Anterior–posterior differences in vertebrate segments: specification of trunk and tail somites in the zebrafish blastula

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During vertebrate embryogenesis, the primary body axis grows posteriorly and is concomitantly segmented into somites, the precursors of the vertebral column, skeletal muscle, and dermis. The somites arise sequentially, with the anterior somites that give rise to the cervical vertebrae created early. The more posterior somites that become the thoracic, lumbar, and sacral vertebrae form at progressively later times. During the axis elongation period, the embryo must parse the somite precursors appropriately so that there are enough cells remaining to make the most posterior somites at the end of somitogenesis. How the embryo allocates these cells is not well understood. However, in this issue, Szeto and Kimelman (2006) address this question by showing that cells are specified to give rise to anterior trunk, posterior trunk, and tail somites. They find that this cell fate decision occurs surprisingly early in zebrafish development, prior to gastrulation, in response to nodal, fgf, and bmp signaling (Fig. 1; Szeto and Kimelman 2006). Their data link the processes of mesoderm induction and patterning with vertebrate segmentation and elucidate a mechanism by which the embryo reserves a somite precursor population for the formation of the more posterior body segments.

While the number of vertebrae varies greatly among different species (Richardson et al. 1998), somite number within a given species is remarkably consistent. The question arises, how does the embryo determine the appropriate somite number and size while the field of cells to be segmented continues to grow posteriorly? Experimentally manipulated Xenopus embryos, with large portions of the blastula physically removed, develop into embryos two-thirds smaller than normal, yet form the same number of segments and at the same rate as unmanipulated sibling embryos. Thus, the embryo “knows” the species-specific number of somites that it needs to generate and divides the available cell population accordingly; that is, there does not appear to be a physical constraint defining the number of cells in a given segment. In these smaller embryos, each segment consists of fewer cells than the somites of their normal-sized siblings (Cooke 1975). Furthermore, knypek, trilobite double mutants, which are much shorter than a wild-type zebrafish embryo due to a severe convergence extension defect, form somites only two cells in length, while zebrafish somites are normally five cells in length (Henry et al. 2000). Consideration of the regulative capacity of vertebrate segmentation led to the proposal that somitogenesis is controlled by a “clock and wavefront” whereby the clock represents a mechanism that causes the somite precursors to oscillate. Cells would only be able to form a segment during a brief period within each cycle of the somite clock. The wavefront represents the progression of tissue maturation and cell differentiation that sweeps head-to-tail along the primary axis of the embryo [Cooke and Zeeman 1975, Cooke 1998]. In this model, a somite forms when the wavefront encounters a group of cells in the correct, permissive phase of the clock. Thus, somite length and rate of formation are dependent on the frequency of the clock/oscillator and the velocity of the wavefront. The regulative capacity of this mechanism allows the embryo to parse cells into segments at a rate that would retain enough cells to populate the most posterior somites. During the past 10 years, molecular evidence for both a clock and a wavefront has emerged [Pourquié 2003, Rida et al. 2004]. However, Szeto and Kimelman (2006) find an additional mechanism by which the zebrafish embryo parses its supply of somite progenitors. They find that the anlagen of the anterior trunk, posterior trunk, and tail somites are specified before gastrulation, 5, 13.5, and 16.5 h before the onset of segmentation of each anlagen, respectively.

nodal, fgf, and bmp signaling specify mesodermal precursor fates

Generally speaking, nodal signaling induces and patterns the mesoderm, fgf and wnt signaling pattern and maintain mesodermal fates, while bmp signaling pat-
terns the ventral–posterior mesoderm [Schier and Talbot 2005; Kimelman 2006]. Fate mapping of the zebrafish blastula shows that the dorsal mesoderm becomes the prechordal plate and notochord, while the more lateral mesoderm becomes the somites, nephros, and heart. The ventral mesoderm gives rise to tail somites and blood (Kimmel et al. 1990; Warga and Nusslein-Volhard 1999). While these maps indicate the typical fate adopted by a cell in a particular location and time, the fate map does not indicate that the cells have been determined [i.e., irreversibly committed to a given fate] or specified [i.e., committed but still capable of altering their fate]. Expanding on previous fate mapping studies, Szeto and Kimelman (2006) find that anterior trunk, posterior trunk, and tail somite anlagen are specified by nodal, fgf, and bmp signaling during the late blastula.

In vertebrates, nodal signaling is required for the induction of both the mesoderm and endoderm. Nodals are members of the TGFβ superfamily and are represented by squint and cyclops in zebrafish [Schier and Talbot 2005]. squint; cyclops double mutants lack all mesoderm and endoderm except for the tail somites (Feldman et al. 1998). Nodals signal through a serine/threonine kinase receptor complex that includes the type I and type II Activin receptors and the EGF-CFC coreceptor. This co-receptor is called one-eyed pinhead in zebrafish, and embryos lacking both the maternal and zygotic function of this gene (MZoep) resemble squint; cyclops double mutants [Fig. 2A; Gritsman et al. 1999]. Before gastrulation, nodal signaling is highest at the margin, the equatorial portion of the embryo where the cells of the embryo proper are juxtaposed to the yolk cell. Due to activation of cyclops expression by squint dorsally, there is higher nodal signaling in the dorsoanterior part of the blastula [Dougan et al. 2003].

Fate mapping studies suggest that the progenitors of the trunk and tail somites are intermingled both during early development and within the tailbud that forms at the end of gastrulation [Fig. 1A; Kimmel et al. 1990; Kanki and Ho 1997; Warga and Nusslein-Volhard 1999]. Szeto and Kimelman [2006] postulated that since only tail somites form in the absence of nodal signaling in MZoep embryos, that they could use this genetic back-

![Figure 1. Specification of trunk and tail mesoderm in the zebrafish.](image1)

![Figure 2. Notable differences between somites along the anterior–posterior axis.](image2)
ground to study the specification of trunk and tail mesodermal progenitor cells (MPCs). For this analysis, they used a cell transplantation technique to take cells from one embryo and place them in a host embryo of a different genetic background or stage of development. They found that if they transplanted MZoep cells from a late-blastula-stage embryo at 5 h post-fertilization (hpf) into a wild-type host at 5 hpf, the transplanted cells only populated the tail somites, whereas wild-type cells transplanted into a wild-type embryo populated all somites. These data indicate that MZoep cells have cell-autonomously adopted a tail MPC fate by 5 hpf. Heterochronic transplants of 5-hpf MZoep cells into a wild-type host embryo at 4 hpf show that the transplanted cells can populate the posterior trunk in addition to the tail. In contrast, if 4-hpf MZoep cells are transplanted into 5-hpf wild-type embryos, the donor cells only populate the tail. These two experiments indicate that the tail and trunk MPC fates are specified between 4 and 5 hpf, before the onset of gastrulation and 5 h before the beginning of somitogenesis [Fig. 1A,B]. This specification signal(s) is missing in MZoep embryos but is present in wild-type embryos.

The trunk-promoting signal may consist of members of the fgf family, at least three of which are expressed along the margin of the zebrafish blastula. fgf signaling promotes dorsal and dorsolateral fates in the zebrafish blastula. Moreover, fgf expression is greatly reduced in MZoep embryos, meaning that fgf is a good candidate for the trunk-inducing signal that is missing in these embryos [Mathieu et al. 2004]. Indeed, Szeto and Kimelman [2006] show that injection of fgf4 mRNA into a MZoep embryo and subsequent transplantation of these cells into a wild-type host result in donor cells populating the posterior trunk, somites 9–15. While this suggests that fgf is a trunk-promoting signal, these cells still do not populate the first nine somites, arguing that an additional signal is needed to specify anterior MPC fates. In fact, anterior trunk MPCs appear to require reception of Nodal. Szeto and Kimelman [2006] injected a constitutively active Nodal receptor into MZoep embryos and found that when transplanted into wild-type embryos, these donor cells populated the anterior nine somites. Thus, direct reception of Nodal is necessary for specification of anterior trunk (somites 1–9), while fgf promotes posterior trunk fates (somites 9–15). Later in development, after the MPC fates have been specified, fgf signaling is needed to maintain the population of both trunk and tail MPCs. Embryos lacking fgf8 and fgf24 retain only portions of the first three to four somites [Fig. 2B; Draper et al. 2003]. Thus, these signaling pathways have distinct effects on the MPCs at different stages of development.

bmps are members of the Tgfβ superfamily and are necessary for specification of posterior tail and ventral fates such as somites and blood [Hammerschmidt and Mullins 2002]. The Bmps are antagonized by the secreted inhibitors Chordin, Noggin, and Follistatin, which are expressed in the dorsal margin, called the shield or Spemann’s Organizer in zebrafish and amphibians, respectively [Schier and Talbot 2005; Kimelman 2006]. During gastrulation, dorsal and lateral mesendoderm undergo convergence toward the dorsal midline. The first 11–12 somites are largely derived of cells that have undergone some dorsal convergence and never passed through the posterior tailbud [Fig. 2C; Kanki and Ho 1997; Jülich et al. 2005]. bmp signaling establishes a “no convergence zone” along the ventral margin and causes many of these cells to remain ventral, enter the tailbud, and ultimately contribute to the somites [Kanki and Ho 1997; Myers et al. 2002]. Expression of the bmp inhibitors noggin, chordin, and follistatin is reduced in MZoep embryos [Gritsman et al. 1999; Ragland and Raible 2004]. Thus, bmps are good candidates for a tail-promoting signal. Szeto and Kimelman [2006] injected bmp2b mRNA into MZoep embryos and transplanted these cells from 4-hpf donors to 4-hpf hosts. Uninjected MZoep cells may populate the posterior trunk, but the bmp2b-expressing cells displayed a stronger bias to populate the tail. These results are consistent with bmp being a tail-promoting signal. Moreover, the trunk-promoting signal fgf expression, suggesting that fgf may specify trunk fates largely by inhibiting the tail-promoting bmp signal [Furthauer et al. 2004].

wnt signaling and tail formation

wnt signaling is also important for establishing and maintaining posterior, ventral mesodermal fates [Schier and Talbot 2005; Kimelman 2006]. In zebrafish, wnt8 and wnt3a have partially redundant roles in tail formation, as morpholino inhibition of both genes results in a loss of tissue posterior to somites 10–12 [Fig. 2D]. wnt8 and wnt3a appear to promote tail formation via caudal homologs, tbx6, and fgf, as expression of each is reduced in embryos lacking wnt8 and wnt3a [Szeto and Kimelman 2004; Shimizu et al. 2005; Thorpe et al. 2005]. It is unclear if wnt signaling can recapitulate the tail-inducing activity of bmps in Szeto and Kimelman’s [2006] MPCR transplantation assay. However, it has been shown that bmp4, cyclops/nodal, and wnt8 can induce the formation of an ectopic tail when mRNAs encoding these genes are coinjected into animal blastomeres that would normally adopt ectodermal fates [Agathon et al. 2003]. Notably in the mouse, wnt signaling is involved in both tail formation and segmentation [Aulehla et al. 2003].

t-box genes and mesodermal patterning

t-box genes are transcription factors that pattern the vertebrate mesoderm. In the zebrafish, no tail (the homolog of brachyury), spadetail, and tbx6 display a complex relationship in patterning the dorsal–ventral and anterior–posterior axes. spadetail, like nodal and fgf, is required for anterior and posterior trunk development. no tail is required for notochord and, like bmp signaling, formation of tail somites. tbx6 appears to act semiredundantly with both no tail and spadetail [Amacher and Kimmel 1998; Griffin et al. 1998; Griffin and Kimelman 2002, 2003; Goering et al. 2003]. Despite the distinct pheno-
signaling, and, in turn, the t-box polarity (Pourquié et al. 2006). Genetic and embryological experiments have uncovered several differences in the specification, formation, and differentiation of the anterior trunk, posterior trunk, and tail somites. Differences in the specification of the anterior paraxial mesoderm have been revealed by genetic experiments in mice and zebrafish. Mice mutant for either of the transcription factors mesogenin or tbx6 form only the anterior trunk somites (Chapman and Papaioannou 1998; Yoon and Wold 2000). Similarly, in zebrafish, Zoep, no tail double mutants lack all but the anterior trunk somites (Fig. 2E; Schier et al. 1997). Since no tail activates both spadetail and fgf8 expression, the loss of posterior mesoderm in Zoep, no tail embryos is a likely due to a combined reduction of nodal, fgf, and t-box function (Draper et al. 2003; Griffin and Kimelman 2003). As discussed above, analysis of t-box genes in zebrafish has uncovered differences in the genetic hierarchy that specifies trunk and tail MPCs, with spadetail and tbx6 involved in specifying anterior and posterior trunk, while no tail and tbx6 specify tail MPCs (Fig. 2F; Kimmel et al. 1989; Griffen et al. 1998; Griffin and Kimelman 2002, Goering et al. 2003).

One consistent difference in anterior trunk somitogenesis observed in mice, zebrafish, and the cephalochordate amphioxus is the more rapid progression of the somite cycle relative to posterior somitogenesis (Tam 1981; Hanneman and Westerfield 1989; Schubert et al. 2001). In the zebrafish, the anterior six somites form every 20 min, while the 24 posterior somites form every 30 min (Fig. 2G; Hanneman and Westerfield 1989). In amphioxus, this temporal difference is even more extreme in that the anterior approximately eight somites form every hour but each subsequent somite cycle is 18 h (Schubert et al. 2001).

In zebrafish, fss mutant embryos and embryos lacking both her1 and her7 show segmentation defects along the entire body axis, indicating that there are common features in the genetic control of trunk and tail somitogenesis (van Eeden et al. 1996; Henry et al. 2002; Oates and Ho 2002). However, numerous genetic studies demonstrate that perturbation of notch signaling in mice, humans, and zebrafish, receptor tyrosine phosphatase θ in zebrafish, or mesp2 and wnt3a in mice leads to a segmentation defect in the posterior but not the anterior trunk somites with the defects occurring posterior to the fifth to ninth somite in zebrafish (Fig. 2H; Rida et al. 2004). Accordingly, the deltaD mutant in zebrafish is called after eight, while the notch1a mutant is named deadly seven (van Eeden et al. 1996; Holley et al. 2000, 2002). Anterior trunk somitogenesis in zebrafish is also resistant to “dominant” perturbation of notch signaling via ectopic expression of an activated Notch, NICD, or a dominant-negative inhibitor of notch signaling, X-Su[H]Drm1 (Wettstein et al. 1997; Takke and Campos-Ortega 1999; Jülicher et al. 2005). In contrast to perturbation of notch pathway function, zebrafish mutants for integrinα5, called before eight, and fibronectin1 affect
the formation of only the first approximately seven somites [Fig. 2H; Jüllich et al. 2005; Koshida et al. 2005]. Zebrafish double mutants between the anterior and posterior specific somite mutants lack all segments. Interestingly, fss/+ embryos or fss/+; aei/deltaD+; des/notch1a/+ embryos display transient segmentation defects centered around the seventh to ninth somites. These observations suggest that the seventh to ninth somites represent a transition zone between anterior and posterior trunk somitogenesis in zebrafish [Jüllich et al. 2005]. It is currently unclear whether these differences in the segmentation program are related to the differences between anterior trunk, posterior trunk, and tail MPCs revealed by Szeto and Kimelman [2006].

In mice and humans, there are also mutations that more severely affect the differentiation of the anterior trunk somites. In the mouse, PDGF-Rs is thought to mediate signaling between the myotome and sclerotome, and mice mutant for this receptor show extensive fusion of the cervical vertebrae but milder defects in thoracic and lumbar vertebrae [Soriano 1997; Tallquist et al. 2000]. This is similar to congenital human defects known as the Klippel-Feil syndrome in which the cervical vertebrae are fused but the rib cage is only moderately affected, if at all. The complementary, posterior segmentation defects in humans are a heterogeneous class including spondylocostal dysostosis, which can be caused by mutations in notch pathway genes [Clarke et al. 1998; Bulman et al. 2000; Pourqué and Kusumi 2001; Sparrow et al. 2006].

Paradoxically, despite these genetic differences between anterior trunk, posterior trunk, and tail somitogenesis, most genes transcribed in a segmental pattern are expressed in all somites. In zebrafish, there is no significant difference in the expression of deltaD, notch1a, integrina5, or fibronectin1 when comparing trunk and tail somites [Bierkamp and Campos-Ortega 1993; Dornseifer et al. 1997; Jüllich et al. 2005; Koshida et al. 2005]. There are a small number of genes that do show differential expression in the tail and trunk somites. For example, a nanos-related gene and soxl1a are only expressed in the anterior trunk somites in zebrafish [Fig. 2I; de Martino et al. 2000; Jüllich et al. 2005]. The segmental expression of snail1a, Engrailed, and myoD arises simultaneously in the anterior trunk somites at the approximately six somite stage, while subsequent expression of these genes arises sequentially as each new somite is generated [Hatta et al. 1991; Ekker et al. 1992; Hammerschmidt and Nüsslein-Volhard 1993; Thisse et al. 1993; Weinberg et al. 1996; Jüllich et al. 2005]. However, none of these differentially expressed genes has been shown to function in the segmentation program. It is also unknown if the localized expression of these genes is related to the earlier specification of the anterior and posterior trunk.

Summary

The findings of Szeto and Kimelman [2006] begin to integrate our understanding of mesoderm patterning with vertebrate segmentation. Using a series of isochochronic and heterochronic cell transplantation experiments, they show that the progenitors of the anterior trunk, posterior trunk, and tail somites are specified during a 1-h period in the zebrafish blastula by nodal, bmp, and fgf signaling. Despite this patterning, fate mapping experiments indicate that these distinct MPC populations intermingle in the late blastula and within the posterior tailbud. At the cellular level, it is not clear how such a pattern is generated nor how cells “remember” when to exit the progenitor pool and enter the segmentation program. We also do not understand how the early specification of anterior trunk, posterior trunk, and tail MPCs relates to the control of segmentation, although the two processes share a dependence on t-box genes, fgf signaling, and wnt signaling. The unresolved questions in the field involve larger issues fundamental to modern developmental biology and molecular medicine. Questions such as how signals are integrated, how one cell population can respond differently to the same signal at different times, and how cells decide to exit their progenitor stem cell niche and begin to differentiate need to be understood to both accurately model vertebrate development and to adroitly engineer stem cells.

Acknowledgments

I thank Bruce Draper, Gilbert Weidinger, and Randy Moon for clarifications, and Tim Brend, Dörthe Jüllich, and Andrew Mara for critical comments on the manuscript. S.A.H. is supported by a grant from NICHD (HD045738-01A1).

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*Genes Dev.* 2006, 20:
Access the most recent version at doi:10.1101/gad.1453706

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