different groups of tissue progenitors fated to arise from different regions of the early gastrula embryo. As Bmps, Wnt8, and other signals elaborate and refine the pattern of the early gastrula, the regulatory interactions among DV-patterning genes change. For example, *vox/vent* and *bmp2b/swirl* are initially expressed independently of each other's action. As embryogenesis proceeds, however, expression of *vox/vent* and *bmp2b/swirl* genes becomes interdependent, apparently through a positive feedback loop established during gastrulation (131, 139, 140, 204). At mid-gastrulation, zygotic Bmp signals are required for normal levels of *vox* and *vent* expression. Conversely, *vox* and *vent* act to promote *bmp2b/swirl* and *bmp4* expression by inhibiting the expression of *chordin*, which blocks a positive autoregulatory activity of BMP signals (110, 277). Although the primary function of *vox/vent/ved* is to repress dorsal genes rather than to induce ventral genes, interruption of this *vox/vent-bmp2b* positive feedback loop is responsible for a reduction of ventral gene expression in embryos lacking *vox* and *vent* at mid-gastrulation. Thus the *vox/vent-bmp2b* positive feedback loop maintains ventral positional identity during gastrulation, and the participation of the extracellular factors Chordin and Bmp incorporates flexibility and sensitivity to the cellular environment into the mechanism that maintains dorsal-ventral identity. For example, a cell moving from ventral to dorsal territories during gastrulation would reduce its expression of *vox* and *vent* in response to increased levels of Chordin and reduced levels of Bmp activity. The reduction of Vox and Vent levels would, in turn, permit the expression of dorsal genes appropriate for the cell's new environment.

MESODERM AND ENDODERM FORMATION: MATERNAL FACTORS

The progenitors of the different germ layers are arranged along the animal-vegetal axis, with mesendoderm progenitors residing at and next to the margin and ectodermal progenitors located more animally (161, 334) (**Figure 3**). The animal-vegetal axis, in contrast to the DV or left-right axes, is already formed during oogenesis (281, reviewed in 237). The egg thus has an animal-vegetal polarity that is highlighted by morphological and molecular markers such as the position of the germinal vesicle and the localization of maternal mRNAs (18, 195). It is unknown how this polarity is generated during oogenesis, but maternal-effect mutants such as *bucky ball* might provide insights into this process (65).

mRNAs that are localized animally or vegetally in wild type fail to do so in *bucky ball* mutants, and cytoplasmic streaming occurs in multiple directions. This phenotype suggests an animal-vegetal polarity defect of the egg.

The animal-vegetal polarity of the egg has to be translated into the induction of mesendoderm at the margin. The molecular basis of this process remains elusive, but several lines of evidence indicate that the yolk cell, and specifically the yolk syncytial layer, contains signals that can induce mesendodermal markers (**Figure 4**). First, transplantation of the yolk cell onto the animal region of the blastoderm can ectopically induce genes that are normally expressed at the margin (212, 213, 232). Second, injection of RNase into the yolk syncytial layer blocks the expression of ventral and lateral mesendodermal markers (44). Dorsal markers are still expressed, probably due to the dorsal determinant β-catenin in dorsal blastomeres. The maternal factors that establish the yolk syncytial layer as a signaling center are unknown. In *Xenopus* the transcription factor VegT has been implicated as a maternal factor that can activate mesoderm inducers (360). *Xenopus VegT* mRNA is localized vegetally and required for mesendoderm induction. In zebrafish *spadetail* is a related T-box gene, but neither its expression nor mutant phenotype suggest any functional similarity to *Xenopus VegT* (99, 156). Maternal *eomesodermin* mRNA is localized vegetally in zebrafish eggs, but there is no evidence that this T-box gene might act in a *VegT*-like fashion (33). No maternal mutant has been isolated yet that blocks mesoderm and endoderm induction in zebrafish.

MESODERM AND ENDODERM FORMATION: ZYGOTIC FACTORS

Nodal Signaling

Members of the Nodal family of TGFβ signals are essential inducers of mesoderm and endoderm in vertebrates (reviewed in 268). Nodal signals are received by EGF-CFC coreceptors and type I and II Activin receptors, which function as serine/threonine kinases. Receptor activation leads to phosphorylation of the transcription factors Smad2 and Smad3. This results in their binding to Smad4, nuclear translocation, and association with additional transcription factors such as FoxH1 and Mixer to regulate target genes. Nodal signaling is antagonized by feedback inhibitors such as Lefty proteins, which are divergent members of the TGFβ family and block EGF-CFC coreceptors (41, 48), and Dapper2, which enhances the degradation of type I Activin receptors (362, but see 335).

Mutant screens in zebrafish have identified several components of the Nodal signaling pathway (**Table 1**). These include the Nodal signals Cyclops (Cyc) and Sqt, the EGF-CFC coreceptor One-eyed pinhead, and FoxH1 [*schmalspur (sur)*] and Mixer [*bonnie & clyde (bon)*] (31, 71, 79, 108, 115, 118, 151, 173, 243, 251, 252, 265, 270, 271, 295, 300, 306, 322, 361). In addition, molecular studies have led to the isolation of zebrafish Lefty and Dapper2 homologues and TARAM-A, a putative Nodal type I receptor (27, 239, 258, 316, 335, 362). In the case of the Leftys and Dapper2, but not TARAM-A, morpholino experiments have revealed essential roles for these proteins (3, 11, 46, 77, 362). Below we summarize the role of Nodal signaling in mesendoderm formation in zebrafish. Recent reviews provide more general discussions of the Nodal signaling pathway and its role during vertebrate development (reviewed in 268, 269).

Absence of Nodal signaling in *cyc;sqt* double mutants or maternal-zygotic *one-eyed pinhead* mutants results in embryos that lack all endoderm and mesoderm, with the exception of a few somites in the tail (79, 102). Mutants also lack trunk spinal cord, but develop forebrain, midbrain, hindbrain, and tail spinal cord. These phenotypes are already presaged before gastrulation by the aberrant expression of genes marking presumptive mesendoderm progenitors in wild type. Consequently, the fate and morphogenetic movement of

marginal cells is affected. Marginal cells do not internalize, and dorsal marginal cells acquire neural fates instead of dorsal mesodermal fates, while ventral and lateral marginal cells contribute exclusively to the tail (36, 78). Conversely, increasing Nodal signaling by loss of Lefty1 and Lefty2 or overexpression of Cyc or Sqt results in the fate transformation of ectodermal cells into mesoderm or endoderm (3, 45, 46, 71, 77, 79, 101, 251, 252, 265).

How does the interplay of *cyc*, *sqt* and *leftys* control mesendoderm formation? Before gastrulation *cyc*, *sqt*, *lefty 1* and *lefty 2* are expressed in the 1–3 cell tiers closest to the margin, overlapping with and vegetally to mesendodermal progenitors (27, 46, 66, 71, 79, 101, 207, 251, 252, 265, 316) (**Figure 6**). In addition, *sqt* is expressed in the yolk syncytial layer (71, 79). Mutant and misexpression studies have suggested a scenario for Nodal-mediated mesendoderm induction (46, reviewed in 268). The *sqt* and *cyc* genes are transcribed in cells closest to the margin, leading to the local generation of Sqt and Cyc proteins. Sqt can move away from the source and induce mesendodermal gene expression in cells at a distance (45). In contrast, Cyc only acts at a short range and induces mesodermal markers locally (45). Nodal signaling also induces the expression of *lefty1* and *lefty2*, which block the Nodal signaling pathway both locally to restrict the expression of *sqt* and at a distance to restrict the response to Sqt (46, 77). Hence, the interaction of Sqt, Cyc, and Leftys determines the extent of mesendoderm formation in zebrafish.

The strongest evidence for this model comes from the analysis of *bhikhari*, a marker for Nodal signaling expressed in 6–10 tiers from the margin. *cyc;sqt* double mutants lack *bhikhari* expression, and in the absence of *squint*, *bhikhari* is expressed only in the first few tiers (45). In contrast, the absence of only *cyc* initially does not affect the extent of *bhikhari* expression. This has suggested that Sqt might act at a long range to induce mesendodermal genes, whereas Cyc has only short-range activity. In support of this model, ectopic clones of Sqt-expressing cells can induce downstream genes in distant cells. In contrast, Cyc-expressing clones only induce downstream genes at a short range (45). Two observations indicate that the long-range effect of Sqt is direct and not mediated via a relay mechanism. First, the activation of the Nodal signaling pathway in a clone of cells induces downstream genes cell autonomously. Second, Sqt can be made in nonresponding cells and apparently move through a field of nonresponding cells to activate gene expression in distant cells (45). These results provide support for a direct long-range effect of Sqt. It is unclear why Sqt is long-range and Cyc short-range, but studies on mouse Nodal indicate that the stability of the mature ligand might be a major determinant of range (178).

Leftys appear to restrict the range of Nodal signaling by two mechanisms (46). First, Leftys dampen Nodal autoregulation, thus limiting the generation of more Nodal. For example, in the absence of Leftys, *sqt* expression extends animally away from the margin and is maintained for a longer time than in wild type. Second, Leftys can act at a long range to inhibit Nodal signaling in distant cells. For example, ectopic expression of Leftys at the animal pole can block Nodal signaling at the margin of the zebrafish embryo. Moreover, depletion of Leftys extends the range of Sqt activity even in the absence of *sqt* autoregulation.

Although the above model accounts for the regulation of Nodal-regulated markers such as *bhikhari*, interactions between *cyc* and *sqt* provide additional complexity. Specifically, Sqt induces the expression of *cyc* on the dorsal side (66). Hence, *sqt* mutants lack Squint and have less Cyc on the dorsal side. The dorsal side is therefore more sensitive to loss of Nodal gene dosage than the lateral and ventral sides. In addition, despite their different ranges, both Cyc and Sqt can induce most mesendoderm derivatives on their own. While *cyc;sqt* double mutants lack all head and trunk mesoderm and endoderm, *cyc* mutants

display only minor defects in prechordal plate formation (66, 115, 317), and *sqt* mutants have quite mild defects in axial mesoderm and endoderm formation (66, 79, 118, 294). Hence, both short- and long-range Nodals can orchestrate most aspects of mesendoderm formation in zebrafish.

Nodal signaling not only induces the extent of mesendoderm, but also seems to pattern it. Partial reduction of Nodal signaling leads to the loss of cell types derived from marginal-most tiers. For instance, at the dorsal margin, high levels of Nodal signaling are required for prechordal plate (anterior axial mesoderm) specification, whereas lower levels are essential for notochord (posterior axial mesoderm) specification (101). Analogously, at the lateral margin where precursors for the myocardium (heart muscle) reside, high levels of Nodal signaling promote ventricular fates whereas lower levels are sufficient to induce atrial fates (145). This has led to the proposal that there might be a gradient of Nodal signaling activity at the margin, leading to the fine patterning of mesodermal and endodermal precursors (101). This conclusion is also supported by fate map studies of *sqt/sqt* and *sqt/sqt;cyc/+* mutants that demonstrate a vegetal shift of cell fates that in wild type are located more animally (66). These mutant combinations also revealed that dorsal mesoderm requires higher levels of Nodal function than ventral and lateral regions; however, differential Nodal signaling does not pattern the mesoderm along the DV axis. In particular, dorsal margin cells are not transformed toward more lateral fates in *sqt/sqt;cyc/+* mutants embryos, but dorsal expression of *cyc* requires Nodal-dependent autoregulation. The specification of endoderm, which derives from blastomeres that are located most marginally, also requires high levels of Nodal signaling (6, 260, 271). These observations suggest that there might be a Nodal activity gradient along the animal-vegetal axis, with the highest levels at the margin inducing endoderm, prechordal plate, and ventricular progenitors, lower levels inducing notochord and other mesodermal fates, and the absence of Nodal signaling allowing neural and tail specification (101, 315). Experiments that block or activate Nodal signaling at different times have suggested that marginal-most cells require sustained Nodal signaling before the onset of gastrulation, whereas cells more distant from the margin require shorter windows of Nodal signaling (10, 101). It is thus conceivable that Nodal signaling induces different fates using both temporal and spatial gradients.

Nodal signaling induces the phosphorylation of Smad2 and Smad3 (reviewed in 268). Smad2 or Smad3 mutants are not available in zebrafish, but mutations in FoxH1 (*sur*) and Mixer (*bon*), transcription factors that can bind to phosphorylated Smad2, have been identified (31, 151, 173, 243, 270, 295, 300, 322). *bon* mutants have severe defects in endoderm formation, whereas maternal-zygotic *sur* mutants have mild defects in axial mesoderm formation (151, 243, 295). Loss of both *bon* and *sur* results in a severe phenotype characterized by absence of prechordal plate, cardiac mesoderm, endoderm, and ventral neuroectoderm (173, 322). Some Nodal-regulated genes are regulated by either Bon or Sur, and others by both Bon and Sur (173). The phenotypes seen upon loss of both Bon and Sur are milder than those seen upon complete loss of Nodal signaling, indicating that additional Smad-associated transcription factors that act as components of the Nodal signaling pathway remain to be identified.

Nodal Signaling and Left-Right Axis Development

Most organs in vertebrates are formed and positioned asymmetrically along the left-right axis. The analyses of *one-eyed pinhead* and *sur* mutants and of morphants for a third Nodal gene, *southpaw*, have established a requirement for Nodal signaling in zebrafish left-right axis formation (43, 191, 356). *southpaw* is expressed in the left lateral plate mesoderm, whereas *one-eyed pinhead* and *sur* are expressed bilaterally (191, 243, 295, 361). Loss

of late *one-eyed pinhead* or *sur* activity, and knock down of *southpaw* lead to a loss or randomization of organ asymmetries (43, 191, 356). For example, the consistent looping of the heart to the right side is randomized or lost in these embryos.

How genes such as *southpaw*, *lefty*, and *pitx2* are specifically activated on the left side is still unclear. Notch signaling and rotating cilia have been implicated in this step of left-right patterning in several vertebrates (235, reviewed in 250). In zebrafish, these cilia are located in Kupffer's vesicle, a specialized organ lined by the descendants of the dorsal forerunner cells (50, 73, 74, 142, 169). Disruption of Kupffer's vesicle development or cilia formation and function results in the randomized activation of left-side specific markers (73, 142, 169). Based on studies in the mouse, it is thought that cilia rotation leads to a leftward flow that results either in the specific activation of mechanoreceptors or the accumulation of a signal on the left that initiates left-side-specific gene expression. Both *southpaw* and the Nodal antagonist *charon* are first expressed symmetrically around Kupffer's vesicle before *southpaw* expression becomes restricted to the left lateral plate mesoderm (114, 191). Inhibition of *charon* expression results in the bilateral activation of *southpaw* in the lateral plate (114). These results suggest a model wherein cilia-mediated flow and *charon* activity bias *southpaw* activation toward the left.

Downstream of Nodal Signaling: Endoderm Formation

Several genes have been identified that are regulated by Nodal signaling and mediate its endoderm-inducing activity, including the Sox gene *casanova*, the GATA gene *faust*, and the homeobox genes *bonnie&clyde* and *mezzo* (5, 42, 61, 150, 151, 245, 255, 256, 264, 302, reviewed in 231). These four genes encode transcription factors and appear to be direct targets of the Nodal signaling pathway, as suggested by experiments using cycloheximide (245). As described above, Bon is also a component of the Nodal signaling pathway.

Of these four genes, *casanova* appears to be the most central and downstream player (61, 150). *casanova* is expressed in a subset of marginal-most cells that are thought to give rise to endoderm. Indeed, loss of *casanova* causes these cells to adopt an aberrant mesodermal fate. In addition, *casanova* is sufficient to induce cells to give rise to endoderm, and *casanova* can induce endoderm in the absence of Nodal signaling. Since the activities of *faust*, *bon*, and *mezzo* depend on *casanova*, the main role of *faust*, *bon*, and *mezzo* may be to induce and maintain *casanova* expression. Casanova activity and maintenance require the POU domain gene *spiel ohne grenzen* (*pou2/Oct4*) (25, 34, 193, 254). In contrast to Casanova, *spiel ohne grenzen* expression is not induced by Nodal, but is ubiquitous and activated both maternally and zygotically. These results suggest that endoderm formation induced by Nodal is predominantly mediated by the induction of Casanova and its interaction with Spiel ohne grenzen.

Downstream of Dorsal Mesoderm Induction: Midline Development

As the embryonic pattern is refined during gastrulation, cells at different positions in the shield and the immediate vicinity adopt different fates, including prechordal plate, notochord, hypochord, adaxial muscle, and floor plate (175, 206, 285). The prechordal plate arises from the marginal-most cells in the shield, under the influence of the highest levels of Nodal signals (101). The cells of the developing prechordal plate specifically express the transcriptional repressor Goosecoid (301). Goosecoid may repress expression of genes that promote other cells types (80), although *goosecoid* has not been analyzed in loss-of-function studies in zebrafish.

The notochord arises from cells at slightly more animal positions within the shield (101, 206). The homeobox gene *flh* and the T-box gene *no tail* (*ntl*) are essential for

notochord development (106, 107, 278, 312). Both genes are expressed in all margin cells in the late blastula (276, 312). By the beginning of gastrulation, *flh* is specifically expressed in notochord precursors, whereas *ntl* is expressed in all margin cells, the developing notochord, and, at later stages, the tail bud. Fate mapping with mutant embryos showed that *flh* is required to prevent notochord precursors from differentiating as muscle, whereas *ntl* acts to prevent notochord precursors from forming floor plate (7, 105, 205, 206). A key target of *flh* is *spadetail* (*spt*), a T-box gene initially expressed in all marginal cells and then repressed in the notochord domain early in gastrulation (8, 99). The *Xenopus* ortholog of *flh*, *Xnot*, encodes a transcriptional repressor, suggesting that Flh may directly repress *spt* transcription (328, 357). Trunk muscle and other ventral-lateral mesodermal derivatives are reduced in *spt* mutants, and it has been proposed that *spt* both activates expression of genes required for muscle differentiation and morphogenesis (e.g., *myod*) and antagonizes the function of *ntl* in notochord development (7, 8, 99, 125, 156).

In addition to the interactions among these transcriptional regulators, local signaling interactions are important to allocate cells in the shield region to particular fates. For example, the Nodal signal Cyc is required during gastrulation for the formation of the medial floor plate (115, 217, 229, 233, 265, 319a, reviewed in 307). The Cyc signal antagonizes the notochord-promoting function of *ntl*, perhaps by inducing the expression of the transcriptional repressor Her9 (105, 176). Repression of *ntl* by Notch signaling and another Hairy/Enhancer of split gene, *her4*, allows cells lateral to the notochord domain to differentiate as hypochord (12, 13, 175). At later stages the growth factor Midkine-a is expressed in the paraxial mesoderm and required for the formation of the posterior medial floor plate (266a). Hedgehog signals from the midline during gastrulation instruct the immediately adjacent adaxial cells to differentiate as slow muscle and the overlying neuroectoderm to form lateral floor plate (29, 169a, 233, 266b, reviewed in 307). These studies indicate that complex, local interactions among several signaling pathways and transcription factors specify different midline cell fates.

FGF Signaling

Members of the FGF family of signals have been implicated in mesoderm formation, neural induction, DV patterning, and anterior-posterior patterning of the embryo (reviewed in 296). Data in zebrafish mainly suggest an early role for FGF signaling in repressing Bmp signaling and a later role in promoting the development of posterior structures. FGFs bind and activate receptor tyrosine kinases. Receptor dimerization leads to *trans*-phosphorylation and the recruitment and activation of a plethora of downstream effectors, including PKC and the ras/MAPK cascade. In contrast to the Nodal and Bmp signaling pathways, zygotic mutant screens have only uncovered a single mutation in a component of the FGF signaling pathway—mutations in *acerebellar* disrupt the *fgf8* gene (30, 253). The dearth of FGF signaling mutants might be due to the overlapping roles or maternal contribution of these gene products. Hence, morpholinos, misexpression or small molecule inhibitors have been used to analyze the role of FGF signaling during embryogenesis. Such studies have identified the type I transmembrane protein Sef ("similar expression to fgf genes") as a novel feedback inhibitor of FGF signaling (84, 323).

The expression patterns of downstream targets for FGF signaling have revealed that the pathway is first active at the dorsal blastoderm margin, then along the entire margin and finally in the tail bud (87, 247, 261, 324) (**Figure 6**). Consistent with this pattern of activity, the earliest role for FGF signaling is during blastula stages, when *fgf3*, *fgf8*, and *fgf24* are expressed at the dorsal margin. Misexpression of FGF signals can inhibit the expression of *bmp2b* and *bmp7* at blastula stages

and lead to the lateralization and dorsalization of the embryo (86, 87, 324). However, blocking only FGF signaling during these stages does not result in a ventralized embryo. In contrast, blocking both Chordin and FGF signaling results in ventralization (87). These results suggest that FGF signaling, Bozozok, and Bmp antagonists such as Noggin and Chordin all contribute to blocking Bmp signaling in dorsal margin blastomeres. Conversely, inactivating the FGF signaling feedback inhibitor Sprouty2 results in the repression of Bmp expression and the dorsalization of the embryo (87). Taken together, these results establish an early role for FGF signaling in restricting Bmp expression and activity.

Following dorsal margin expression, several FGF ligands become expressed in the entire margin at the onset of gastrulation (35, 67, 85, 253). Moreover, downstream targets like *pea3*, *erm*, and *sprouty4* are induced in broad domains in neighboring cells (87, 247, 261, 324). These downstream genes appear to be activated at different thresholds of FGF activity, with *sprouty4* being the target most sensitive to low levels of FGF signaling and expressed in most-distant cells (275). These results suggest that FGF signals form a vegetal-to-animal activity gradient. Indeed, tagged FGF can be detected in intracellular vesicles at a distance from the source. This localization is dependent on receptor-mediated endocytosis, which leads to the clearance of the ligand from extracellular space. As a consequence, blocking endocytosis results in an increased FGF signaling range (275).

These results suggest a long-range and graded FGF signaling activity at early gastrulation stages, but it is unclear what role this activity might have. At the onset of gastrulation mesendoderm formation and patterning are initiated correctly in the apparent absence of FGF signaling, and only at later gastrulation stages is the expression of genes such as *tbx6*, *spt*, and *ntl* lost (67, 84, 87, 98, 100, 200, 247, 323, 324). It is therefore conceivable that very early FGF signaling only has consequences at later stages of gastrulation (see below), or that it acts redundantly with other pathways, e.g., the Nodal signaling pathway. The latter possibility is raised by double mutant studies of FGF and Nodal signaling components (100, 200). For example, partial inhibition of FGF signaling and blocking zygotic activity of One-eyed pinhead, a Nodal coreceptor, disrupts posterior development and leads to the death of dorsal mesoderm cells by the end of gastrulation. However, it remains unclear when Nodal and FGF signaling interact.

An alternative scenario is that FGF signaling during gastrulation primes and maintains cells for posterior development. For example, severe posterior truncations are generated in embryos exposed to the FGFR inhibitor SU5402, expressing a dominant-negative FGF receptor or lacking full *fgf8* and *fgf24* activity (67, 98, 100, 200). Tail and posterior trunk mesoderm do not form in these embryos and mesodermal markers such as the T-box genes *ntl* and *spt* cease to be expressed at later gastrulation stages. These results suggest that FGF signaling is required for the maintenance of a pool of mesoderm progenitors during gastrulation. A role for FGF signaling in the formation of posterior cell types is also seen in the nervous system (167, 170, 172, 189, 257). FGF signaling is required for the expression of posterior neural markers during gastrulation. Strikingly, this neural inducing role of FGFs is independent of the organizer or the inhibition of Bmp signaling.

Retinoic Acid Signaling

Retinoic acid signaling acts during gastrula stages in the posteriorization of the neuroectoderm and the formation of myocardial progenitors (**Figure 6**). Retinoic acid binds to its receptors, members of the nuclear hormone receptor family, leading to the regulation of downstream genes. Retinoic acid is synthesized by RALDH and hydrolyzed by Cyp26/P450RA1. Studies on retinoic acid function in zebrafish have used exposure to retinoic acid, pharmacological inhibition of

retinoic acid receptors, mutations in *raldh2* or *cyp26a1*, and morpholinos against *cyp26a1* (24, 70, 97, 122, 172, 185). These studies have suggested that the expression of RALDH in the posterior mesoderm during gastrulation generates a source of retinoic acid that induces posterior hindbrain and spinal cord markers, in particular specific subsets of *Hox* genes. Conversely, *cyp26a1* is expressed in more anteriorly located precursors of the neuroectoderm and thought to generate a retinoic acid-free zone that is thus protected from retinoic acid-mediated posteriorization.

A role for retinoic acid has also been found in the mesoderm. Blocking retinoic acid signaling during gastrulation stages increases the number of myocardial progenitors (144). This is not simply due to an expansion of the myocardial progenitor region but appears to be a consequence of an increase in the density of myocardial progenitors within a normally sized field containing these precursors.

MicroRNAs

MicroRNAs are ~22 nucleotides long nonprotein-coding RNAs that regulate gene expression at the posttranscriptional level (reviewed in 9, 20). MicroRNAs have been implicated in many processes, but the roles of microRNAs in zebrafish embryogenesis have only been tested recently (92, 338, 339). A large-scale analysis of the expression of more than 100 microRNAs has revealed very specific patterns during embryonic and larval stages (338). Maternal-zygotic mutants for the RNaseIII enzyme Dicer cannot process microRNA precursors and thus lack mature microRNAs (92). The resulting phenotype is quite mild. Mutant embryos develop normal axes and are regionalized correctly. The major cell types are specified, and no dramatic modulation of embryonic signaling pathways has been observed. The main defects are in morphogenetic processes such as a delay in epiboly, impairment of ventricle inflation, and abnormal somite differentiation (92). These results suggest that microRNAs have subtle roles during zebrafish axis formation and might be involved in the differentiation of multiple cell types at later stages.

GASTRULATION MOVEMENTS

The movements of epiboly, internalization, convergence, and extension transform the radially symmetric blastula into the gastrula embryo with clear DV and anterior-posterior axes (333, reviewed in 2, 138, 155, 181, 215, 298) (**Figure 2**). Mutant analysis has indicated that these processes can be genetically separated, e.g., defects in internalization do not lead to an obligatory disruption of epiboly or convergence.

Epiboly

Epiboly describes the process of spreading and thinning of the embryo during blastula and gastrula stages and results in the envelopment of the yolk by the embryo. Microfilament and microtubule networks in the yolk cell are thought to contribute to epiboly (47, 299, 305, 321). The cellular basis for epiboly appears to be at least in part a process of radial intercalation—deeply positioned cells move outward between more superficial cells, resulting in a thinning and spreading of the embryo (**Figure 2**). This process has been well documented during gastrula stages, when two cell layers can be distinguished in the epiblast. An epithelial-like exterior layer underlies the enveloping layer and an inner layer overlies the hypoblast (137). During epiboly, cells from the inner layer intercalate between cells of the outer layer and flatten to dimensions typical in that layer. Hence, both cell intercalation and cell flattening can contribute to epiboly.

Zygotic screens have isolated mutations in only a single locus that affects epiboly (135, 137, 202, 300). Mutations in the adhesion molecule E-cadherin (*half-baked*) severely affect epiboly and arrest the vegetal spreading of deep cells during gastrulation (137, 289). Mutant cells can intercalate but often deintercalate into the deeper layer and do not

flatten. These results suggest that E-cadherin is required to bind cells together so that they can form a flattened spread-out layer that envelops the yolk. How epiboly is regulated is still poorly understood, but blocking the T-box transcription factor Eomesodermin or its target *mtx2*, a homeobox gene, blocks epiboly (32).

Consistent with an adhesive role for E-cadherin, more severe depletion using morpholinos instead of *hab* mutants results in the deadhesion of cells and disintegration of the embryo already during early cleavage divisions (14). An additional factor that might be involved in cell (de)adhesion, but not epiboly, is the EGF-CFC protein One-eyed pinhead. During late-blastoderm stages, *one-eyed pinhead* mutant cells appear less motile and more cohesive (332). It remains unclear how this contributes to morphogenesis and if this process is dependent on Nodal signaling.

The isolation of four maternal mutants (*betty boop*, *poky*, *slow*, *bedazzled*) suggests that many components required for epiboly are provided maternally (330). These mutants display premature constriction of the margin (*betty boop*) and slower or delayed epiboly (*poky*, *slow*, *bedazzled*).

Internalization

Internalization describes the process by which mesendodermal precursors located at the margin move inside, resulting in an embryo with an outer epiblast layer and an inner hypoblast layer (159, 333). There has been some debate about the exact cellular mechanism that underlies internalization (2). It was proposed that internalization is caused by involution, the inward flow of a sheet of cells, or ingression, the inward movement of individual cells (36, 53, 77, 214, 285). Imaging and embryological studies suggest that synchronized ingression underlies internalization (2). Cells move coherently toward the margin, where they begin to form protrusions, lose coherence with their neighbors, and ingress. This "flow of individuals" results in internalization.

The molecules that drive internalization are largely unknown. Complete absence of Nodal signaling blocks all internalization movements (36, 78). Conversely, upregulation of the pathway in the absence of Lefty leads to prolonged and increased internalization (77). A single cell that is mutant for the Nodal coreceptor One-eyed pinhead can initially be internalized when placed at the margin of a wild-type embryo but then it egresses (36). This result suggests that the flow of internalizing wild-type cells can carry but not keep neighboring cells inside. Activation of the Nodal pathway in a single cell can lead to the ingression of this cell even when neighboring cells do not internalize (36, 55). This effect might be caused by the differential adhesion between cells that have an active or inactive Nodal signaling pathway (214), but the Nodal downstream genes that mediate internalization are elusive.

Convergence and Extension

Convergence and extension are defined as the narrowing of embryonic tissues mediolaterally (convergence) and their elongation anterioposteriorly (extension). These movements are driven by a number of directed and coordinated cell behaviors that lead to the accumulation of cells on the dorsal side and the formation of an axis (reviewed in 146, 222, 331). Several distinct cell behaviors underlie convergence and extension in zebrafish, including the directed migration of internalized cells toward the animal pole and toward the dorsal side and the mediolateral intercalation of dorsal and lateral cells.

Immediately after internalization, hypoblast cells move away from the margin. This has been best studied on the dorsal side, where prechordal plate (anterior axial mesoderm) precursors migrate anteriorly. It has been proposed that a β-catenin-Stat3-Liv1-Snail1 pathway regulates prechordal plate migration (211, 354, 355). In this model, β-catenin activates an unknown ligand for the JAK/STAT pathway, culminating in the phosphorylation

and consequent activation of the transcription factor Stat3. Stat3 then activates the expression of the zinc transporter Liv1. Increased levels of zinc might allow the nuclear accumulation of the zinc finger transcription factor Snail1. It has been proposed that Snail1 might promote the epithelial-to-mesenchymal transition of anterior axial mesoderm cells, but such changes in cell behavior have not been observed in wild-type embryos (2, 214). Further genetic and cell biological studies are required to more thoroughly test the β-catenin-Stat3-Liv1-Snail1 model.

The PDGF/PI3K/PKB pathway has also been implicated in the migration of prechordal mesendoderm precursors (216). Pharmacological block of the PDGF receptor or phosphoinositide 3-kinase inhibits the formation of polarized processes on prechordal plate progenitors and the localization of protein kinase B and F-actin to the leading edge. Despite these defects, cells maintain their directional, albeit slower, migration.

A third pathway regulating prechordal plate migration is the noncanonical Wnt signaling pathway. As discussed in more detail below, this pathway has a key role in polarizing cells in all animals (reviewed in 163). Blocking *wnt11* activity during prechordal cell migration results in slower and less directed movements and the abnormal orientation of cellular protrusions (119, 325). Because the correlation of direction of movement and direction of protrusions is not absolute, it is not yet known if there is a causative link between these two *wnt11*-dependent processes.

The pathways described above ultimately have to regulate cell behavior by modulating cytoskeletal or adhesive properties. Indeed, two proteins implicated in actin dynamics have been implicated in the migration of prechordal plate cells (51). A *cap1* homolog and *quattro*, which encodes a guanine nucleotide exchange factor, are expressed in the anterior mesendoderm. *Quattro* morpholinos disrupt the anteriorly directed convergence and aggregation of prechordal plate, and *cap1* is required for the migration of the aggregated cluster toward the animal pole. These observations identify restricted actin-regulatory molecules in the control of cell movements during gastrulation. In addition, there also appears to be a minor role for E-cadherin in the elongation and migration of dorsal hypoblast cells (214).

A hallmark of gastrulation is the polarity of cell movements and cell shapes. Some of the molecular mechanisms underlying this process appear to be conserved in all animals. In particular, components implicated in planar polarity formation in *Drosophila* are also involved in the control of cell polarization and convergence and extension movements in zebrafish and other vertebrates (reviewed in 163, 298, 331). Several components of the noncanonical Wnt signaling pathway have been identified as convergence and extension genes in zebrafish, including the Wnt signals Wnt5 (*pipetail*), Wnt11 (*silberblick*), and Wnt4; the Wnt receptor Fz2; the putative Wnt coreceptor Glypican4 (*knypek*); and the cytoplasmic signal transducer Dishevelled (119, 152, 201, 249, 309, 320). In addition, modulators of the pathway have been identified, including Van gogh-like 2/Strabismus (*trilobite*) and Prickle (37, 132, 236, 283, 326). Additional components identified in *Drosophila*, such as Flamingo and Diversin, also play important roles in zebrafish (82, 279). Downstream mediators that have been shown to play a role in zebrafish include ROK, Rac, and RhoA (17, 197, 201).

The transparency of the zebrafish embryo has been employed to great effect to study the cellular role of planar polarity genes. The main conclusion from these studies is that planar polarity proteins are required for the proper polarization of cells during directed dorsal migration and mediolateral intercalation (reviewed in 215, 222). In this process, cells intercalate between their medial and lateral neighbors, similar to the cell behavior during frog gastrulation, or migrate directionally first toward the animal pole and then toward the dorsal side, a movement not observed in frogs. Planar polarity signaling

regulates both the length-to-width ratio of cells and their orientation with respect to the embryonic axes. This polarization is also thought to be required for the persistent migration of cells. For example, both the length-to-width ratio and mediolateral alignment of paraxial ectodermal cells is reduced in *trilobite* (Van gogh-like 2/Strabismus) mutants (132). Hence, cells are more rounded and more randomly oriented compared with the more elongated and more uniformly oriented wild-type cells. Concomitantly, *trilobite* mutant cells move with reduced net dorsal speed along less direct trajectories when compared with wild-type cells. It has thus been proposed that *trilobite* and other planar cell polarity genes allow for the medial-lateral cell polarization that is required for the persistent dorsal migration of cells along straight paths. The connections between cell behavior and movement are still correlative, but planar cell polarity signaling clearly controls both the shape and movement of cells.

Planar polarity signaling in zebrafish is not only required for the polarization of cells but also controls cell division orientation (95). Epiblast cells in dorsal tissues preferentially divide along the animal-vegetal axis of the embryo. Inhibition of the establishment of this animal-vegetal polarity by blocking *wnt11*, *dishevelled*, or *trilobite* disrupts this orientation and thus reduces the extension of the axis.

Although convergence and extension can be linked, they can also be independent. In *ntl* mutants, convergence but not extension of axial mesoderm is affected (93). In dorsalized *swirl/bmp2b* mutants, convergence of lateral cell populations is reduced, whereas their extension is normal or even increased (221). Moreover, the absence of the polysaccharide hyaluronan blocks the convergence but not the extension of lateral mesoderm (17).

In zebrafish there might also be an attractant on the dorsal side that guides cells. Specifically, misexpression of β-catenin on the ventral side not only induces ectopic dorsal fates but also redirects cells ventrally. Hence, there might be β-catenin–regulated genes that provide the directionality of convergence and extension movements. Although components of the noncanonical Wnt signaling pathway are required for directional movement of cells, they are unlikely to act as chemoattractants in this process. For example, ubiquitous expression of *wnt5* or *wnt11* is able to rescue *wnt11* mutants, arguing against a localized Wnt signal that controls cell polarity and migration (152). Instead, a signal regulated by Stat3 might provide polarity cues (211, 354). As described above, phospho-STAT3 accumulates specifically on the dorsal side in response to β-catenin stabilization. Blocking STAT3 function using morpholinos results in severe reduction of convergence and extension movement. This effect on lateral cells is non-cell autonomous. It has thus been proposed that STAT3 activates an as-yet unidentified factor that guides DV cell polarity. This interpretation is complicated by the fact that convergence and extension are also reduced in the *quattro* and *cap1* morphants described above, probably secondarily to the abnormal migration of anterior axial mesoderm (51). In this case, it might not be the absence of a signal but abnormal morphogenesis of axial mesoderm that impairs convergence and extension.

Despite the central role of planar cell polarity signaling during convergence and extension, several additional molecules have been implicated in gastrulation movements or cell polarity, including Gα12/13 (184), hyaluronan (17), Cyclooxygenase-1 (38), Widerborst [a B′ regulatory subunit of protein phosphatase 2A (111)], Estrogen receptor-related α (19), Scribble 1 (329), Fyn/Yes (134), Nemo-like kinase (318), Ephrins (39, 230), and Slit (359). Most of these factors have been implicated in gastrulation movements based on overexpression or morpholino analysis. Future genetic studies will be required to firmly establish a role for these molecules and to determine how they interact with other factors controlling gastrulation.

THE BIOGRAPHIES OF CELLS

The previous sections discussed how the fates and movements of cells are dependent on their position in the embryo and how signaling pathways, transcription factors, and other molecules influence these decisions. We are now in a position to attempt a synthesis of these observations and describe how different cells receive and interpret these diverse inputs during early development to generate specific cell types and move to specific positions. These "childhood biographies of cells" not only allow us to integrate the findings described above, but they also serve to inform strategies in stem cell research (reviewed in 284). A major application of vertebrate embryology and genetics is to drive multipotent cells to a particular fate for therapeutic purposes. In turn, these in vitro studies provide a critical test of how completely we understand embryonic development.

The Dorsal Margin: Making Prechordal Plate

Prechordal plate progenitors are located at the dorsal margin and become marked as dorsal when β-catenin is stabilized soon after fertilization (see above). After mid-blastula transition, β-catenin activates *sqt* and *cyc* expression in prechordal plate precursors, resulting in the full activation of the Nodal signaling pathway in these cells. In contrast, Bmp and Wnt signaling are suppressed by the β-catenin-mediated activation of Chordin, Dkk1, and other antagonists of Bmp and Wnt signaling, and because β-catenin activates repressors such as Boz, which represses *wnt* and *bmp* gene expression on the dorsal side. β-catenin might also activate transcription factors such as Goosecoid that directly specify dorsal fates. Hence, it might be sufficient to activate β-catenin and full Nodal signaling and block all other signaling pathways to specify prechordal plate precursors. This leads to the activation of prechordal plate-specific genes (e.g., *goosecoid*) and to the internalization and migration of progenitors toward the animal pole. This migration is controlled in part by the STAT3 pathway, Cap1, Quattro, Wnt11 signaling, and PDGF/PI3K signaling.

The Dorsal Margin: Making Notochord

Like prechordal plate progenitors, notochord precursors are initially marked by β-catenin, which activates *sqt* and *cyc* expression next to and potentially in notochord precursors. This results in the partial activation of the Nodal signaling pathway in these cells. Hence, it might be sufficient to activate β-catenin and intermediate levels of Nodal signaling and block all other signaling pathways to generate notochord precursors. This leads to the induction of *flh* and *ntl*, which encode transcription factors that specify notochord identity, and the internalization, convergence, and extension of notochord progenitors. This process is regulated by *ntl* (convergence) and noncanonical Wnt signaling (convergence and extension). According to this model, the level or timing of Nodal signaling is the key factor that distinguishes prechordal plate and notochord progenitors.

The Margin: Making Endoderm

A subset of the cells that are located at the margin become endoderm (reviewed in 231). These cells are exposed to an unknown signal from the yolk syncytial layer and are also likely to contain maternally provided mRNAs encoding transcription factors of unknown identity. Before gastrulation, and shortly after the activation of *sqt* at the dorsal margin, all endodermal precursors express Cyc and Sqt, resulting in the full activation of the Nodal signaling pathway in these cells. Depending on the DV position, Bmp, FGF, or Wnt signaling is also activated in endodermal precursors; these pathways do not influence endodermal fate specification per se, but might modulate the type of endoderm that is formed. Hence, it might be sufficient to fully activate

Nodal signaling to generate endoderm progenitors. This eventually leads to the induction of the transcription factor Casanova, which in conjunction with the transcription factor Spiel ohne grenzen might be sufficient to specify endoderm progenitors. Since high Nodal signaling is involved in both prechordal plate and endoderm specification, additional factors (e.g., β-catenin and its downstream genes) might be required to specifically induce prechordal plate cells. It remains unclear why only some cells at the margin are induced to express *casanova* and form endoderm, whereas neighboring cells form mesoderm.

The Lateral Margin: Making Heart Muscle

Cells at the lateral margin give rise to the cardiomyocytes of the heart (reviewed in 358). Nodal and Bmp signaling are required for this process, and at later stages FGF signaling is also thought to contribute to myocardium formation. These signaling pathways lead to the induction of *nkx2.5*, a marker for cells that can give rise to heart muscle, and downstream genes such as *hand2*. In contrast, retinoic acid signaling limits the number of cells in this region that are selected to form cardiomyocyte progenitors.

The Ventral Margin: Making Blood and Tail Somites

Cells at the ventral margin give rise to multiple cell types, including blood and tail somites. Many signaling pathways are active in this region, including Wnt, Bmp, FGF, and Nodal. Both the Wnt and Bmp pathways are most active in the ventral margin region, and this coincidence is apparently required for proper expression of ventral margin genes such as *tbx6* (311). Conversely, ventral margin cells can induce an ectopic tail, and this activity can be mimicked by the local application of Bmp, Wnt, and Nodal signals (4). Despite this activity, Nodal signals are not required to make tail somites, suggesting that high-level activity of Wnt, Bmp, and potentially FGF signaling may be sufficient to generate tail mesodermal identity. This leads to the activation of downstream transcription factors [e.g., members of the T-box and caudal-related gene families (56, 288)] and planar polarity signaling, which regulate tail morphogenesis (196).

Development of blood also requires Wnt, Bmp, and FGF signals, but in addition is dependent on Nodal signals (reviewed in 57). Indeed, Nodal signals may be a factor in determining why some ventral margin cells form blood while others form tail somites. The *sqt* and *cyc* genes are expressed at the ventral margin just prior to the onset of gastrulation, and mutational analysis shows that Nodal signals are essential for blood but not tail somites. Thus Nodal signals at certain levels or times in development may allocate a subset of ventral margin cells to a blood fate. It is not clear how Nodal signals might drive ventral margin cells toward blood fates, but they could instruct blood progenitors to involute early in gastrulation or trigger the expression of certain target genes that specify blood identity.

Lateral and Ventral Ectoderm: Making Spinal Cord

The precursors of the spinal cord are located laterally and ventrally between the margin and the animal pole. These cells originate distant to the organizer and, in contrast to other neuronal progenitors, do not require organizer-derived inhibitors to be specified. BMP and FGF signaling promote spinal cord development, whereas Nodal signaling counteracts it. Wnt and retinoic acid signaling are also involved in this process by posteriorizing the neuroectoderm. Dorsal spinal cord and neural crest progenitors, which are located more laterally at neural plate stages, appear to be specified by higher levels of Bmp signaling than ventral progenitors, which are located more medially at neural plate stages (21, 226).

The Animal Region: Making Forebrain and Midbrain-Hindbrain Boundary

Cells in the animal dorsal region become forebrain progenitors. It appears that forebrain specification requires the absence of all known signaling pathways. Indeed, blocking Nodal, Bmp, FGF, Wnt, and retinoic acid signaling does not affect forebrain formation and in some cases results in the expansion of forebrain territory. Absence of these signaling pathways is achieved by the absence of the signals that might suppress forebrain formation and the expression of inhibitors of these signaling pathways. For example, Dickkopf and Tlc are both inhibitors of Wnt signaling expressed in the prechordal plate underlying the forebrain territory and at the anterior border of the forebrain region, respectively. These and other factors (Tcf3, Chordin, Noggin, Lefty) inhibit signaling and allow forebrain formation. It is still controversial if forebrain formation is indeed the default state of development. For example, FGF signaling has been proposed to be required for neural induction, including the forebrain, but genetic evidence in zebrafish is not available (304).

The precursors of the midbrain-hindbrain boundary are located dorsally and at an intermediate position between the animal pole and margin. It appears that these cells are induced by lack of Bmp, Nodal, FGF, and retinoic acid signaling but require intermediate levels of Wnt signaling (259).

COMPARATIVE ASPECTS

How applicable are the findings in zebrafish to other vertebrates and vice versa? Vertebrate embryos share a similar body plan, and the fate map of one species can be morphed into the one of another (298). It has therefore been expected that the underlying molecular mechanisms are also shared. The past 10 years have seen dramatic progress in our molecular understanding of zebrafish, frog, chick and mouse embryogenesis, and we can now ask if the molecular mechanisms underlying vertebrate axis formation are conserved. At a superficial level, the answer is yes. First, the same signaling pathways and transcription factors are employed during the early embryogenesis of all vertebrates (reviewed in 59, 112, 192, 298, 303, 313). For example, the Nodal, Bmp, FGF, and Wnt signaling pathways are active in all vertebrates during blastula or gastrula stages, and transcription factors such as homeodomain and T-box proteins are widely employed. Second, interference with these regulators can result in similar phenotypes. For example, lack of Nodal signaling severely compromises mesoderm and endoderm induction (reviewed in 268), loss of FGF signaling affects posterior development (reviewed in 296), and mutations in *Brachyury/ntl* result in notochord defects and posterior truncations in all model vertebrates (reviewed in 297).

Despite these similarities there are also intriguing differences, in particular between frog and fish on one side and mouse on the other. For example, noncanonical Wnt signaling is required for proper gastrulation in fish and frog, but mice that lack components of this pathway display only mild posterior truncations and spina bifida (reviewed in 163, 331). However, in general, the loss-of-function phenotypes of particular signaling pathways are more severe in mouse than in zebrafish or frog (reviewed in 313). For example, β-catenin is required for the formation of dorsal structures in fish and frog but not for mesoderm and endoderm formation (reviewed in 129). In contrast, *β-catenin* mutant mice lack anterior-posterior polarity and do not develop embryonic mesoderm and endoderm (103). Similarly, mouse *wnt3* mutants do not form mesodermal and endodermal progenitors (187). In fish and frog Bmp signaling is necessary for the development of ventral structures but not for mesoderm and endoderm formation (reviewed in 219). In contrast, mouse cells mutant for Bmp receptors cannot form mesoderm and endoderm (210, reviewed in 219). Lack of FGF signaling in frog and

fish results in posterior truncation in frog and fish, similar to partial loss-of-function phenotypes in mouse (reviewed in 296). However, in *fgf8* mouse mutants endoderm and mesoderm progenitors cannot gastrulate properly, resulting in the loss of these cell types (310). Hence, there are clear differences between fish/frog and mouse in the requirement for key signaling pathways.

We suggest that these differences arise from the much more pronounced role of reciprocal signaling interactions in mouse than in fish or frog. Although there is some cross-regulation of FGF, Bmp, Wnt, and Nodal signaling in fish, there appears to be a striking interdependence of these signaling pathways in mouse. For example, Bmp, Wnt, and Nodal signals maintain each others' expression before the onset of mouse gastrulation (reviewed in 192, 313). Hence, interference with one pathway will affect the activity of the others, leading to more pronounced phenotypes. In fish and frog embryos, these pathways might not only be more independent but even act redundantly, suppressing potentially more severe phenotypes.

We propose that reciprocal signaling manifests itself more prominently in mouse than in fish or frog because of three major differences in the early embryogenesis of these organisms. First, mouse embryos undergo dramatic growth, whereas the volume of fish and frog embryos does not significantly change until organogenesis. In mouse, the need to coordinate growth and patterning may be met by extensive cross-regulatory interactions among various signaling pathways. This cross-regulation might explain the apparently similar phenotypes in mouse embryos that have mutations in different signaling pathways (reviewed in 313).

Second, mouse embryos require extraembryonic tissues for implantation and anterior-posterior patterning (reviewed in 192, 313). These tissues serve as signaling centers, and they are in turn regulated by different signals and their antagonists. For example, the Nodal signaling pathway patterns the extraembryonic (visceral) endoderm and in turn is regulated by extraembryonic ectoderm (reviewed in 192, 269). There is a role for an extraembryonic structure in the fish (the yolk syncytial layer), but it appears to be less important than mouse extraembryonic tissues and does not apparently require reciprocal signals from embryonic signaling centers. Hence, reciprocal signaling appears essential to coordinate embryonic and extraembryonic development in mouse, whereas embryonic development in fish occurs in the context of extraembryonic structures that are largely pre-established during oogenesis.

Third, fish and frog embryos strongly rely on maternal determinants to guide axis formation. The egg is already polarized, zygotic transcription is only initiated after the 500-cell stage, and in frog maternal factors such as Wnt11, β-catenin, VegT, and Ectodermin are required for axis formation (69, 116, 314, 360). In contrast, mammals appear not to rely on localized maternal determinants and initiate zygotic transcription as early as the two-cell stage. There seem to be asymmetries during early cleavages, but they do not necessarily translate into orientations of specific axes (89, 242). Hence, the mouse embryo has to "self-organize," a process likely to require cross-regulatory interactions between patterning signals.

Taken together, these observations suggest that the dramatic growth, the importance of extraembryonic tissues, and the lack of a prepattern in mouse embryos necessitate complex cross-regulatory interactions between tissues and signaling pathways. Interference with one tissue or pathway can thus have dramatic effects on other tissues or pathways. In contrast, fish and frog eggs contain detailed patterning information, embryos grow little, and extraembryonic tissues play minor roles. In this case, the different signaling pathways are less interdependent and can specify distinct tissue types. These developmental differences might also drive the very rapid development of frog and fish embryos compared with

the relatively slow development of mouse embryos.

PERSPECTIVES

After 15 years of zebrafish molecular genetics we have attained a basic outline of the molecular bases of zebrafish axis formation, but many important questions remain. First, many of the key components involved in axis formation are not yet identified. For example, we lack any systematic knowledge of the maternal factors that contribute to vertebrate embryogenesis; RNAi experiments in other systems suggest that many modulators of specific signaling pathways are still unidentified (54); moreover, it is almost completely unknown which genes are regulated by the signals and transcription factors that set up the vertebrate body plan. Maternal or sensitized screens, reverse genetics, RNAi or morpholinos, small molecule inhibitors, and microarray experiments are likely to lead to the isolation of additional factors involved in axis formation. We speculate, however, that it is unlikely that many new signaling pathways required for axis formation will be identified. The genetic screens in zebrafish have by now reached at least 50% saturation and have not isolated any novel signaling pathways required for axis formation, although these screens did define new roles for and new modulators of known pathways (e.g., 102, 141, 348). Similarly, recent misexpression screens in *Xenopus* have not identified novel signaling pathways. It is conceivable that signaling pathways that have more subtle roles [e.g., during gastrulation movements (17, 38, 184)] remain to be discovered, but we predict that most progress will be made in the isolation of maternal upstream factors, signaling modulators, and downstream mediators.

Second, our understanding of the cell biological and molecular bases of vertebrate embryogenesis is still poor. How do signals move through the embryo? How do cells read and respond to these extracellular inputs over time? How are cytoskeletal and adhesive properties changed in response to specific signals? How is chromatin modified as cells become specified? What are the subcellular changes when progenitors become neurons, muscles, blood, and other cell types? The development of probes that allow the in vivo imaging of subcellular processes promises a detailed cell biological and dynamic view of cell movements and differentiation (reviewed in 203). The transparency of the zebrafish makes this organism particularly well suited to address these questions.

Ultimately, we need to understand how all these inputs are integrated into regulatory hierarchies and networks (reviewed in 183). It has become clear that individual cells receive multiple and diverse inputs depending on their position and history, but we are largely ignorant about how these inputs are translated into flexible but ultimately robust outcomes. This knowledge will not only provide a basis to understand human birth defects and guide our efforts in stem cell manipulations but might also uncover the regulatory logic that drives vertebrate embryogenesis.

SUMMARY POINTS

1. A combination of embryological, genetic, and molecular approaches has provided an outline of the molecular basis of zebrafish axis formation.
2. During oogenesis the animal-vegetal axis is specified and dorsal determinants are deposited into the egg.
3. Nodal, FGF, Bmp, Wnt, and retinoic acid signals provide positional information and activate transcription factors that specify cell fates during gastrulation.

4. Planar cell polarity signaling is required for the gastrulation movements of convergence extension.

FUTURE ISSUES

1. Identify additional factors involved in zebrafish axis formation and gastrulation.
2. Develop in vivo probes to study the subcellular and molecular basis of zebrafish embryogenesis.
3. Determine how cells integrate multiple inputs to acquire specific fates and movements.

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LITERATURE CITED

1. Abdelilah S, Solnica-Krezel L, Stainier DY, Driever W. 1994. Implications for dorsoventral axis determination from the zebrafish mutation *janus*. *Nature* 370:468–71
2. Adams RJ, Kimmel CB. 2004. Morphogenetic cellular flows during zebrafish gastrulation. See Ref. 303a, pp. 305–16
3. Agathon A, Thisse B, Thisse C. 2001. Morpholino knock-down of *antivin1* and *antivin2* upregulates nodal signaling. *Genesis* 30:178–82
4. Agathon A, Thisse C, Thisse B. 2003. The molecular nature of the zebrafish tail organizer. *Nature* 424:448–52
5. Alexander J, Rothenberg M, Henry GL, Stainier DY. 1999. *Casanova* plays an early and essential role in endoderm formation in zebrafish. *Dev. Biol.* 215:343–57
6. Alexander J, Stainier DY. 1999. A molecular pathway leading to endoderm formation in zebrafish. *Curr. Biol.* 9:1147–57
7. Amacher SL, Draper BW, Summers BR, Kimmel CB. 2002. The zebrafish T-box genes *no tail* and *spadetail* are required for development of trunk and tail mesoderm and medial floor plate. *Development* 129:3311–23
8. Amacher SL, Kimmel CB. 1998. Promoting notochord fate and repressing muscle development in zebrafish axial mesoderm. *Development* 125:1397–406
9. Ambros V. 2004. The functions of animal microRNAs. *Nature* 431:350–55
10. Aoki TO, David NB, Minchiotti G, Saint-Etienne L, Dickmeis T, et al. 2002. Molecular integration of *casanova* in the Nodal signalling pathway controlling endoderm formation. *Development* 129:275–86
11. Aoki TO, Mathieu J, Saint-Etienne L, Rebagliati MR, Peyrieras N, Rosa FM. 2002. Regulation of nodal signalling and mesendoderm formation by TARAM-A, a TGFβ-related type I receptor. *Dev. Biol.* 241:273–88

12. Appel B, Fritz A, Westerfield M, Grunwald DJ, Eisen JS, Riley BB. 1999. Delta-mediated specification of midline cell fates in zebrafish embryos. *Curr. Biol.* 9:247–56
13. Appel B, Marasco P, McClung LE, Latimer AJ. 2003. *lunatic fringe* regulates Delta-Notch induction of hypochord in zebrafish. *Dev. Dyn.* 228:281–86
14. Babb SG, Marrs JA. 2004. E-cadherin regulates cell movements and tissue formation in early zebrafish embryos. *Dev. Dyn.* 230:263–77
15. Bakkers J, Camacho-Carvajal M, Nowak M, Kramer C, Danger B, Hammerschmidt M. 2005. Destabilization of ΔNp63α by Nedd4-mediated ubiquitination and Ubc9-mediated sumoylation, and its implications on dorsoventral patterning of the zebrafish embryo. *Cell Cycle* 4:790–800
16. Bakkers J, Hild M, Kramer C, Furutani-Seiki M, Hammerschmidt M. 2002. Zebrafish Δ*Np63* is a direct target of Bmp signaling and encodes a transcriptional repressor blocking neural specification in the ventral ectoderm. *Dev. Cell* 2:617–27
17. Bakkers J, Kramer C, Pothof J, Quaedvlieg NE, Spaink HP, Hammerschmidt M. 2004. Has2 is required upstream of Rac1 to govern dorsal migration of lateral cells during zebrafish gastrulation. *Development* 131:525–37
18. Bally-Cuif L, Dubois L, Vincent A. 1998. Molecular cloning of *Zcoe2*, the zebrafish homolog of *Xenopus Xcoe2* and mouse *EBF-2*, and its expression during primary neurogenesis. *Mech. Dev.* 77:85–90
19. Bardet PL, Horard B, Laudet V, Vanacker JM. 2005. The ERRα orphan nuclear receptor controls morphogenetic movements during zebrafish gastrulation. *Dev. Biol.* 281:102–11
20. Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281–97
21. Barth KA, Kishimoto Y, Rohr KB, Seydler C, Schulte-Merker S, Wilson SW. 1999. Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* 126:4977–87
22. Bauer H, Lele Z, Rauch GJ, Geisler R, Hammerschmidt M. 2001. The type I serine/threonine kinase receptor Alk8/Lost-a-fin is required for Bmp2b/7 signal transduction during dorsoventral patterning of the zebrafish embryo. *Development* 128:849–58
23. Baxendale S, Davison C, Muxworthy C, Wolff C, Ingham PW, Roy S. 2004. The B-cell maturation factor Blimp-1 specifies vertebrate slow-twitch muscle fiber identity in response to Hedgehog signaling. *Nat. Genet.* 36:88–93
24. Begemann G, Schilling TF, Rauch GJ, Geisler R, Ingham PW. 2001. The zebrafish *neckless* mutation reveals a requirement for *raldh2* in mesodermal signals that pattern the hindbrain. *Development* 128:3081–94
25. Belting HG, Hauptmann G, Meyer D, Abdelilah-Seyfried S, Chitnis A, et al. 2001. *spiel ohne grenzen/pou2* is required during establishment of the zebrafish midbrain-hindbrain boundary organizer. *Development* 128:4165–76
26. Birely J, Schneider VA, Santana E, Dosch R, Wagner DS, et al. 2005. Genetic screens for genes controlling motor nerve-muscle development and interactions. *Dev. Biol.* 280:162–76
27. Bisgrove BW, Essner JJ, Yost HJ. 1999. Regulation of midline development by antagonism of *lefty* and *nodal* signaling. *Development* 126:3253–62
28. Blader P, Rastegar S, Fischer N, Strähle U. 1997. Cleavage of the BMP-4 antagonist chordin by zebrafish tolloid. *Science* 278:1937–40

29. Blagden CS, Currie PD, Ingham PW, Hughes SM. 1997. Notochord induction of zebrafish slow muscle mediated by Sonic hedgehog. *Genes Dev.* 11:2163–75
30. Brand M, Heisenberg CP, Jiang YJ, Beuchle D, Lun K, et al. 1996. Mutations in zebrafish genes affecting the formation of the boundary between midbrain and hindbrain. *Development* 123:179–90
31. Brand M, Heisenberg CP, Warga RM, Pelegri F, Karlstrom RO, et al. 1996. Mutations affecting development of the midline and general body shape during zebrafish embryogenesis. *Development* 123:129–42
32. Bruce AE, Howley C, Fox MD, Ho RK. 2005. T-box gene *eomesodermin* and the homeobox-containing Mix/Bix gene *mtx2* regulate epiboly movements in the zebrafish. *Dev. Dyn.* 233:105–14
33. Bruce AE, Howley C, Zhou Y, Vickers SL, Silver LM, et al. 2003. The maternally expressed zebrafish T-box gene *eomesodermin* regulates organizer formation. *Development* 130:5503–17
34. Burgess S, Reim G, Chen W, Hopkins N, Brand M. 2002. The zebrafish *spiel-ohne-grenzen (spg)* gene encodes the POU domain protein Pou2 related to mammalian Oct4 and is essential for formation of the midbrain and hindbrain, and for pre-gastrula morphogenesis. *Development* 129:905–16
35. Cao Y, Zhao J, Sun Z, Zhao Z, Postlethwait J, Meng A. 2004. *fgf17b*, a novel member of Fgf family, helps patterning zebrafish embryos. *Dev. Biol.* 271:130–43
36. Carmany-Rampey A, Schier AF. 2001. Single-cell internalization during zebrafish gastrulation. *Curr. Biol.* 11:1261–65
37. Carreira-Barbosa F, Concha ML, Takeuchi M, Ueno N, Wilson SW, Tada M. 2003. Prickle 1 regulates cell movements during gastrulation and neuronal migration in zebrafish. *Development* 130:4037–46
38. Cha YI, Kim SH, Solnica-Krezel L, Dubois RN. 2005. Cyclooxygenase-1 signaling is required for vascular tube formation during development. *Dev. Biol.* 282:274–83
39. Chan J, Mably JD, Serluca FC, Chen JN, Goldstein NB, et al. 2001. Morphogenesis of prechordal plate and notochord requires intact Eph/ephrin B signaling. *Dev. Biol.* 234:470–82
40. Chang C, Holtzman DA, Chau S, Chickering T, Woolf EA, et al. 2001. Twisted gastrulation can function as a BMP antagonist. *Nature* 410:483–87
41. Chen C, Shen MM. 2004. Two modes by which Lefty proteins inhibit nodal signaling. *Curr. Biol.* 14:618–24
42. Chen JN, Haffter P, Odenthal J, Vogelsang E, Brand M, et al. 1996. Mutations affecting the cardiovascular system and other internal organs in zebrafish. *Development* 123:293–302
43. Chen JN, van Eeden FJ, Warren KS, Chin A, Nusslein-Volhard C, et al. 1997. Left-right pattern of cardiac *BMP4* may drive asymmetry of the heart in zebrafish. *Development* 124:4373–82
44. Chen S, Kimelman D. 2000. The role of the yolk syncytial layer in germ layer patterning in zebrafish. *Development* 127:4681–89
45. Chen Y, Schier AF. 2001. The zebrafish Nodal signal squint functions as a morphogen. *Nature* 411:607–10
46. Chen Y, Schier AF. 2002. Lefty proteins are long-range inhibitors of squint-mediated nodal signaling. *Curr. Biol.* 12:2124–28
47. Cheng JC, Miller AL, Webb SE. 2004. Organization and function of microfilaments during late epiboly in zebrafish embryos. *Dev. Dyn.* 231:313–23

In vivo demonstration that a Nodal signal can act at a long range.

48. Cheng SK, Olale F, Brivanlou AH, Schier AF. 2004. Lefty blocks a subset of TGFβ signals by antagonizing EGF-CFC coreceptors. *PLoS Biol.* 2:e30
49. Connors SA, Trout J, Ekker M, Mullins MC. 1999. The role of *tolloid/mini fin* in dorsoventral pattern formation of the zebrafish embryo. *Development* 126:3119–30
50. Cooper MS, D'Amico LA. 1996. A cluster of noninvoluting endocytic cells at the margin of the zebrafish blastoderm marks the site of embryonic shield formation. *Dev. Biol.* 180:184–98
51. Daggett DF, Boyd CA, Gautier P, Bryson-Richardson RJ, Thisse C, et al. 2004. Developmentally restricted actin-regulatory molecules control morphogenetic cell movements in the zebrafish gastrula. *Curr. Biol.* 14:1632–38
52. Dale L, Howes G, Price BM, Smith JC. 1992. Bone morphogenetic protein 4: a ventralizing factor in early *Xenopus* development. *Development* 115:573–85
53. D'Amico LA, Cooper MS. 2001. Morphogenetic domains in the yolk syncytial layer of axiating zebrafish embryos. *Dev. Dyn.* 222:611–24
54. DasGupta R, Kaykas A, Moon RT, Perrimon N. 2005. Functional genomic analysis of the Wnt-Wingless signaling pathway. *Science* 308:826–33
55. David NB, Rosa FM. 2001. Cell autonomous commitment to an endodermal fate and behaviour by activation of Nodal signalling. *Development* 128:3937–47
56. Davidson AJ, Ernst P, Wang Y, Dekens MP, Kingsley PD, et al. 2003. *cdx4* mutants fail to specify blood progenitors and can be rescued by multiple *hox* genes. *Nature* 425:300–6
57. Davidson AJ, Zon LI. 2004. The 'definitive' (and 'primitive') guide to zebrafish hematopoiesis. *Oncogene* 23:7233–46
58. Dekens MP, Pelegri FJ, Maischein HM, Nusslein-Volhard C. 2003. The maternal-effect gene *futile cycle* is essential for pronuclear congression and mitotic spindle assembly in the zebrafish zygote. *Development* 130:3907–16
59. De Robertis EM, Kuroda H. 2004. Dorsal-ventral patterning and neural induction in *Xenopus* embryos. *Annu. Rev. Cell. Dev. Biol.* 20:285–308
60. Dick A, Hild M, Bauer H, Imai Y, Maifeld H, et al. 2000. Essential role of Bmp7 (*snail-house*) and its prodomain in dorsoventral patterning of the zebrafish embryo. *Development* 127:343–54
61. Dickmeis T, Mourrain P, Saint-Etienne L, Fischer N, Aanstad P, et al. 2001. A crucial component of the endoderm formation pathway, CASANOVA, is encoded by a novel *sox*-related gene. *Genes Dev.* 15:1487–92
62. Dorsky RI, Itoh M, Moon RT, Chitnis A. 2003. Two *tcf3* genes cooperate to pattern the zebrafish brain. *Development* 130:1937–47
63. Dorsky RI, Sheldahl LC, Moon RT. 2002. A transgenic Lef1/β-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. *Dev. Biol.* 241:229–37
64. Dosch R, Gawantka V, Delius H, Blumenstock C, Niehrs C. 1997. Bmp-4 acts as a morphogen in dorsoventral mesoderm patterning in *Xenopus*. *Development* 124:2325–34
65. Dosch R, Wagner DS, Mintzer KA, Runke G, Wiemelt AP, Mullins MC. 2004. Maternal control of vertebrate development before the midblastula transition: mutants from the zebrafish I. *Dev. Cell* 6:771–80
66. Dougan ST, Warga RM, Kane DA, Schier AF, Talbot WS. 2003. The role of the zebrafish *nodal*-related genes *squint* and *cyclops* in patterning of mesendoderm. *Development* 130:1837–51

67. Draper BW, Stock DW, Kimmel CB. 2003. Zebrafish *fgf24* functions with *fgf8* to promote posterior mesodermal development. *Development* 130:4639–54

Summary of the large-scale Boston screen for zebrafish mutants.

68. Driever W, Solnica-Krezel L, Schier AF, Neuhauss SC, Malicki J, et al. 1996. A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* 123:37–46

69. Dupont S, Zacchigna L, Cordenonsi M, Soligo S, Adorno M, et al. 2005. Germ-layer specification and control of cell growth by Ectodermin, a Smad4 ubiquitin ligase. *Cell* 121:87–99

70. Emoto Y, Wada H, Okamoto H, Kudo A, Imai Y. 2005. Retinoic acid-metabolizing enzyme Cyp26a1 is essential for determining territories of hindbrain and spinal cord in zebrafish. *Dev. Biol.* 278:415–27

71. Erter CE, Solnica-Krezel L, Wright CV. 1998. *Zebrafish nodal-related 2* encodes an early mesendodermal inducer signaling from the extraembryonic yolk syncytial layer. *Dev. Biol.* 204:361–72

72. 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* 128:3571–83

73. Essner JJ, Amack JD, Nyholm MK, Harris EB, Yost HJ. 2005. Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. *Development* 132:1247–60

74. Essner JJ, Vogan KJ, Wagner MK, Tabin CJ, Yost HJ, Brueckner M. 2002. Conserved function for embryonic nodal cilia. *Nature* 418:37–38

75. Fekany K, Yamanaka Y, Leung T, Sirotkin HI, Topczewski J, et al. 1999. The zebrafish *bozozok* locus encodes Dharma, a homeodomain protein essential for induction of gastrula organizer and dorsoanterior embryonic structures. *Development* 126:1427–38

76. Fekany-Lee K, Gonzalez E, Miller-Bertoglio V, Solnica-Krezel L. 2000. The homeobox gene *bozozok* promotes anterior neuroectoderm formation in zebrafish through negative regulation of BMP2/4 and Wnt pathways. *Development* 127:2333–45

77. Feldman B, Concha ML, Saude L, Parsons MJ, Adams RJ, et al. 2002. Lefty antagonism of Squint is essential for normal gastrulation. *Curr. Biol.* 12:2129–35

78. Feldman B, Dougan ST, Schier AF, Talbot WS. 2000. Nodal-related signals establish mesendodermal fate and trunk neural identity in zebrafish. *Curr. Biol.* 10:531–34

Discovery that Nodal signals are essential for mesoderm and endoderm induction in zebrafish.

79. Feldman B, Gates MA, Egan ES, Dougan ST, Rennebeck G, et al. 1998. Zebrafish organizer development and germ-layer formation require nodal-related signals. *Nature* 395:181–85

80. Ferreiro B, Artinger M, Cho K, Niehrs C. 1998. Antimorphic goosecoids. *Development* 125:1347–59

81. Fisher S, Amacher SL, Halpern ME. 1997. Loss of *cerebum* function ventralizes the zebrafish embryo. *Development* 124:1301–11

82. Formstone CJ, Mason I. 2005. Combinatorial activity of Flamingo proteins directs convergence and extension within the early zebrafish embryo via the planar cell polarity pathway. *Dev. Biol.* 282:320–35

83. Fujii R, Yamashita S, Hibi M, Hirano T. 2000. Asymmetric p38 activation in zebrafish: its possible role in symmetric and synchronous cleavage. *J. Cell Biol.* 150:1335–48

84. Furthauer M, Lin W, Ang SL, Thisse B, Thisse C. 2002. Sef is a feedback-induced antagonist of Ras/MAPK-mediated FGF signalling. *Nat. Cell Biol.* 4:170–74

85. Furthauer M, Reifers F, Brand M, Thisse B, Thisse C. 2001. *sprouty4* acts in vivo as a feedback-induced antagonist of FGF signaling in zebrafish. *Development* 128:2175–86

86. Furthauer M, Thisse C, Thisse B. 1997. A role for FGF-8 in the dorsoventral patterning of the zebrafish gastrula. *Development* 124:4253–64
87. Furthauer M, Van Celst J, Thisse C, Thisse B. 2004. Fgf signalling controls the dorsoventral patterning of the zebrafish embryo. *Development* 131:2853–64
88. Gaiano N, Amsterdam A, Kawakami K, Allende M, Becker T, Hopkins N. 1996. Insertional mutagenesis and rapid cloning of essential genes in zebrafish. *Nature* 383:829–32
89. Gardner RL, Davies TJ. 2003. The basis and significance of pre-patterning in mammals. *Philos. Trans. R. Soc. London Ser. B* 358:1331–38; discussion 8–9
90. Gates MA, Kim L, Egan ES, Cardozo T, Sirotkin HI, et al. 1999. A genetic linkage map for zebrafish: comparative analysis and localization of genes and expressed sequences. *Genome Res.* 9:334–47
91. Geisler R, Rauch GJ, Baier H, van Bebber F, Bross L, et al. 1999. A radiation hybrid map of the zebrafish genome. *Nat. Genet.* 23:86–89
92. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, et al. 2005. MicroRNAs regulate brain morphogenesis in zebrafish. *Science* 308:833–38
93. Glickman NS, Kimmel CB, Jones MA, Adams RJ. 2003. Shaping the zebrafish notochord. *Development* 130:873–87
94. Golling G, Amsterdam A, Sun Z, Antonelli M, Maldonado E, et al. 2002. Insertional mutagenesis in zebrafish rapidly identifies genes essential for early vertebrate development. *Nat. Genet.* 31:135–40
95. Gong Y, Mo C, Fraser SE. 2004. Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. *Nature* 430:689–93
96. Gonzalez EM, Fekany-Lee K, Carmany-Rampey A, Erter C, Topczewski J, et al. 2000. Head and trunk in zebrafish arise via coinhibition of BMP signaling by *bozozok* and *chordino*. *Genes Dev.* 14:3087–92
97. Grandel H, Lun K, Rauch GJ, Rhinn M, Piotrowski T, et al. 2002. Retinoic acid signalling in the zebrafish embryo is necessary during pre-segmentation stages to pattern the anterior-posterior axis of the CNS and to induce a pectoral fin bud. *Development* 129:2851–65
98. Griffin K, Patient R, Holder N. 1995. Analysis of FGF function in normal and *no tail* zebrafish embryos reveals separate mechanisms for formation of the trunk and the tail. *Development* 121:2983–94
99. Griffin KJ, Amacher SL, Kimmel CB, Kimelman D. 1998. Molecular identification of *spadetail*: regulation of zebrafish trunk and tail mesoderm formation by T-box genes. *Development* 125:3379–88
100. Griffin KJ, Kimelman D. 2003. Interplay between FGF, *one-eyed pinhead*, and T-box transcription factors during zebrafish posterior development. *Dev. Biol.* 264:456–66
101. Gritsman K, Talbot WS, Schier AF. 2000. Nodal signaling patterns the organizer. *Development* 127:921–32
102. Gritsman K, Zhang J, Cheng S, Heckscher E, Talbot WS, Schier AF. 1999. The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. *Cell* 97:121–32
103. Haegel H, Larue L, Ohsugi M, Fedorov L, Herrenknecht K, Kemler R. 1995. Lack of β-catenin affects mouse development at gastrulation. *Development* 121:3529–37
104. Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, et al. 1996. The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development* 123:1–36

Together with (361) discovery of EGF-CFC proteins as extracellular factors required for Nodal signaling.

Summary of the large-scale Tuebingen screen for zebrafish mutants.

105. Halpern ME, Hatta K, Amacher SL, Talbot WS, Yan YL, et al. 1997. Genetic interactions in zebrafish midline development. *Dev. Biol.* 187:154–70
106. Halpern ME, Ho RK, Walker C, Kimmel CB. 1993. Induction of muscle pioneers and floor plate is distinguished by the zebrafish *no tail* mutation. *Cell* 75:99–111
107. Halpern ME, Thisse C, Ho RK, Thisse B, Riggleman B, et al. 1995. Cell-autonomous shift from axial to paraxial mesodermal development in zebrafish *floating head* mutants. *Development* 121:4257–64
108. Hammerschmidt M, Pelegri F, Mullins MC, Kane DA, Brand M, et al. 1996. Mutations affecting morphogenesis during gastrulation and tail formation in the zebrafish, *Danio rerio*. *Development* 123:143–51
109. Hammerschmidt M, Pelegri F, Mullins MC, Kane DA, van Eeden FJ, et al. 1996. *dino* and *mercedes*, two genes regulating dorsal development in the zebrafish embryo. *Development* 123:95–102
110. Hammerschmidt M, Serbedzija GN, McMahon AP. 1996. Genetic analysis of dorsoventral pattern formation in the zebrafish: requirement of a BMP-like ventralizing activity and its dorsal repressor. *Genes Dev.* 10:2452–61
111. Hannus M, Feiguin F, Heisenberg CP, Eaton S. 2002. Planar cell polarization requires Widerborst, a B′ regulatory subunit of protein phosphatase 2A. *Development* 129:3493–503
112. Harland R, Gerhart J. 1997. Formation and function of Spemann's organizer. *Annu. Rev. Cell. Dev. Biol.* 13:611–67
113. Hashimoto H, Itoh M, Yamanaka Y, Yamashita S, Shimizu T, et al. 2000. Zebrafish Dkk1 functions in forebrain specification and axial mesendoderm formation. *Dev. Biol.* 217:138–52
114. Hashimoto H, Rebagliati M, Ahmad N, Muraoka O, Kurokawa T, et al. 2004. The Cerberus/Dan-family protein Charon is a negative regulator of Nodal signaling during left-right patterning in zebrafish. *Development* 131:1741–53
115. Hatta K, Kimmel CB, Ho RK, Walker C. 1991. The *cyclops* mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* 350:339–41
116. Heasman J, Crawford A, Goldstone K, Garner-Hamrick P, Gumbiner B, et al. 1994. Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell* 79:791–803
117. Heisenberg CP, Houart C, Take-Uchi M, Rauch GJ, Young N, 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.* 15:1427–34
118. Heisenberg CP, Nusslein-Volhard C. 1997. The function of *silberblick* in the positioning of the eye anlage in the zebrafish embryo. *Dev. Biol.* 184:85–94
119. Heisenberg CP, Tada M, Rauch GJ, Saude L, Concha ML, et al. 2000. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405:76–81
120. Helde KA, Wilson ET, Cretekos CJ, Grunwald DJ. 1994. Contribution of early cells to the fate map of the zebrafish gastrula. *Science* 265:517–20
121. Hemmati-Brivanlou A, Melton D. 1997. Vertebrate embryonic cells will become nerve cells unless told otherwise. *Cell* 88:13–17
122. Hernandez RE, Rikhof HA, Bachmann R, Moens CB. 2004. *vhnf1* integrates global RA patterning and local FGF signals to direct posterior hindbrain development in zebrafish. *Development* 131:4511–20

Discovery that non-canonical Wnt signaling is required for proper gastrulation movements.

123. Hernandez-Lagunas L, Choi IF, Kaji T, Simpson P, Hershey C, et al. 2005. Zebrafish *narrowminded* disrupts the transcription factor *prdm1* and is required for neural crest and sensory neuron specification. *Dev. Biol.* 278:347–57
124. Hild M, Dick A, Rauch GJ, Meier A, Bouwmeester T, et al. 1999. The *smad5* mutation *somitabun* blocks Bmp2b signaling during early dorsoventral patterning of the zebrafish embryo. *Development* 126:2149–59
125. Ho RK, Kane DA. 1990. Cell-autonomous action of zebrafish *spt-1* mutation in specific mesodermal precursors. *Nature* 348:728–30
126. Ho RK, Kimmel CB. 1993. Commitment of cell fate in the early zebrafish embryo. *Science* 261:109–11
127. Houart C, Caneparo L, Heisenberg C, Barth K, Take-Uchi M, Wilson S. 2002. Establishment of the telencephalon during gastrulation by local antagonism of Wnt signaling. *Neuron* 35:255–65
128. Houart C, Westerfield M, Wilson SW. 1998. A small population of anterior cells patterns the forebrain during zebrafish gastrulation. *Nature* 391:788–92
129. Houston DW, Wylie C. 2004. The role of Wnts in gastrulation. See Ref. 303a, pp. 521–38
130. Hukriede NA, Joly L, Tsang M, Miles J, Tellis P, et al. 1999. Radiation hybrid mapping of the zebrafish genome. *Proc. Natl. Acad. Sci. USA* 96:9745–50
131. 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* 128:2407–20
132. Jessen JR, Topczewski J, Bingham S, Sepich DS, Marlow F, et al. 2002. Zebrafish *trilobite* identifies new roles for Strabismus in gastrulation and neuronal movements. *Nat. Cell Biol.* 4:610–15
133. Jesuthasan S, Stahle U. 1997. Dynamic microtubules and specification of the zebrafish embryonic axis. *Curr. Biol.* 7:31–42
134. Jopling C, den Hertog J. 2005. Fyn/Yes and non-canonical Wnt signalling converge on RhoA in vertebrate gastrulation cell movements. *EMBO Rep.* 6:426–31
135. Kane DA, Hammerschmidt M, Mullins MC, Maischein HM, Brand M, et al. 1996. The zebrafish epiboly mutants. *Development* 123:47–55
136. Kane DA, Kimmel CB. 1993. The zebrafish midblastula transition. *Development* 119:447–56
137. Kane DA, McFarland KN, Warga RM. 2005. Mutations in *half baked*/E-cadherin block cell behaviors that are necessary for teleost epiboly. *Development* 132:1105–16
138. Kane DA, Warga RM. 2004. Teleost gastrulation. See Ref. 303a, pp. 157–70
139. Kawahara A, Wilm T, Solnica-Krezel L, Dawid IB. 2000. Antagonistic role of *vega1* and *bozozok/dharma* homeobox genes in organizer formation. *Proc. Natl. Acad. Sci. USA* 97:12121–26
140. Kawahara A, Wilm T, Solnica-Krezel L, Dawid IB. 2000. Functional interaction of *vega2* and *goosecoid* homeobox genes in zebrafish. *Genesis* 28:58–67
141. Kawakami A, Nojima Y, Toyoda A, Takahoko M, Satoh M, et al. 2005. The zebrafish-secreted matrix protein You/Scube2 is implicated in long-range regulation of hedgehog signaling. *Curr. Biol.* 15:480–88
142. Kawakami Y, Raya A, Raya RM, Rodriguez-Esteban C, Belmonte JC. 2005. Retinoic acid signalling links left-right asymmetric patterning and bilaterally symmetric somitogenesis in the zebrafish embryo. *Nature* 435:165–71

143. Kawano Y, Kypta R. 2003. Secreted antagonists of the Wnt signalling pathway. *J. Cell Sci.* 116:2627–34
144. Keegan BR, Feldman JL, Begemann G, Ingham PW, Yelon D. 2005. Retinoic acid signaling restricts the cardiac progenitor pool. *Science* 307:247–49
145. Keegan BR, Meyer D, Yelon D. 2004. Organization of cardiac chamber progenitors in the zebrafish blastula. *Development* 131:3081–91
146. Keller R. 2002. Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* 298:1950–54
147. 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* 127:3899–911
148. Kelly GM, Erezyilmaz DF, Moon RT. 1995. Induction of a secondary embryonic axis in zebrafish occurs following the overexpression of β-catenin. *Mech. Dev.* 53:261–73
149. Kelly GM, Greenstein P, Erezyilmaz DF, Moon RT. 1995. Zebrafish *wnt8* and *wnt8b* share a common activity but are involved in distinct developmental pathways. *Development* 121:1787–99
150. Kikuchi Y, Agathon A, Alexander J, Thisse C, Waldron S, et al. 2001. *casanova* encodes a novel Sox-related protein necessary and sufficient for early endoderm formation in zebrafish. *Genes Dev.* 15:1493–505
151. Kikuchi Y, Trinh LA, Reiter JF, Alexander J, Yelon D, Stainier DY. 2000. The zebrafish *bonnie and clyde* gene encodes a Mix family homeodomain protein that regulates the generation of endodermal precursors. *Genes Dev.* 14:1279–89
152. 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.* 120:467–76
153. Kim CH, Oda T, Itoh M, Jiang D, Artinger KB, et al. 2000. Repressor activity of Headless/Tcf3 is essential for vertebrate head formation. *Nature* 407:913–16
154. Kimmel CB. 1989. Genetics and early development of zebrafish. *Trends. Genet.* 5:283–88
155. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. 1995. Stages of embryonic development of the zebrafish. *Dev. Dyn.* 203:253–310
156. Kimmel CB, Kane DA, Walker C, Warga RM, Rothman MB. 1989. A mutation that changes cell movement and cell fate in the zebrafish embryo. *Nature* 337:358–62
157. Kimmel CB, Law RD. 1985. Cell lineage of zebrafish blastomeres. II. Formation of the yolk syncytial layer. *Dev. Biol.* 108:86–93
158. Kimmel CB, Warga RM. 1986. Tissue specific cell lineages originate in the gastrula of the zebrafish. *Science* 231:356–68
159. Kimmel CB, Warga RM. 1987. Cell lineages generating axial muscle in the zebrafish embryo. *Nature* 327:234–37
160. Kimmel CB, Warga RM. 1987. Indeterminate cell lineage of the zebrafish embryo. *Dev. Biol.* 124:269–80
161. Kimmel CB, Warga RM, Schilling TF. 1990. Origin and organization of the zebrafish fate map. *Development* 108:581–94
162. Kishimoto Y, Lee KH, Zon L, Hammerschmidt M, Schulte-Merker S. 1997. The molecular nature of zebrafish *swirl*: BMP2 function is essential during early dorsoventral patterning. *Development* 124:4457–66

This paper spelled out the experimental advantages and potential of the zebrafish as a vertebrate model system, inspiring other investigators to join the zebrafish field.

First description and analysis of a zebrafish mutant affecting early embryogenesis.

First systematic fate map of the zebrafish blastula.

163. Klein TJ, Mlodzik M. 2005. Planar cell polarization: An emerging model points in the right direction. *Annu. Rev. Cell. Dev. Biol.* 21:155–76
164. Knapik EW, Goodman A, Ekker M, Chevrette M, Delgado J, et al. 1998. A microsatellite genetic linkage map for zebrafish (*Danio rerio*). *Nat. Genet.* 18:338–43
165. Koos DS, Ho RK. 1998. The *nieuwkoid* gene characterizes and mediates a Nieuwkoop-center-like activity in the zebrafish. *Curr. Biol.* 8:1199–206
166. Koos DS, Ho RK. 1999. The *nieuwkoid/dharma* homeobox gene is essential for *bmp2b* repression in the zebrafish pregastrula. *Dev. Biol.* 215:190–207
167. Koshida S, Shinya M, Nikaido M, Ueno N, Schulte-Merker S, et al. 2002. Inhibition of BMP activity by the FGF signal promotes posterior neural development in zebrafish. *Dev. Biol.* 244:9–20
168. Kramer C, Mayr T, Nowak M, Schumacher J, Runke G, et al. 2002. Maternally supplied Smad5 is required for ventral specification in zebrafish embryos prior to zygotic Bmp signaling. *Dev. Biol.* 250:263–79
169. Kramer-Zucker AG, Olale F, Haycraft CJ, Yoder BK, Schier AF, Drummond IA. 2005. Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. *Development* 132:1907–21
169a. Krauss S, Concordet JP, Ingham PW. 1993. A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. *Cell* 75:1431–44
170. 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* 131:3581–92
171. Kudoh T, Dawid IB. 2001. Role of the *iroquois3* homeobox gene in organizer formation. *Proc. Natl. Acad. Sci. USA* 98:7852–57
172. Kudoh T, Wilson SW, Dawid IB. 2002. Distinct roles for Fgf, Wnt and retinoic acid in posteriorizing the neural ectoderm. *Development* 129:4335–46
173. Kunwar PS, Zimmerman S, Bennett JT, Chen Y, Whitman M, Schier AF. 2003. Mixer/Bon and FoxH1/Sur have overlapping and divergent roles in Nodal signaling and mesendoderm induction. *Development* 130:5589–99
174. Larrain J, Oelgeschlager M, Ketpura NI, Reversade B, Zakin L, De Robertis EM. 2001. Proteolytic cleavage of Chordin as a switch for the dual activities of Twisted gastrulation in BMP signaling. *Development* 128:4439–47
175. Latimer AJ, Dong X, Markov Y, Appel B. 2002. Delta-Notch signaling induces hypochord development in zebrafish. *Development* 129:2555–63
176. Latimer AJ, Shin J, Appel B. 2005. *her9* promotes floor plate development in zebrafish. *Dev. Dyn.* 232:1098–104
176a. Lecuit T, Brook WJ, Ng M, Calleja M, Sun H, Cohen SM. 1996. Two distinct mechanisms for long-range patterning by Decapentaplegic in the *Drosophila* wing. *Nature* 381:387–93
177. Lee H, Kimelman D. 2002. A dominant-negative form of p63 is required for epidermal proliferation in zebrafish. *Dev. Cell* 2:607–16
178. Le Good JA, Joubin K, Giraldez AJ, Ben-Haim N, Beck S, et al. 2005. Nodal stability determines signaling range. *Curr. Biol.* 15:31–36
179. 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* 1:103–14
180. Lele Z, Nowak M, Hammerschmidt M. 2001. Zebrafish *admp* is required to restrict

Discovery of the inducing activity of hedgehog in vertebrates.

the size of the organizer and to promote posterior and ventral development. *Dev. Dyn.* 222:681–87
181. Leptin M. 2005. Gastrulation movements: the logic and the nuts and bolts. *Dev. Cell* 8:305–20
182. Leung T, Bischof J, Soll I, Niessing D, Zhang D, et al. 2003. *bozozok* directly represses *bmp2b* transcription and mediates the earliest dorsoventral asymmetry of *bmp2b* expression in zebrafish. *Development* 130:3639–49
183. Levine M, Davidson EH. 2005. Gene regulatory networks for development. *Proc. Natl. Acad. Sci. USA* 102:4936–42
184. Lin F, Sepich DS, Chen S, Topczewski J, Yin C, et al. 2005. Essential roles of Gα12/13 signaling in distinct cell behaviors driving zebrafish convergence and extension gastrulation movements. *J. Cell Biol.* 169:777–87
185. Linville A, Gumusaneli E, Chandraratna RA, Schilling TF. 2004. Independent roles for retinoic acid in segmentation and neuronal differentiation in the zebrafish hindbrain. *Dev. Biol.* 270:186–99
186. Little SC, Mullins MC. 2004. Twisted gastrulation promotes BMP signaling in zebrafish dorsal-ventral axial patterning. *Development* 131:5825–35
187. Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. 1999. Requirement for *Wnt3* in vertebrate axis formation. *Nat. Genet.* 22:361–65
188. Logan CY, Nusse R. 2004. The Wnt signaling pathway in development and disease. *Annu. Rev. Cell. Dev. Biol.* 20:781–810
189. Londin ER, Niemiec J, Sirotkin HI. 2005. Chordin, FGF signaling, and mesodermal factors cooperate in zebrafish neural induction. *Dev. Biol.* 279:1–19
190. Long Q, Meng A, Wang H, Jessen JR, Farrell MJ, Lin S. 1997. *GATA-1* expression pattern can be recapitulated in living transgenic zebrafish using GFP reporter gene. *Development* 124:4105–11
191. Long S, Ahmad N, Rebagliati M. 2003. The zebrafish *nodal*-related gene *southpaw* is required for visceral and diencephalic left-right asymmetry. *Development* 130:2303–16
192. Lu CC, Brennan J, Robertson EJ. 2001. From fertilization to gastrulation: axis formation in the mouse embryo. *Curr. Opin. Genet. Dev.* 11:384–92
193. Lunde K, Belting HG, Driever W. 2004. Zebrafish *pou5f1/pou2*, homolog of mammalian *Oct4*, functions in the endoderm specification cascade. *Curr. Biol.* 14:48–55
194. Lyons DA, Pogoda HM, Voas MG, Woods IG, Diamond B, et al. 2005. *erbb3* and *erbb2* are essential for schwann cell migration and myelination in zebrafish. *Curr. Biol.* 15:513–24
195. Maegawa S, Yasuda K, Inoue K. 1999. Maternal mRNA localization of zebrafish *DAZ*-like gene. *Mech. Dev.* 81:223–26
196. Marlow F, Gonzalez EM, Yin C, Rojo C, Solnica-Krezel L. 2004. No tail co-operates with non-canonical Wnt signaling to regulate posterior body morphogenesis in zebrafish. *Development* 131:203–16
197. Marlow F, Topczewski J, Sepich D, Solnica-Krezel L. 2002. Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr. Biol.* 12:876–84
198. Martinez-Barbera JP, Toresson H, Da Rocha S, Krauss S. 1997. Cloning and expression of three members of the zebrafish Bmp family: *Bmp2a*, *Bmp2b* and *Bmp4*. *Gene* 198:53–59
199. Martyn U, Schulte-Merker S. 2003. The ventralized *ogon* mutant phenotype is caused

by a mutation in the zebrafish homologue of Sizzled, a secreted Frizzled-related protein. *Dev. Biol.* 260:58–67
200. Mathieu J, Griffin K, Herbomel P, Dickmeis T, Strähle U, et al. 2004. Nodal and Fgf pathways interact through a positive regulatory loop and synergize to maintain mesodermal cell populations. *Development* 131:629–41
201. Matsui T, Raya A, Kawakami Y, Callol-Massot C, Capdevila J, et al. 2005. Noncanonical Wnt signaling regulates midline convergence of organ primordia during zebrafish development. *Genes Dev.* 19:164–75
202. McFarland KN, Warga RM, Kane DA. 2005. Genetic locus *half baked* is necessary for morphogenesis of the ectoderm. *Dev. Dyn.* 233:390–406
203. Megason SG, Fraser SE. 2003. Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development. *Mech. Dev.* 120:1407–20
204. Melby AE, Beach C, Mullins M, Kimelman D. 2000. Patterning the early zebrafish by the opposing actions of *bozozok* and *vox/vent*. *Dev. Biol.* 224:275–85
205. Melby AE, Kimelman D, Kimmel CB. 1997. Spatial regulation of *floating head* expression in the developing notochord. *Dev. Dyn.* 209:156–65
206. Melby AE, Warga RM, Kimmel CB. 1996. Specification of cell fates at the dorsal margin of the zebrafish gastrula. *Development* 122:2225–37
207. Meno C, Gritsman K, Ohishi S, Ohfuji Y, Heckscher E, et al. 1999. Mouse Lefty2 and zebrafish antivin are feedback inhibitors of nodal signaling during vertebrate gastrulation. *Mol. Cell.* 4:287–98
208. Miller-Bertoglio V, Carmany-Rampey A, Furthauer M, Gonzalez EM, Thisse C, et al. 1999. Maternal and zygotic activity of the zebrafish *ogon* locus antagonizes BMP signaling. *Dev. Biol.* 214:72–86
209. Mintzer KA, Lee MA, Runke G, Trout J, Whitman M, Mullins MC. 2001. *Lost-a-fin* encodes a type I BMP receptor, Alk8, acting maternally and zygotically in dorsoventral pattern formation. *Development* 128:859–69
210. Mishina Y, Crombie R, Bradley A, Behringer RR. 1999. Multiple roles for activin-like kinase-2 signaling during mouse embryogenesis. *Dev. Biol.* 213:314–26
211. Miyagi C, Yamashita S, Ohba Y, Yoshizaki H, Matsuda M, Hirano T. 2004. STAT3 noncell-autonomously controls planar cell polarity during zebrafish convergence and extension. *J. Cell Biol.* 166:975–81
212. Mizuno T, Yamaha E, Kuroiwa A, Takeda H. 1999. Removal of vegetal yolk causes dorsal deficencies and impairs dorsal-inducing ability of the yolk cell in zebrafish. *Mech. Dev.* 81:51–63
213. Mizuno T, Yamaha E, Wakahara M, Kuroiwa A, Takeda H. 1996. Mesoderm induction in zebrafish. *Nature* 383:131–32
214. Montero JA, Carvalho L, Wilsch-Brauninger M, Kilian B, Mustafa C, Heisenberg CP. 2005. Shield formation at the onset of zebrafish gastrulation. *Development* 132:1187–98
215. Montero JA, Heisenberg CP. 2004. Gastrulation dynamics: cells move into focus. *Trends Cell Biol.* 14:620–27
216. Montero JA, Kilian B, Chan J, Bayliss PE, Heisenberg CP. 2003. Phosphoinositide 3-kinase is required for process outgrowth and cell polarization of gastrulating mesendodermal cells. *Curr. Biol.* 13:1279–89
217. Muller F, Albert S, Blader P, Fischer N, Hallonet M, Strähle U. 2000. Direct action of the nodal-related signal cyclops in induction of *sonic hedgehog* in the ventral midline of the CNS. *Development* 127:3889–97

218. Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, Brand M, et al. 1996. Genes establishing dorsoventral pattern formation in the zebrafish embryo: the ventral specifying genes. *Development* 123:81–93
219. Munoz-Sanjuan I, Brivanlou AH. 2004. Modulation of BMP signaling during vertebrate gastrulation. See Ref. 303a, pp. 475–90
220. Muraoka O, Ichikawa H, Shi H, Okumura S, Taira E, et al. 2000. Kheper, a novel ZFH/δEF1 family member, regulates the development of the neuroectoderm of zebrafish (*Danio rerio*). *Dev. Biol.* 228:29–40
221. Myers DC, Sepich DS, Solnica-Krezel L. 2002. Bmp activity gradient regulates convergent extension during zebrafish gastrulation. *Dev. Biol.* 243:81–98
222. Myers DC, Sepich DS, Solnica-Krezel L. 2002. Convergence and extension in vertebrate gastrulae: cell movements according to or in search of identity? *Trends Genet*. 18:447–55
223. Nasevicius A, Ekker SC. 2000. Effective targeted gene 'knockdown' in zebrafish. *Nat. Genet.* 26:216–20
224. Neave B, Holder N, Patient R. 1997. A graded response to BMP-4 spatially coordinates patterning of the mesoderm and ectoderm in the zebrafish. *Mech. Dev.* 62:183–95
225. Nellen D, Burke R, Struhl G, Basler K. 1996. Direct and long-range action of a DPP morphogen gradient. *Cell* 85:357–68
226. Nguyen VH, Schmid B, Trout J, Connors SA, Ekker M, Mullins MC. 1998. Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a *bmp2b/swirl* pathway of genes. *Dev. Biol.* 199:93–110
227. Nikaido M, Tada M, Saji T, Ueno N. 1997. Conservation of BMP signaling in zebrafish mesoderm patterning. *Mech. Dev.* 61:75–88
228. Nojima H, Shimizu T, Kim CH, Yabe T, Bae YK, et al. 2004. Genetic evidence for involvement of maternally derived Wnt canonical signaling in dorsal determination in zebrafish. *Mech. Dev.* 121:371–86
229. Norton WH, Mangoli M, Lele Z, Pogoda HM, Diamond B, et al. 2005. Monorail/Foxa2 regulates floorplate differentiation and specification of oligodendrocytes, serotonergic raphe neurones and cranial motoneurones. *Development* 132:645–58
230. Oates AC, Lackmann M, Power MA, Brennan C, Down LM, et al. 1999. An early developmental role for eph-ephrin interaction during vertebrate gastrulation. *Mech. Dev.* 83:77–94
231. Ober EA, Field HA, Stainier DY. 2003. From endoderm formation to liver and pancreas development in zebrafish. *Mech. Dev.* 120:5–18
232. Ober EA, Schulte-Merker S. 1999. Signals from the yolk cell induce mesoderm, neuroectoderm, the trunk organizer, and the notochord in zebrafish. *Dev. Biol.* 215:167–81
233. Odenthal J, van Eeden FJ, Haffter P, Ingham PW, Nusslein-Volhard C. 2000. Two distinct cell populations in the floor plate of the zebrafish are induced by different pathways. *Dev. Biol.* 219:350–63
234. Oelgeschlager M, Larrain J, Geissert D, De Robertis EM. 2000. The evolutionarily conserved BMP-binding protein Twisted gastrulation promotes BMP signalling. *Nature* 405:757–63
235. Okada Y, Takeda S, Tanaka Y, Belmonte JC, Hirokawa N. 2005. Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* 121:633–44
236. Park M, Moon RT. 2002. The planar cell-polarity gene *stbm* regulates cell behaviour and cell fate in vertebrate embryos. *Nat. Cell Biol.* 4:20–25

237. Pelegri F. 2003. Maternal factors in zebrafish development. *Dev. Dyn.* 228:535–54
238. Pelegri F, Maischein HM. 1998. Function of zebrafish β-catenin and TCF-3 in dorsoventral patterning. *Mech. Dev.* 77:63–74
239. Peyrieras N, Strähle U, Rosa F. 1998. Conversion of zebrafish blastomeres to an endodermal fate by TGF-β-related signaling. *Curr. Biol.* 8:783–86
240. Piccolo S, Agius E, Lu B, Goodman S, Dale L, De Robertis EM. 1997. Cleavage of Chordin by Xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell* 91:407–16
241. Piccolo S, Sasai Y, Lu B, De Robertis EM. 1996. Dorsoventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* 86:589–98
242. Plusa B, Hadjantonakis AK, Gray D, Piotrowska-Nitsche K, Jedrusik A, et al. 2005. The first cleavage of the mouse zygote predicts the blastocyst axis. *Nature* 434:391–95
243. Pogoda HM, Solnica-Krezel L, Driever W, Meyer D. 2000. The zebrafish forkhead transcription factor FoxH1/Fast1 is a modulator of nodal signaling required for organizer formation. *Curr. Biol.* 10:1041–49
244. Postlethwait JH, Johnson SL, Midson CN, Talbot WS, Gates M, et al. 1994. A genetic linkage map for the zebrafish. *Science* 264:699–703
245. Poulain M, Lepage T. 2002. Mezzo, a paired-like homeobox protein is an immediate target of Nodal signalling and regulates endoderm specification in zebrafish. *Development* 129:4901–14
246. Pyati UJ, Webb AE, Kimelman D. 2005. Transgenic zebrafish reveal stage-specific roles for Bmp signaling in ventral and posterior mesoderm development. *Development* 132:2333–43
247. Raible F, Brand M. 2001. Tight transcriptional control of the ETS domain factors Erm and Pea3 by Fgf signaling during early zebrafish development. *Mech. Dev.* 107:105–17
248. Ramel MC, Lekven AC. 2004. Repression of the vertebrate organizer by Wnt8 is mediated by Vent and Vox. *Development* 131:3991–4000
249. Rauch GJ, Hammerschmidt M, Blader P, Schauerte HE, Strähle U, et al. 1997. Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harbor Symp. Quant. Biol.* 62:227–34
250. Raya A, Belmonte JC. 2004. Sequential transfer of left-right information during vertebrate embryo development. *Curr. Opin. Genet. Dev.* 14:575–81
251. Rebagliati MR, Toyama R, Fricke C, Haffter P, Dawid IB. 1998. Zebrafish *nodal*-related genes are implicated in axial patterning and establishing left-right asymmetry. *Dev. Biol.* 199:261–72
252. Rebagliati MR, Toyama R, Haffter P, Dawid IB. 1998. *cyclops* encodes a nodal-related factor involved in midline signaling. *Proc. Natl. Acad. Sci. USA* 95:9932–37
253. Reifers F, Bohli H, Walsh EC, Crossley PH, Stainier DY, Brand M. 1998. *Fgf8* is mutated in zebrafish *acerebellar* (*ace*) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. *Development* 125:2381–95
254. Reim G, Mizoguchi T, Stainier DY, Kikuchi Y, Brand M. 2004. The POU domain protein Spg (Pou2/Oct4) is essential for endoderm formation in cooperation with the HMG domain protein Casanova. *Dev. Cell* 6:91–101
255. Reiter JF, Alexander J, Rodaway A, Yelon D, Patient R, et al. 1999. Gata5 is required for the development of the heart and endoderm in zebrafish. *Genes Dev.* 13:2983–95
256. Reiter JF, Kikuchi Y, Stainier DY. 2001. Multiple roles for Gata5 in zebrafish endoderm formation. *Development* 128:125–35

257. Rentzsch F, Bakkers J, Kramer C, Hammerschmidt M. 2004. Fgf signaling induces posterior neuroectoderm independently of Bmp signaling inhibition. *Dev. Dyn.* 231:750–57
258. Renucci A, Lemarchandel V, Rosa F. 1996. An activated form of type I serine/threonine kinase receptor TARAM-A reveals a specific signalling pathway involved in fish head organiser formation. *Development* 122:3735–43
259. Rhinn M, Lun K, Luz M, Werner M, Brand M. 2005. Positioning of the midbrain-hindbrain boundary organizer through global posteriorization of the neuroectoderm mediated by Wnt8 signaling. *Development* 132:1261–72
260. Rodaway A, Takeda H, Koshida S, Broadbent J, Price B, et al. 1999. Induction of the mesendoderm in the zebrafish germ ring by yolk cell-derived TGF-β family signals and discrimination of mesoderm and endoderm by FGF. *Development* 126:3067–78
261. Roehl H, Nusslein-Volhard C. 2001. Zebrafish *pea3* and *erm* are general targets of FGF8 signaling. *Curr. Biol.* 11:503–7
262. Ross JJ, Shimmi O, Vilmos P, Petryk A, Kim H, et al. 2001. Twisted gastrulation is a conserved extracellular BMP antagonist. *Nature* 410:479–83
263. Ryu SL, Fujii R, Yamanaka Y, Shimizu T, Yabe T, et al. 2001. Regulation of *dharma/bozozok* by the Wnt pathway. *Dev. Biol.* 231:397–409
264. Sakaguchi T, Kuroiwa A, Takeda H. 2001. A novel sox gene, *226D7*, acts downstream of Nodal signaling to specify endoderm precursors in zebrafish. *Mech. Dev.* 107:25–38
265. Sampath K, Rubinstein AL, Cheng AM, Liang JO, Fekany K, et al. 1998. Induction of the zebrafish ventral brain and floorplate requires cyclops/nodal signalling. *Nature* 395:185–89
266. Saude L, Woolley K, Martin P, Driever W, Stemple DL. 2000. Axis-inducing activities and cell fates of the zebrafish organizer. *Development* 127:3407–17
266a. Schäfer M, Rembold M, Wittbrodt J, Schartl M, Winkler C. 2005. Medial floor plate formation in zebrafish consists of two phases and requires trunk-derived Midkine-a. *Genes Dev.* 19:897–902
266b. Schauerte HE, van Eeden FJ, Fricke C, Odenthal J, Strähle U, Haffter P. 1998. *Sonic hedgehog* is not required for the induction of medial floor plate cells in the zebrafish. *Development* 125:2983–93
267. Schier AF. 2001. Axis formation and patterning in zebrafish. *Curr. Opin. Genet. Dev.* 11:393–404
268. Schier AF. 2003. Nodal signaling in vertebrate development. *Annu. Rev. Cell. Dev. Biol.* 19:589–621
269. Schier AF. 2004. Nodal signaling during gastrulation. See Ref. 303a, pp. 491–504
270. Schier AF, Neuhauss SC, Harvey M, Malicki J, Solnica-Krezel L, et al. 1996. Mutations affecting the development of the embryonic zebrafish brain. *Development* 123:165–78
271. Schier AF, Neuhauss SC, Helde KA, Talbot WS, Driever W. 1997. The *one-eyed pinhead* gene functions in mesoderm and endoderm formation in zebrafish and interacts with *no tail*. *Development* 124:327–42
272. Schmid B, Furthauer M, Connors SA, Trout J, Thisse B, et al. 2000. Equivalent genetic roles for *bmp7/snailhouse* and *bmp2b/swirl* in dorsoventral pattern formation. *Development* 127:957–67
273. Schmidt JE, Suzuki A, Ueno N, Kimelman D. 1995. Localized BMP-4 mediates dorsal/ventral patterning in the early *Xenopus* embryo. *Dev. Biol.* 169:37–50
274. 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.* 57:191–98

275. Scholpp S, Brand M. 2004. Endocytosis controls spreading and effective signaling range of Fgf8 protein. *Curr. Biol.* 14:1834–41
276. Schulte-Merker S, Ho RK, Herrmann BG, Nusslein-Volhard C. 1992. The protein product of the zebrafish homologue of the mouse *T* gene is expressed in nuclei of the germ ring and the notochord of the early embryo. *Development* 116:1021–32
277. Schulte-Merker S, Lee KJ, McMahon AP, Hammerschmidt M. 1997. The zebrafish organizer requires *chordino*. *Nature* 387:862–63
278. Schulte-Merker S, van Eeden FJ, Halpern ME, Kimmel CB, Nusslein-Volhard C. 1994. *no tail* (*ntl*) is the zebrafish homologue of the mouse *T* (*Brachyury*) gene. *Development* 120:1009–15
279. Schwarz-Romond T, Asbrand C, Bakkers J, Kuhl M, Schaeffer HJ, et al. 2002. The ankyrin repeat protein Diversin recruits Casein kinase Iε to the β-catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling. *Genes Dev.* 16:2073–84
280. Scott IC, Blitz IL, Pappano WN, Maas SA, Cho KW, Greenspan DS. 2001. Homologues of Twisted gastrulation are extracellular cofactors in antagonism of BMP signalling. *Nature* 410:475–78
281. Selman K, Wallace RA, Sarka A, Qi X. 1993. Stages of oocyte development in the zebrafish, *Brachydanio rerio*. *J. Morphol.* 218:203–24
282. Sepich DS, Calmelet C, Kiskowski M, Solnica-Krezel L. 2005. Initiation of convergence and extension movements during zebrafish gastrulation. *Dev. Dyn.* In press
283. Sepich DS, Myers DC, Short R, Topczewski J, Marlow F, Solnica-Krezel L. 2000. Role of the zebrafish *trilobite* locus in gastrulation movements of convergence and extension. *Genesis* 27:159–73
284. Shaywitz DA, Melton DA. 2005. The molecular biography of the cell. *Cell* 120:729–31
285. Shih J, Fraser SE. 1995. Distribution of tissue progenitors within the shield region of the zebrafish gastrula. *Development* 121:2755–65
286. Shih J, Fraser SE. 1996. Characterizing the zebrafish organizer: microsurgical analysis at the early-shield stage. *Development* 122:1313–22
287. Shimell MJ, Ferguson EL, Childs SR, O'Connor MB. 1991. The *Drosophila* dorsal-ventral patterning gene *tolloid* is related to human *bone morphogenetic protein 1*. *Cell* 67:469–81
288. Shimizu T, Bae YK, Muraoka O, Hibi M. 2005. Interaction of Wnt and *caudal*-related genes in zebrafish posterior body formation. *Dev. Biol.* 279:125–41
289. Shimizu T, Yabe T, Muraoka O, Yonemura S, Aramaki S, et al. 2005. E-cadherin is required for gastrulation cell movements in zebrafish. *Mech. Dev.* 122:747–63
290. Shimizu T, Yamanaka Y, Nojima H, Yabe T, Hibi M, Hirano T. 2002. A novel repressor-type homeobox gene, *ved*, is involved in *dharma/bozozok*-mediated dorsal organizer formation in zebrafish. *Mech. Dev.* 118:125–38
291. Shimizu T, Yamanaka Y, Ryu SL, Hashimoto H, Yabe T, et al. 2000. Cooperative roles of Bozozok/Dharma and Nodal-related proteins in the formation of the dorsal organizer in zebrafish. *Mech. Dev.* 91:293–303
292. Shinya M, Eschbach C, Clark M, Lehrach H, Furutani-Seiki M. 2000. Zebrafish Dkk1, induced by the pre-MBT Wnt signaling, is secreted from the prechordal plate and patterns the anterior neural plate. *Mech. Dev.* 98:3–17
293. Sidi S, Goutel C, Peyrieras N, Rosa FM. 2003. Maternal induction of ventral fate by zebrafish *radar*. *Proc. Natl. Acad. Sci. USA* 100:3315–20

294. Sirotkin HI, Dougan ST, Schier AF, Talbot WS. 2000. *bozozok* and *squint* act in parallel to specify dorsal mesoderm and anterior neuroectoderm in zebrafish. *Development* 127:2583–92
295. Sirotkin HI, Gates MA, Kelly PD, Schier AF, Talbot WS. 2000. *fast1* is required for the development of dorsal axial structures in zebrafish. *Curr. Biol.* 10:1051–54
296. Sivak J, Amaya E. 2004. FGF signaling during gastrulation. See Ref. 303a, pp. 463–74
297. Smith JC. 2004. Role of T-box genes during gastrulation. See Ref. 303a, pp. 571–80
298. Solnica-Krezel L. 2005. Conserved patterns of cell movements during vertebrate gastrulation. *Curr. Biol.* 15:R213–28
299. Solnica-Krezel L, Driever W. 1994. Microtubule arrays of the zebrafish yolk cell: organization and function during epiboly. *Development* 120:2443–55
300. Solnica-Krezel L, Stemple DL, Mountcastle-Shah E, Rangini Z, Neuhauss SC, et al. 1996. Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish. *Development* 123:67–80
301. Stachel SE, Grunwald DJ, Myers PZ. 1993. Lithium perturbation and goosecoid expression identify a dorsal specification pathway in the pregastrula zebrafish. *Development* 117:1261–74
302. Stainier DY, Fouquet B, Chen JN, Warren KS, Weinstein BM, et al. 1996. Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. *Development* 123:285–92
303. Stern CD. 2004. Gastrulation in the chick. See Ref. 303a, pp. 219–32
303a. Stern CD, ed. 2004. *Gastrulation: From Cells to Embryo*. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
304. Stern CD. 2005. Neural induction: old problem, new findings, yet more questions. *Development* 132:2007–21
305. Strähle U, Jesuthasan S. 1993. Ultraviolet irradiation impairs epiboly in zebrafish embryos: evidence for a microtubule-dependent mechanism of epiboly. *Development* 119:909–19
306. Strähle U, Jesuthasan S, Blader P, Garcia-Villalba P, Hatta K, Ingham PW. 1997. *one-eyed pinhead* is required for development of the ventral midline of the zebrafish (*Danio rerio*) neural tube. *Genes Funct.* 1:131–48
307. Strähle U, Lam CS, Ertzer R, Rastegar S. 2004. Vertebrate floor-plate specification: variations on common themes. *Trends Genet.* 20:155–62
308. Streisinger G, Walker C, Dower N, Knauber D, Singer F. 1981. Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature* 291:293–96
309. Sumanas S, Kim HJ, Hermanson S, Ekker SC. 2001. Zebrafish *frizzled 2* morphant displays defects in body axis elongation. *Genesis* 30:114–18
310. Sun X, Meyers EN, Lewandoski M, Martin GR. 1999. Targeted disruption of *Fgf8* causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev.* 13:1834–46
311. Szeto DP, Kimelman D. 2004. Combinatorial gene regulation by Bmp and Wnt in zebrafish posterior mesoderm formation. *Development* 131:3751–60
312. Talbot WS, Trevarrow B, Halpern ME, Melby AE, Farr G, et al. 1995. A homeobox gene essential for zebrafish notochord development. *Nature* 378:150–57
313. Tam PPL, Gad JM. 2004. Gastrulation in the mouse embryo. See Ref. 303a, pp. 233–62
314. Tao Q, Yokota C, Puck H, Kofron M, Birsoy B, et al. 2005. Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* 120:857–71

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Discovery of a novel transcription factor required for patterning the organizer region.

315. Thisse B, Wright CV, Thisse C. 2000. Activin- and Nodal-related factors control anteroposterior patterning of the zebrafish embryo. *Nature* 403:425–28
316. Thisse C, Thisse B. 1999. Antivin, a novel and divergent member of the TGFβ superfamily, negatively regulates mesoderm induction. *Development* 126:229–40
317. Thisse C, Thisse B, Halpern ME, Postlethwait JH. 1994. *Goosecoid* expression in neurectoderm and mesendoderm is disrupted in zebrafish *cyclops* gastrulas. *Dev. Biol.* 164:420–29
318. Thorpe CJ, Moon RT. 2004. *nemo-like kinase* is an essential co-activator of Wnt signaling during early zebrafish development. *Development* 131:2899–909
319. Thorpe CJ, Weidinger G, Moon RT. 2005. Wnt/β-catenin regulation of the Sp1-related transcription factor *sp5l* promotes tail development in zebrafish. *Development* 132:1763–72
319a. Tian J, Yam C, Balasundaram G, Wang H, Gore A, Sampth K. 2003. A temperature-sensitive mutation in the *nodal*-related gene *cyclops* reveals that the floor plate is induced during gastrulation in zebrafish. *Development* 130:3331–42
319b. Tiso N, Filippi A, Pauls S, Bortolussi M, Argenton F. 2002. BMP signalling regulates anteroposterior endoderm patterning in zebrafish. *Mech. Dev.* 118:29–37
320. Topczewski J, Sepich DS, Myers DC, Walker C, Amores A, et al. 2001. The zebrafish glypican knypek controls cell polarity during gastrulation movements of convergent extension. *Dev. Cell* 1:251–64
321. Topczewski J, Solnica-Krezel L. 1999. Cytoskeletal dynamics of the zebrafish embryo. *Methods Cell Biol.* 59:205–26
322. Trinh LA, Meyer D, Stainier DY. 2003. The Mix family homeodomain gene *bonnie and clyde* functions with other components of the Nodal signaling pathway to regulate neural patterning in zebrafish. *Development* 130:4989–98
323. Tsang M, Friesel R, Kudoh T, Dawid IB. 2002. Identification of Sef, a novel modulator of FGF signalling. *Nat. Cell Biol.* 4:165–69
324. Tsang M, Maegawa S, Kiang A, Habas R, Weinberg E, Dawid IB. 2004. A role for MKP3 in axial patterning of the zebrafish embryo. *Development* 131:2769–79
325. Ulrich F, Concha ML, Heid PJ, Voss E, Witzel S, et al. 2003. Slb/Wnt11 controls hypoblast cell migration and morphogenesis at the onset of zebrafish gastrulation. *Development* 130:5375–84
326. Veeman MT, Slusarski DC, Kaykas A, Louie SH, Moon RT. 2003. Zebrafish Prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr. Biol.* 13:680–85
327. von Bubnoff A, Cho KW. 2001. Intracellular BMP signaling regulation in vertebrates: pathway or network? *Dev. Biol.* 239:1–14
328. 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.* 7:355–66
329. Wada H, Iwasaki M, Sato T, Masai I, Nishiwaki Y, et al. 2005. Dual roles of zygotic and maternal Scribble1 in neural migration and convergent extension movements in zebrafish embryos. *Development* 132:2273–85
330. Wagner DS, Dosch R, Mintzer KA, Wiemelt AP, Mullins MC. 2004. Maternal control of development at the midblastula transition and beyond: mutants from the zebrafish II. *Dev. Cell* 6:781–90
331. Wallingford JB, Fraser SE, Harland RM. 2002. Convergent extension: the molecular

control of polarized cell movement during embryonic development. *Dev. Cell* 2:695–706
332. Warga RM, Kane DA. 2003. One-eyed pinhead regulates cell motility independent of Squint/Cyclops signaling. *Dev. Biol.* 261:391–411
333. Warga RM, Kimmel CB. 1990. Cell movements during epiboly and gastrulation in zebrafish. *Development* 108:569–80
334. Warga RM, Nusslein-Volhard C. 1999. Origin and development of the zebrafish endoderm. *Development* 126:827–38
335. Waxman JS, Hocking AM, Stoick CL, Moon RT. 2004. Zebrafish Dapper1 and Dapper2 play distinct roles in Wnt-mediated developmental processes. *Development* 131:5909–21
336. Weaver C, Kimelman D. 2004. Move it or lose it: axis specification in *Xenopus*. *Development* 131:3491–99
337. 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
338. Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, et al. 2005. MicroRNA expression in zebrafish embryonic development. *Science.* 309:310–11
339. Wienholds E, Koudijs MJ, van Eeden FJ, Cuppen E, Plasterk RH. 2003. The microRNA-producing enzyme Dicer1 is essential for zebrafish development. *Nat. Genet.* 35:217–18
340. Wienholds E, Schulte-Merker S, Walderich B, Plasterk RH. 2002. Target-selected inactivation of the zebrafish *rag1* gene. *Science* 297:99–102
341. Willot V, Mathieu J, Lu Y, Schmid B, Sidi S, et al. 2002. Cooperative action of ADMP- and BMP-mediated pathways in regulating cell fates in the zebrafish gastrula. *Dev. Biol.* 241:59–78
342. Wilm TP, Solnica-Krezel L. 2005. Essential roles of a zebrafish *prdm1/blimp1* homolog in embryo patterning and organogenesis. *Development* 132:393–404
343. Wilson PA, Lagna G, Suzuki A, Hemmati-Brivanlou A. 1997. Concentration-dependent patterning of the *Xenopus* ectoderm by BMP4 and its signal transducer Smad1. *Development* 124:3177–84
344. Wolenski JS, Hart NH. 1987. Scanning electron microscope studies of sperm incorporation into the zebrafish (*Brachydanio*) egg. *J. Exp. Zool.* 243:259–73
345. Woo K, Fraser SE. 1995. Order and coherence in the fate map of the zebrafish nervous system. *Development* 121:2595–609
346. Woo K, Fraser SE. 1997. Specification of the zebrafish nervous system by nonaxial signals. *Science* 277:254–57
347. Woo K, Fraser SE. 1998. Specification of the hindbrain fate in the zebrafish. *Dev. Biol.* 197:283–96
348. Woods IG, Talbot WS. 2005. The *you* gene encodes an EGF-CUB protein essential for Hedgehog signaling in zebrafish. *PLoS Biol.* 3:e66
349. Xiao T, Roeser T, Staub W, Baier H. 2005. A GFP-based genetic screen reveals mutations that disrupt the architecture of the zebrafish retinotectal projection. *Development* 132:2955–67
350. Xie J, Fisher S. 2005. Twisted gastrulation enhances BMP signaling through chordin dependent and independent mechanisms. *Development* 132:383–91
351. Yabe T, Shimizu T, Muraoka O, Bae YK, Hirata T, et al. 2003. Ogon/secreted frizzled functions as a negative feedback regulator of Bmp signaling. *Development* 130:2705–16

352. Yamamoto Y, Oelgeschlager M. 2004. Regulation of bone morphogenetic proteins in early embryonic development. *Naturwissenschaften* 91:519–34
353. Yamanaka Y, Mizuno T, Sasai Y, Kishi M, Takeda H, et al. 1998. A novel homeobox gene, *dharma*, can induce the organizer in a non-cell-autonomous manner. *Genes Dev.* 12:2345–53
354. Yamashita S, Miyagi C, Carmany-Rampey A, Shimizu T, Fujii R, et al. 2002. Stat3 controls cell movements during zebrafish gastrulation. *Dev. Cell* 2:363–75
355. Yamashita S, Miyagi C, Fukada T, Kagara N, Che YS, Hirano T. 2004. Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizer. *Nature* 429:298–302
356. Yan YT, Gritsman K, Ding J, Burdine RD, Corrales JD, et al. 1999. Conserved requirement for *EGF-CFC* genes in vertebrate left-right axis formation. *Genes Dev.* 13:2527–37
357. Yasuo H, Lemaire P. 2001. Role of Goosecoid, Xnot and Wnt antagonists in the maintenance of the notochord genetic programme in *Xenopus* gastrulae. *Development* 128:3783–93
358. Yelon D. 2001. Cardiac patterning and morphogenesis in zebrafish. *Dev. Dyn.* 222:552–63
359. Yeo SY, Little MH, Yamada T, Miyashita T, Halloran MC, et al. 2001. Overexpression of a slit homologue impairs convergent extension of the mesoderm and causes cyclopia in embryonic zebrafish. *Dev. Biol.* 230:1–17
360. Zhang J, Houston DW, King ML, Payne C, Wylie C, Heasman J. 1998. The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. *Cell* 94:515–24
361. Zhang J, Talbot WS, Schier AF. 1998. Positional cloning identifies zebrafish *one-eyed pinhead* as a permissive EGF-related ligand required during gastrulation. *Cell* 92:241–51
362. Zhang L, Zhou H, Su Y, Sun Z, Zhang H, et al. 2004. Zebrafish Dpr2 inhibits mesoderm induction by promoting degradation of nodal receptors. *Science* 306:114–17

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