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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 Nusslein-Volhard and Driever groups con- ¨ ducted 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.

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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

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).

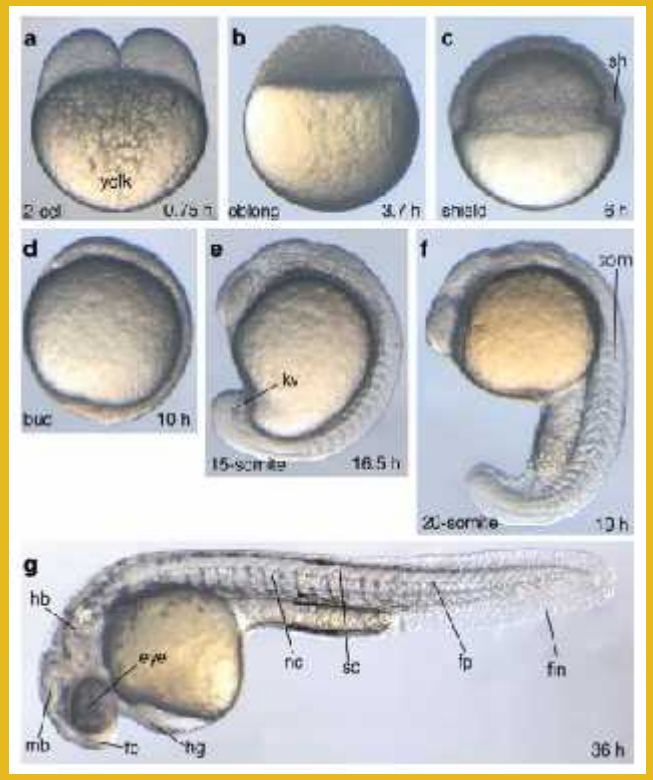

Figure 1 :Zebrafish embryogenesis. Living zebrafish embryos are shown at the indicated developmental stages. Approximate developmental ages in hours postfertilization (h) are shown. Embryos are oriented: (a, b) animal pole to top; (c) animal pole to top, dorsal to the right; (d–f) anterior to the top, 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.

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

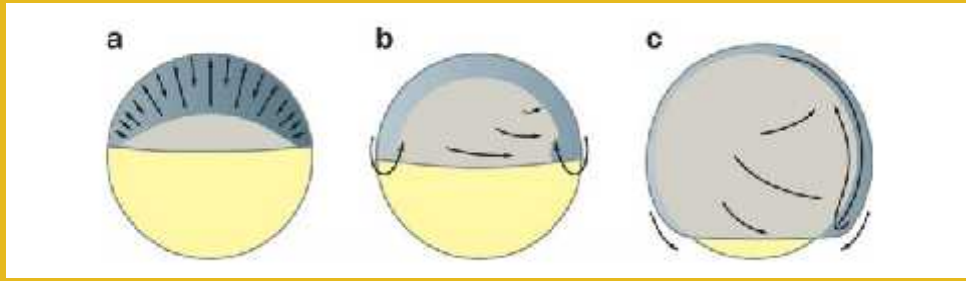

Figure 2: Gastrulation movements. (a) Dome stage. Cells intercalate radially, contributing to epiboly. (b) Shield stage. Cells at the margin internalize and migrate toward the animal pole. Cells converge dorsally, with lateral mesodermal cells starting convergence at later stages than cells closer to the shield (282). (c) 90% epiboly stage. Epiboly, internalization, convergence and extension continue. Modified from Reference 138.

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 re gion. 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.

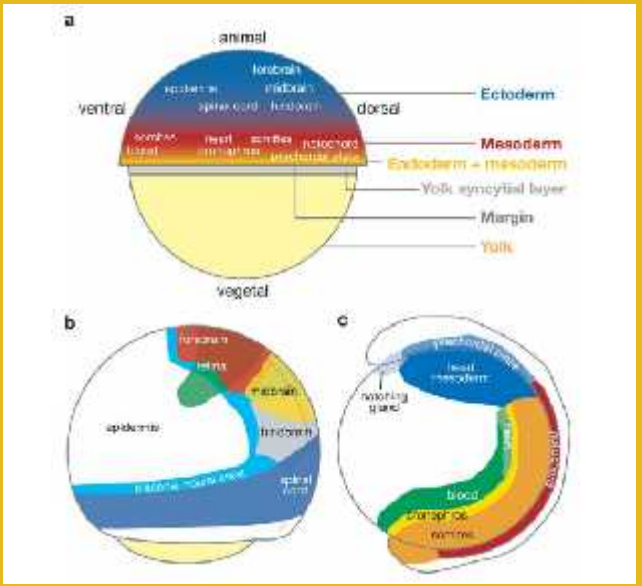

Figure 3 :Zebrafish fate maps. (a) Fate map at 50% epiboly stage, the onset of gastrulation. Lateral view, dorsal to the right, animal pole to the top. Germ layers are arranged along the animal-vegetal axis. Different mesodermal and ectodermal fates are arranged along the dorsal-ventral axis. For details 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.

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 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 neighbouring 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).

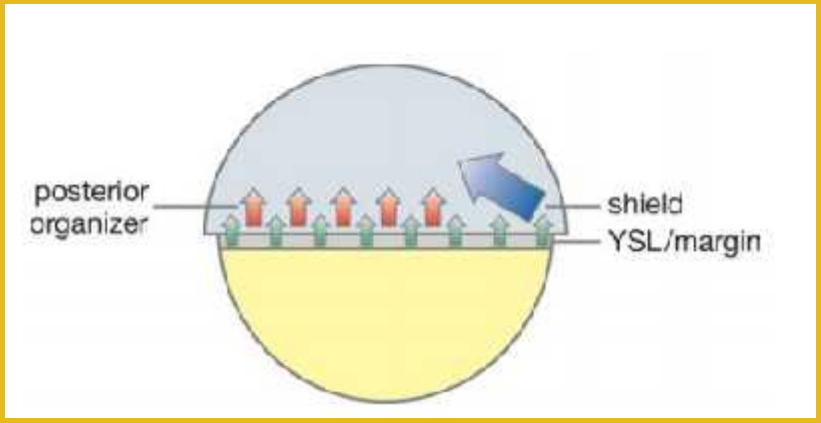

Figure 4 :Zebrafish organizing centers. Lateral view, dorsal to the right, animal pole to the top. Yolk syncytial layer (YSL) can induce mesendodermal fates upon transplantation (green arrows). Posterior organizer is located at the ventral and lateral margin and can induce tail, posterior trunk, and hindbrain tissue upon transplantation (red arrows). Shield corresponds to Spemann-Mangold organizer and can induce dorsal and anterior structures upon transplantation (blue arrow).

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)

ß-catenin

Evidence suggests that maternal ß-catenin acts to establish the dorsal-ventral axis in zebrafish. ß-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 GSK3ß and other components targets ß-catenin protein for degradation, thereby allowing only a low level of ß-catenin to accumulate. Activation of the canonical Wnt signalling pathway inhibits the ß-catenin degradation complex, stabilizing ß-catenin and allowing it to enter the nucleus, where it activates transcription of canonical Wnt target genes. In the zebrafish embryo, ß-catenin accumulates specifically in nuclei of dorsal margin blastomeres as early as the 128-cell stage (66, 274). This asymmetric nuclear localization of ß-catenin is an early marker of the dorsal ventral axis (Figure 5). As in the amphibian embryo, overexpression of ß-catenin leads to axis duplication (148). Moreover, ß-catenin seems to be required for dorsal axis formation, as overexpression of proteins that inhibit ßcatenin's action as a transcriptional activator (cadherin or a dominant negative form of Tcf3 that binds ß-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 ß-catenin and lead to ventralized embryos (147, 228). Soon after the mid-blastula transition, ßcatenin 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 ßcatenin targets act to inhibit the action of ventralizing factors or, in the case of Sqt, induce mesendodermal fates at the dorsal margin.

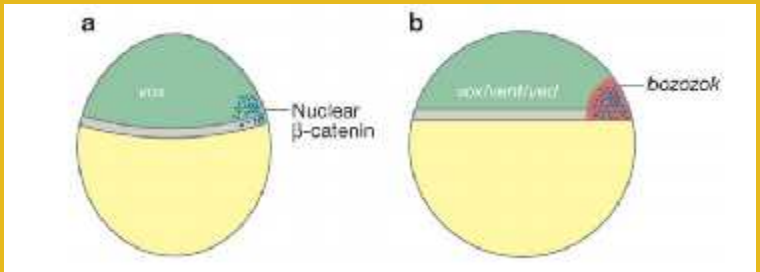

Figure 5 :Transcriptional interactions patterning the dorsal-ventral axis. Lateral view, dorsal to the right, animal pole to the top. (a) ß-catenin is stabilized on the dorsal side during cleavage stages. Soon after mid-blastula transition, vox is expressed ubiquitously. (b) ß-catenin activates bozozok (boz), which represses vox, vent, and ved expression in dorsal blastomeres.

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 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).

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 Bmp2bBmp7 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 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 Np63 and kheper, both of which encode transcriptional repressors (15, 16, 177, 220). The ventrally expressed Np63 gene is required for development of the epidermis and is directly activated by Bmps. Kheper, a zinc fingerhomeobox 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 dorsalventral 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 of Bmp signaling activity across the dorsalventral axis.

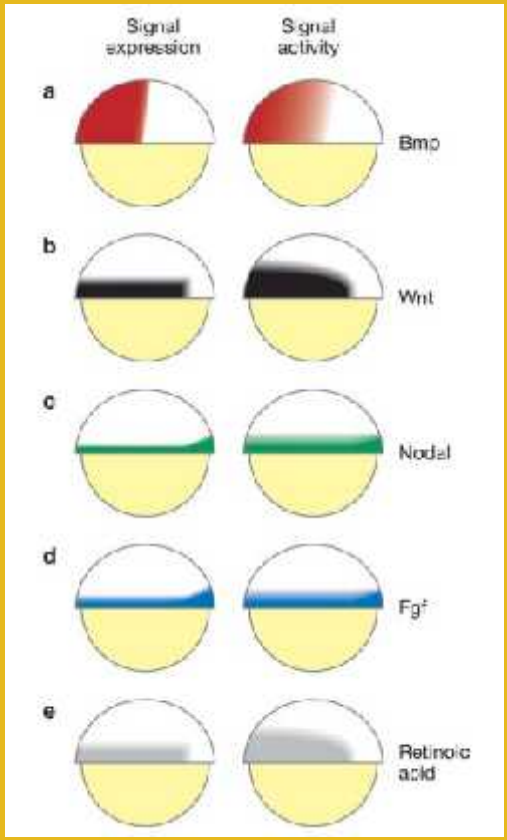

Figure 6: Signals patterning the embryo. Late-blastula stage, lateral view, dorsal to the right, animal pole to the top. Signal expression is based on published reports, but signaling activities are speculative and based on the potential range of signals and the expression pattern and range of antagonists. 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.

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 Msmad5, mutants) (168). The dorsalized phenotype of Msmad5 mutants is apparent before the zygotic bmp mutant phenotype, suggesting that the Msmad5 phenotype reflects more than a simple function as a transcriptional mediator of zygotic bmp2b and bmp7 (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 Msmad5 phenotype (293). Bmp4 and bmp7 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 morpholinoinhibition 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 proposedthat 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, insufficient 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 midgastrula 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 non-autonomously 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/ventbmp2b 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.