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Tales of tails (and trunks): forming the posterior body in vertebrate embryos

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Summary

A major question in developmental biology is how the early embryonic axes are established. Recent studies using different model organisms and mammalian in vitro systems have revealed the surprising result that most of the early posterior embryonic body forms from a Wnt regulated bipotential neuromesodermal progenitor population that escapes early germ layer patterning. Part of the regulatory network that drives the maintenance and differentiation of these progenitors has recently been determined, but much remains to be discovered. This review discusses some of the common features present in all vertebrates, as well as unique aspects that different species utilize to establish their anterior-posterior (A-P) axis.

1. Formation of the posterior body

In all vertebrates the posterior body comprises everything except the head and is subdivided into two major domains; from the head to the anus or cloaca is the trunk, and from the anus or cloaca to the most posterior end is the tail (Figure 1B). The tissues present in both regions of the embryonic posterior body include the somites, which produce musculature, dermis, bone and cartilage, the neural tube, the notochord, and the vasculature (Figure 1A), whereas the trunk region includes all of the aforementioned tissues as well as the gut, which terminates at the anus, and the pronephros, which ends at the cloaca. During early development the posterior body grows progressively from the anterior to posterior, which is seen most obviously with the somites that form at regular intervals starting with the most anterior somite, just posterior to the head, and ending with the most posterior somite (Benazeraf and Pourquie, 2013; Holley, 2007). Although this period when the axis of the embryo elongates and the anterior-posterior (A-P) axis of the vertebrate embryo is completely established has different names in different model systems, here it will be referred to as the somitogenesis stages. This progressive mode of growth from anterior to posterior is observed in a broad range of species, and while not used in long germ-band insects such as the widely studied *Drosophila*, is found in the less well-studied but more ubiquitous short germ-band insects and may represent the ancestral mode of posterior body development (Martin and Kimelman, 2009).

The formation of the posterior body has fascinated scientists for well over a century and consequently there is an enormous body of work investigating this issue, yet as recent studies have shown, there is much more to learn and many new tools from ES cells to improved transgenic technology are now available to advance our understanding. However, because of the different morphologies of various vertebrate embryos, as well as the types of

studies performed in each of the common model organisms, it is often difficult to piece together shared features from the literature. In this review I will focus on the mechanisms that regulate differentiation of the early embryonic progenitors during A-P axis formation, emphasizing features that unite the vertebrates from fish to humans while also pointing out interesting species specific differences that reflect evolutionary changes.

2. Differing morphological features versus a common mechanism

Until relatively recently, one of the major ways to study embryos was to focus on morphological features visible under white light, but these structures often represent physical adaptations used by different species of embryos and are less revealing of the underlying mechanism than is often ascribed to them. For example, much of the literature refers to the tailbud, a group of cells located at the most posterior end of the growing embryonic body during the post-gastrula stages. However, despite the name, the tailbud does not necessarily produce the true tail. For example, the zebrafish tailbud forms just as gastrulation ends and contributes cells to both the trunk and tail (Kanki and Ho, 1997) whereas the chick tailbud forms later and does represent the cells forming the tail (Criley, 1969). Other visibly defined features in posterior growth include a post-gastrula region of amniote embryos (e.g. chick and mouse) comprising the caudal end of the node and the rostral end of the primitive streak called the node-streak border (Wilson et al., 2009), but this feature is not found in non-amniotes (e.g. fish and frog). Similarly, cells of the chordoneural hinge play an essential role in embryonic development in many species (Wilson et al., 2009), but this structure does not exist in fish which have the equivalent cells located in a somewhat different region (Martin and Kimelman, 2012). Thus, while these different and species-specific features provide useful landmarks for investigators, recent evidence discussed below reveals that all vertebrate embryos use a common mechanism to form the posterior body.

3. The neuromesodermal progenitor

While it is clear that cells at the very posterior end of the extending body contribute to a variety of cell types (Figure 1A), a long-standing and major question has been whether these derive from a pluripotent population or from a set of progenitors with very restricted fates. As with many issues in biology, the answer lies somewhere in the middle. The general view has been that germ layer patterning occurs very early in development, and one of the major accomplishments in developmental biology over the past 30 years has been defining the many signals and pathways that establish these fates (Kimelman, 2006; Ozair et al., 2013; Zorn and Wells, 2009). However, recent work has clearly shown that progenitors of the posterior body remain in a multi-potent state during the somitogenesis stages. A cell lineage study in frogs intriguingly suggested that the cells at the most posterior end of the embryo could contribute to multiple tissues such as muscle, notochord, neural and epidermis, but this study was limited by an inability of the investigators to label single cells (Davis and Kirschner, 2000). However, a lineage study using transgenic mouse embryos definitively showed that many cells of the embryonic posterior body remain in a bipotential neuromesodermal state late into development that produces cells contributing to the somites and spinal cord, whereas endoderm, epidermis, and cranial and cardiac mesoderm segregate early as independent lineages (Figure 2, Tzouanacou et al., 2009). The existence of this

neuromesodermal population has also been found in fish, demonstrating that this is a conserved feature of vertebrate embryonic development (Martin and Kimelman, 2012). Very recent studies have shown that it is possible to produce the neuromesodermal progenitors (NMPs) in vitro using mouse and human stem cells (Gouti et al., 2014; Tsakiridis et al., 2014; Turner et al., 2014), opening up the possibility of detailed genomic analysis of the neuromesodermal state.

Of the other tissues in the posterior body the data is still incomplete. Some of the tail vasculature comes from the posterior progenitor population, at least in fish (Martin and Kimelman, 2012), but most of the vasculature originates separately in the embryo (Saha et al., 2004). The origin of the notochord is also uncertain (Tzouanacou et al., 2009), although a recent study in mouse indicates that the NMPs and notochord are separate populations (Garriock et al., 2015). In the non-amniotes the notochord originates from the dorsal marginal zone, separate from the lateral and ventral marginal zones where most of the posterior body originates (Dale and Slack, 1987; Kimelman and Martin, 2012; Warga and Nusslein-Volhard, 1999), and these different cells are only brought near each other by the mechanics of gastrulation, consistent with an ancestral separation of notochord and NMP fates. With the recent availability of in vitro models (Gouti et al., 2015), the exact nature of multipotency in the mammalian NMPs should now be testable.

4. Stem or Progenitor cells?

While it is now clear that there are multipotent progenitor cells that form the posterior body, an important question is whether or not these progenitor cells are also stem cells, with self-renewing capacity. In amniotes, the evidence is increasingly in favor of a stem-cell capacity (Nicolas et al., 1996; Selleck and Stern, 1992; Wilson et al., 2009). For example, the chordoneural hinge regions of mouse and chick embryos could be transplanted into the primitive streak region of earlier host embryos through multiple passages and they continued to contribute cells to the somites, neural tube and notochord (Cambray and Wilson, 2002; McGrew et al., 2008; Tam and Tan, 1992). In non-amniotes the issue is not resolved since these types of transplantation experiments have thus far not been technically feasible. Since it is not yet clear whether lower vertebrates have self-renewing capacity, at this point it is safest to say that all vertebrates have a population of posterior progenitors that at least in amniotes has stem-like self-renewing capabilities.

In amniotes, the progenitor cells that contribute to the medial and lateral regions of the somites have separate origins. Cells destined for the medial region of somites (closer to the midline) have stem cell properties based on lineage studies whereas the lateral somite cells have a much more restricted developmental potential (Freitas et al., 2001; Iimura et al., 2007; Selleck and Stern, 1991). What regulates these two different modes of development is unclear, but potentially tied to signaling events occurring during gastrulation. It has been suggested that this division of the somitic mesodermal precursors is preserved in the lower vertebrates (Iimura et al., 2007) since zebrafish have a discrete population of medial muscle cells that form next to the notochord (the adaxial cells, Stickney et al., 2000). However, there is no evidence that the zebrafish adaxial cells, which form a small amount of slow muscle in the embryo unlike the bulk of the somites that form fast muscle, have stem-cell properties.

Moreover, the adaxial progenitors have little proliferative capacity compared to the fast muscle progenitors (Bouldin et al., 2014; Hirsinger et al., 2004), and there is as of yet no evidence that they have both neural and mesodermal potential. Thus, it may be that the common vertebrate ancestor had NMPs with a limited proliferative potential that formed all of the somites, and that amniotes developed both NMPs with stem-like self-renewing capacity and non-NMP derived cells to form the lateral parts of the somites.

5. Brachyury and the NMP niche

The *brachyury* mouse mutant was discovered almost 90 years ago as recessive gene causing a short tail phenotype in heterozygotes (*brachyury* is Greek for short tail, Dobrovolskaia-Zavadskaia, 1927), and a lack of notochord and an almost complete absence of somitic mesoderm in homozygotes (Chesley, 1935; Gluecksohn-Schoenheimer, 1944). Since this gene has different names in different species (e.g. *T* in the mouse), I will use the name *brachyury* to refer to it in all vertebrates. In zebrafish, a *brachyury* mutant was discovered that produced homozygous embryos with a normal head and trunk but lacking a tail, suggesting that it has a less important role in lower vertebrates (Halpern et al., 1993; Schulte-Merker et al., 1994). Later studies revealed that fish have a second *brachyury* gene, and that embryos unable to express both genes recapitulate the mouse mutant phenotype, demonstrating a conserved and crucial ancestral role for this gene among the vertebrates (Martin and Kimelman, 2008).

Brachyury is the founding member of the T-box transcription factor family (Herrmann et al., 1990) and is co-expressed with *sox2* in the NMPs (Figure 3, Garriock et al., 2015; Martin and Kimelman, 2012; Olivera-Martinez et al., 2012; Tsakiridis et al., 2014), allowing the NMPs to adopt either a mesodermal fate (*brachyury*+/*sox2*-) or a neural fate (*brachyury*-/*sox2*+) when they begin to differentiate. Despite *brachyury* being essential for formation of the notochord and almost all somite mesoderm, a cell transplantation study revealed the surprising result that individual cells lacking *brachyury* function are able to normally differentiate into muscle cells (Martin and Kimelman, 2008), although they are not able to differentiate into notochord (Halpern et al., 1997). These results revealed that the major function of *brachyury* is non-cell autonomous, and led to the idea that *brachyury* is essential in the whole embryo for forming the somites because it creates the niche that allows the NMPs to remain as progenitors (Martin and Kimelman, 2010). That key targets of Brachyury are specific Wnt ligands (Martin and Kimelman, 2008), together with previous results showing that *brachyury* is regulated by Wnt (Arnold et al., 2000; Vonica and Gumbiner, 2002; Yamaguchi et al., 1999), demonstrates that Brachyury and Wnt participate in an autoregulatory loop required to maintain the NMPs (Figure 3).

A second important function of Brachyury is to induce expression of the retinoic acid (RA) degrading enzyme Cyp26a in order to prevent RA, which is produced within the somites, from spreading posteriorly and thus disrupting the Brachyury-Wnt autoregulatory loop within the NMPs since RA inhibits *brachyury* expression (Iulianella et al., 1999; Martin and Kimelman, 2010; Sakai et al., 2001). Although Cyp26a would not be typically thought of as a non-cell autonomous factor since it is not secreted, the NMP cells must work as a

community to degrade RA, preventing it from altering the fate of the NMPs during the stages of axis elongation.

6. Wnt signaling and the neuromesodermal fate choice

How do the NMP cells decide which fate to choose between neural and mesodermal? Evidence from fish to embryonic stem (ES) cells now strongly supports the view that Wnt is a critical regulator of this decision. In fish embryos, single NMP cells with Wnt signaling blocked become neural, whereas NMP cells expressing a constitutively active form of the downstream Wnt effector β -catenin (ca- β -catenin) are directed to the mesodermal fate (Martin and Kimelman, 2012). Similar results have been observed in mammalian ES cells (Gouti et al., 2015; Gouti et al., 2014). Moreover, mice lacking either the key posterior Wnt, Wnt3a, β -catenin, or the Wnt-responsive transcription factors Tcf1 and Lef1, show an expansion of neural tissue and a severe deficit in mesoderm (Cunningham et al., 2015; Dunty et al., 2008; Galceran et al., 1999; Garriock et al., 2015; Nowotschin et al., 2012; Takada et al., 1994; Yoshikawa et al., 1997), all of which support a key role for Wnt signaling in suppressing neural fate and activating the mesodermal fate. Recent gain of function studies in mouse are, however, somewhat contradictory. One study showed that overexpression of Wnt3a inhibits neural tissue formation but does not promote mesoderm (Jurberg et al., 2014) while another showed that overexpression of ca- β -catenin promotes mesoderm but does not fully block neural fates (Garriock et al., 2015). These discrepancies may be due to the domain-specific expression levels of the different promoters used in the two studies (Jurberg et al., 2014), as well as the nature of the Wnt pathway activators since ca- β -catenin is not subject to the types of feedback control that the Wnt ligand is (MacDonald et al., 2009). While it remains possible that there are some differences among the vertebrates in precisely how signaling controls differentiation of the NMPs, thus far the preponderance of the evidence indicates that as the posterior body extends, NMPs are making neural or mesodermal choices depending on their exposure to Wnt signaling. We have proposed that this system allows the embryo to allocate cells to either the mesodermal or neural lineages as the body extends by dynamically regulating Wnt levels in the most posterior region of the embryo, ensuring that at every axial position the embryo produces the correct ratio of spinal cord to somite-derived mesoderm (Martin and Kimelman, 2010).

7. Wnt signaling and establishment of the NMPs

Wnt signaling has an additional important role in the establishment of the NMPs, being essential for their formation in vivo (Garriock et al., 2015) and for their formation in vitro together with Fgf (Gouti et al., 2014; Tsakiridis et al., 2014; Turner et al., 2014). How is Wnt able to both establish the NMPs and direct NMPs toward a mesodermal fate? In zebrafish, we have found that Wnt signaling is present in the NMP containing progenitor zone, and then increased in the cells that have just begun the commitment to mesoderm (Bouldin et al., 2015). Based on this and other data, we propose that NMP cells are maintained by an intermediate level of Wnt, and then if they see elevated levels of Wnt they commit to mesoderm, whereas if they see decreased Wnt they adopt the neural fate (Figure 3). Intriguingly, mathematical modeling and experiments in *Xenopus* have shown that cells respond to fold-changes in Wnt signaling not absolute levels (Goentoro and Kirschner,

2009), which would allow cells to change fate as they move between regions with different levels of Wnt signaling even if the absolute levels of Wnt signaling changes as the axis elongates. The new in vitro models of mammalian NMPs (Gouti et al., 2015) will provide an excellent system to determine if activation of the mesodermal program in mammalian embryos is similarly regulated by fold-changes in the level of Wnt pathway activation.

8. Tbx6/16 and the acquisition of mesodermal fate

If cells leave the progenitor zone and are no longer exposed to Wnt signaling they maintain *sox2* expression and commit to the neural fate, which has been well covered in an excellent recent review and is therefore not discussed here (Gouti et al., 2015). In contrast, if cells leaving the progenitor zone are exposed to high Wnt signaling they adopt the mesodermal fate by activating expression of particular members of the *tbx6/16* family, which all derive from a common ancestral gene (Ahn et al., 2012), but which have varying roles depending on the species. For example, mouse embryos lacking *tbx6* or zebrafish embryos lacking *tbx16* have a severe deficit in somite-derived mesoderm and an enlarged tailbud that contains the cells that normally would contribute to the somites (Chapman et al., 2003; Chapman and Papaioannou, 1998; Griffin et al., 1998; Kimmel et al., 1989). Remarkably, mouse *tbx6* mutants form ectopic neural tubes (Chapman and Papaioannou, 1998), and both mouse *tbx6* mutants and zebrafish *tbx16* mutants upregulate the neural marker *sox2*, demonstrating that cells leaving the NMPs adopt a neural fate at the expense of mesoderm when *tbx6/16* gene function is absent (Figure 3, Bouldin et al., 2015; Takemoto et al., 2011).

In addition to being necessary for the activation of genes involved in somite mesoderm formation (Bouldin et al., 2015; Chapman and Papaioannou, 1998; Griffin and Kimelman, 2002; Takemoto et al., 2011), Tbx6/16 directly activates expression of the *delta* and *mesp* genes that regulate formation of the boundaries between somites (Garnett et al., 2009; Jahangiri et al., 2012; White and Chapman, 2005; Yasuhiko et al., 2008). In fish, Tbx16 acts as a dual function activator/repressor, and through its repressive function is able to inhibit *sox2*, thus blocking the neural fate (Figure 3, Bouldin et al., 2015). In mouse, detailed analysis has identified a small fragment of the *sox2* enhancer (enhancer N1) that mediates Sox2 repression within the newly forming mesoderm downstream of Tbx6 (Takemoto et al., 2006; Takemoto et al., 2011). In vitro analysis indicates that Tbx6 does not bind *sox2* enhancer N1, and thus in mouse the repression of *sox2* may be indirect (Kondoh and Takemoto, 2012; Takemoto et al., 2011), although the function of Tbx6 in vivo and on the whole *sox2* promoter has thus far not yet been studied. However, zebrafish does not obviously have the N1 enhancer element (Okuda et al., 2006), and so it is possible that the mechanism of *sox2* regulation by Tbx6/16 has diverged over time.

Tbx6/16 turn off the progenitor fate by also inhibiting *brachyury* expression (Chapman and Papaioannou, 1998; Griffin and Kimelman, 2002), although in this case the repression is indirect, mediated by inhibition of *wnt* gene expression (Bouldin et al., 2015; Kondoh and Takemoto, 2012; Takemoto et al., 2011). Thus, as cells adopt the mesodermal fate, Tbx6/16 locks them into the mesodermal fate by turning off the neural fate and progenitor state (Figure 3). Why is this important? Wnt signaling is confined to the most posterior end of the embryo, which is clearly essential because forced expansion of Wnt signaling anteriorly

causes serious defects in mesodermal differentiation, somite formation and A/P patterning of the somites (Aulehla et al., 2008; Dunty et al., 2008; Garriock et al., 2015; Jurberg et al., 2014). Thus, while Wnt signaling directly turns on *tbx16* in the most posterior end of the fish embryo (Bouldin et al., 2015), and potentially also *tbx6* in the mouse (Dunty et al., 2008), cells rapidly leave the Wnt zone as they move anteriorly. These cells therefore could potentially revert to a progenitor state or switch to becoming neural if they didn't continue to experience high Wnt levels. However, using the regulatory logic shown in Figure 3, once the cells commit to mesoderm they will not adopt another fate.

9. Mesogenin1 and mesodermal fate

In addition to *tbx6/16*, another important mesodermal regulator activated by Wnt in the newly forming mesoderm is *mesogenin1* (*msgn1*, Nowotschin et al., 2012; Wittler et al., 2007; Yoon and Wold, 2000). In the mouse, the *msgn1* mutant phenotype is similar to *tbx6* mutants with an enlarged tailbud and a loss of all but the anterior somites (Yoon and Wold, 2000). Interestingly, *Msgn1* is at least partially necessary for *tbx6* expression and *Tbx6* is at least partially necessary for *msgn1* expression, demonstrating that these factors act in an autoregulatory loop and that both are necessary for mesoderm formation (Figure 4, Chalamalasetty et al., 2014; Nowotschin et al., 2012; Wittler et al., 2007). *Msgn1* overexpression and loss of function studies show that it is unable to suppress neural fates even as it promotes mesoderm formation from the NMPs (Chalamalasetty et al., 2014; Nowotschin et al., 2012), although it does act to inhibit *wnt3a* expression, presumably indirectly (Nowotschin et al., 2012). Thus, it has both similarities and differences to *Tbx6* (Figure 4).

In zebrafish the regulatory logic is somewhat different since loss of *msgn1* has a quite mild phenotype causing an average 15% reduction in the size of the somites (Fior et al., 2012; Yabe and Takada, 2012). While *tbx16* mutants have a complete lack of trunk somites, they do produce small tail somites, whereas a loss of *tbx16* and *msgn1* causes a complete absence of all somites (Fior et al., 2012; Yabe and Takada, 2012). This demonstrates that *msgn1* in fish, as in mouse, can control mesoderm differentiation. The reason for the trunk/tail difference is that *Tbx16* is necessary for *msgn1* expression during the trunk forming stages but not during the tail stages of somitogenesis (Griffin and Kimelman, 2002). Thus, unlike in mouse, *Msgn1* has a subsidiary role to the *Tbx6/16* factors, which is most clearly revealed when *Tbx16* is not present (Figure 4). The role of *msgn1* in other vertebrates remains to be determined.

10. Cdx contributes to posterior development

The *Caudal*-related genes (*cdx* in vertebrates) also play essential roles in axial elongation. In mouse, three *Cdx* genes are likely to be involved directly in this process through the direct regulation of *Wnt3a*, *Brachyury*, and *Cyp26a1* (Savory et al., 2009; Young et al., 2009), each of which promotes the NMP state (Figure 4). At least two of the *Cdx* genes are also direct targets of Wnt signaling (Lickert et al., 2000; Pilon et al., 2006), and so the *Cdx* factors operate in an autoregulatory loop that parallels the one between Wnt and *Brachyury*. Intriguingly, whereas *Cdx2*^{+/-}; *Cdx4*^{-/-} embryos have strong axis extension defects,

individual mutant cells with this genotype behave normally in a wild-type background, indicating that a major role for the *Cdx* factors is to non-cell autonomously regulate axis extension (Bialecka et al., 2010), which is very reminiscent of the essential non-cell autonomous role for *Brachyury*, discussed above.

In zebrafish, knockdown of the two *cdx* genes expressed during the gastrula and somitogenesis stages causes strong posterior defects, similar to those observed when Wnt signaling is inhibited (Davidson and Zon, 2006; Shimizu et al., 2005; Thorpe et al., 2005). Similar results are observed in *Xenopus*, where experiments indicate that the *Cdx* genes, as in mouse, act in a positive feedback loop with Wnt signaling (Faas and Isaacs, 2009). Thus, the evidence indicates that in all vertebrates the *Cdx* genes play a key role in preserving the signaling environment necessary for maintenance of the NMPs.

11. Termination of body elongation

The posterior body will continue to form somites and spinal cord as long as there are NMPs to produce these tissues. However, at some point the progenitors are exhausted and the extension of the body terminates, completing formation of the embryonic A-P axis. In zebrafish, the NMPs are proliferative during the gastrula stage but then become quiescent during the somitogenesis stages (Bouldin et al., 2014) so termination of the axis may simply occur when the last of the NMPs begins differentiation. Evidence in chick indicates that as the embryo reaches the full extent of axis elongation, *brachyury* expression disappears from the NMPs, leading them to switch to a neural fate (Olivera-Martinez et al., 2012). The cause of *brachyury* down-regulation was attributed to an increase of RA at the posterior end of the embryo during the late stages of axis elongation, which in turn causes inhibition of Fgf signaling in the posterior end of the embryo, and consequent *brachyury* down-regulation. However, in fish and mouse, blocking RA synthesis does not affect termination of axis formation (Begemann et al., 2001; Cunningham et al., 2011) so this mechanism may be specific to only some species.

In addition, a recent study in chick has shown that a subset of posterior *Hox* genes can inhibit Wnt (and Fgf) signaling, with the most posterior *hox* genes having the greatest strength of repression (Denans et al., 2015). Since Wnt and Fgf promote *brachyury* expression and since *Brachyury* promotes expression of the RA degrading enzyme Cyp26a (Martin and Kimelman, 2010), the most posterior *Hox* genes in chick could cause a rise in RA in the NMPs, and thus the two models for axis termination in chick could be connected (Figure 5, Denans et al., 2015; Olivera-Martinez et al., 2012). Intriguingly, *Hoxb13* mutant mice have supernumerary vertebrae and an overgrowth of the posterior spinal cord, although this was attributed to increased proliferation and decreased apoptosis (Economides et al., 2003). It will be interesting to examine the signaling and transcription changes during the stages of axis termination in these mice in light of the recent studies in chick.

11. Conclusions and future directions

The discovery of the bipotential NMPs has opened up a major new way of understanding how the vertebrate posterior axis elongates with the discovery that these cells escape the

early mechanisms of germ layer patterning. The use of several model systems has been instrumental in elucidating common features of posterior growth in the vertebrates, demonstrating similar mechanisms operating from fish to humans. With the recent development of mammalian in vitro systems as well as constant improvements in in vivo approaches, it will now be possible to make rapid progress in understanding both common features and species-specific differences in the regulation of these progenitors as they make their different cell fate decisions. But these decisions must also be coupled with precise cell movements to actually form the posterior body, and this is an exciting area that is being aided with improving optics and methodology (Benazeraf and Pourquie, 2013; Lawton et al., 2013; Uriu et al., 2014). Combining an understanding of intercellular signaling, genomic regulation and morphogenesis is certain to bring exciting new discoveries to the long-standing question of how the vertebrate embryonic body forms.

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Figure 1. Morphological features of the vertebrate posterior body

(A) A generic cross section through the post-anal region of a vertebrate embryo. In the pre-anal region, the pronephric tubules and gut tube would also be present. (B) The posterior of the embryo is divided into pre-anal trunk and post-anal tail. Shown is a zebrafish embryo but the same division applies to all vertebrates.

Figure 2. Maintenance of the neuromesodermal progenitors

Lineage labeling studies in the mouse show that while many germ layer decisions are made early, a neuromesodermal progenitor population continues to produce both neural and mesodermal cells during the somitogenesis stages (Tzouanacou et al., 2009). The times at which the gastrula stage fate decisions are made is approximate.

Figure 3. Model for regulation of mesoderm formation in zebrafish

Sox2-expressing NMP cells are in a zone of moderate Wnt signaling (light orange) that maintains Brachyury. As cells leave this region, if they are not exposed to continued Wnt signaling, they express Sox2 but not Brachyury and become neural. Instead, if they are exposed to high levels of Wnt (dark orange) they express Tbx16, which represses Sox2. Tbx16 also represses Wnts so as cells continue to move anteriorly (left side), they shut off Brachyury and turn on mesodermal differentiation genes, thus locking in the mesodermal choice.

Figure 4. Comparison of the regulatory logic of fish and mouse posterior cell fate choices

A schematic showing the regulatory relationships among signaling and transcription factors discussed in this review. Tbx6 is shown here to cause repressive effects indirectly, but as with Tbx16, it may turn out to be able to directly repress target genes. Fgf is omitted for simplicity.

Figure 5. Model for termination of axis extension in chick

During somitogenesis, the progenitor domain excludes RA. Near the end of somitogenesis, the most posterior *Hox* genes, possibly together with other molecular changes, causes RA to expand posteriorly, thus inhibiting the progenitor cell fate.