Anterior-posterior patterning and segmentation of the vertebrate head

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Synopsis Segmentation of the vertebrate head emerges out of earlier processes that establish the anterior-posterior (A-P) axis. Recent genetic studies and comparisons across species have led to a better understanding of the links between A-P patterning and segmentation. These point to similar signals acting on both head and trunk, such as retinoic acid and fibroblast growth factors. These form interacting networks of diffusible morphogen gradients that pattern both hindbrain rhombomeres and mesodermal somites. New computational models, particularly for retinoic acid, have revealed how morphogen gradients are established and made robust to changes in signaling levels. However, the orientations of these gradients, as well as how they interact to generate segments, differ remarkably between germ layers and body regions. Thus, the vertebrate head is, in part, built through modifications of the same processes that link A-P patterning and segmentation in the trunk, but fundamental differences in how these processes are deployed lend further doubt to the notion that head and trunk segments are homologous.

Introduction

The idea that the vertebrate head is fundamentally segmented dates back to the early 19th century with the vertebral theories of Goethe (1820) and others (de Beer 1937; Northcutt 2008). Comparative embryologists emphasized the segmentation of mesoderm and its relationship to the mesodermal somites of the trunk and tail (Balfour 1878; Goodrich 1930). However, the head contains several segmental structures derived largely, or entirely, from ectoderm, namely the rhombomeres of the hindbrain and arches of the pharyngeal apparatus, which are difficult to align with any mesodermal organization. One feature that is shared in common with all of these tissues is an earlier dependence on graded signals that pattern the anterior-posterior (A-P) axis. In this article, I discuss new evidence showing how networks of morphogen gradients are established and deployed in distinct ways in the cranial region.

One main reason for a renewed interest in head segmentation has come from studies on the embryonic hindbrain (Lumsden and Keynes 1989; Fraser et al. 1990; Trevarrow et al. 1990; reviewed by Moens and Prince 2002; Lumsden 2004). These have provided clear evidence for a rhombomeric organization underlying the pattern of cranial nerves, which correlates with the migratory pathways of cranial neural-crest cells that contribute to the pharyngeal arches. In contrast, the spinal cord does not display an obvious, intrinsic, early segmental patterning. Rather, spinal nerve segmentation is dictated by surrounding somites (Keynes and Stern 1984; Eisen and Pike 1991; Ensini et al. 1998). Molecular developmental biology has shown segmental patterns of gene expression in rhombomeres and arches, and requirements for Hox genes in both sets of structures, but these have failed to resolve the issue of the basic segmental plan underlying the organization of the vertebrate head (reviewed by Schilling and Knight 2001). At least two fundamental questions remain: (1) how are A-P patterning and segmentation linked and (2) how do these mechanisms differ between germ layers (i.e., ectoderm and mesoderm) and body regions (i.e., head and trunk)?

A-P patterning of head segments

In vertebrates, regional induction of cell fates along the A-P axis is perhaps best understood for the neural plate. Tissue derived from the dorsal mesoderm of Spemann's organizer in amphibians induces neural identity and also regionally specifies neural tissue as future brain or spinal cord (Mangold 1933). The first signs of segmentation in the brain emerge morphologically soon after gastrulation as a series of bulges along the A-P axis of the neural tube,

including 7-8 hindbrain rhombomeres (Fig. 1). Rhombomeres are serially reiterated, each containing the same basic cell types (e.g., commissural inter-neurons, branchiomotor neurons), but also have distinct identities and contribute to different cranial nerves (Lumsden 2004). In the embryo, each rhombomere also forms a compartment, within which cell lineages are confined and separated by distinct boundary regions (Fraser et al. 1990).

Rhombomere identities are determined, at least in part, by a combinatorial code of trancription factors such as the Hox genes (Fig. 1; reviewed by Moens and Prince 2002; Lumsden 2004). Stripes of rhomb-omere-specific Hox gene expression domains appear in the neural ectoderm by the end of gastrulation and these are positioned by early signals along the A-P axis (Fig. 1A). Based primarily on studies in fish and amphibians, three signals in particular form an A-P patterning network: Wnt signals initially repress expression of anterior genes, after which Wnts, Fibroblast growth factors (Fgfs) and the vitamin A

derivative, retinoic acid (RA), activate posterior genes in a concentration-dependent manner (Cho and De Robertis 1990; Sive et al. 1990; Holowacz and Sokol 1999; Domingos et al. 2001; Kudoh et al. 2002). Fgf and RA, in particular, are good candidates for the molecular link between A-P patterning and segmentation, potentially acting as graded "mor-phogens" that establish boundaries of segments at distinct concentration thresholds.

Rhombomeric segmentation is also closely tied to the segmental formation and migration of cranial neural-crest cells that contribute to the pharyngeal arches (Fig. 1B). These migrate in three major streams, each with a distinct rhombomeric origin (stream 1, r2–3; stream 2, r4–5; stream 3, r6–7). Transplantations of rhombomeres along the A-P axis in avian embryos, grafted together with adjacent neural-crest cells bound for one of the three streams, lead to arch transformations in which the segmental fates of surrounding tissues are reorganized accord-ing to the identities of the transplanted/donor cells
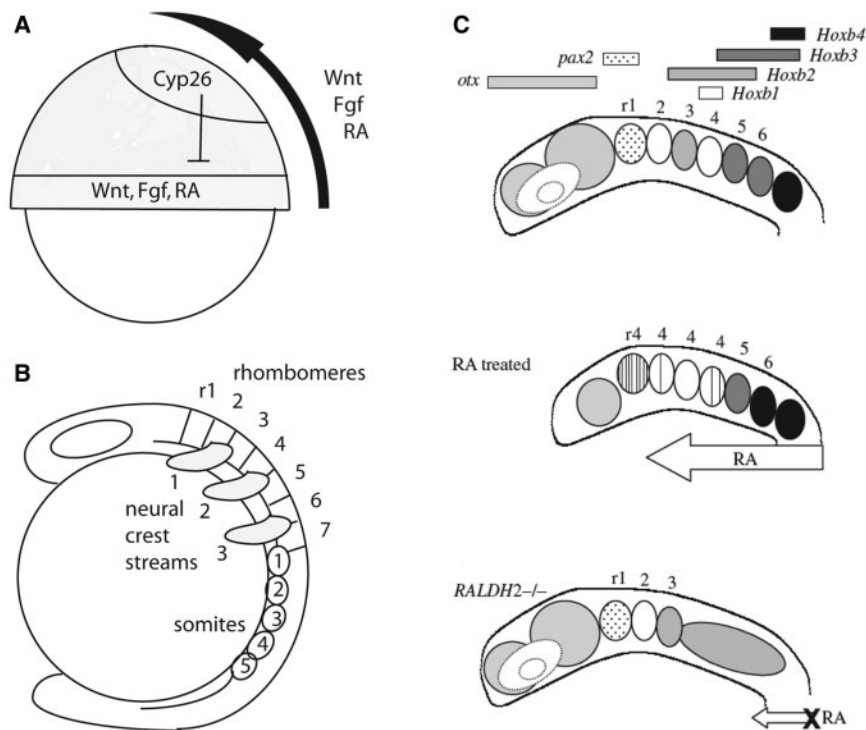
Fig. 1 A-P patterning signals and hindbrain segmentation. (A) Diagrams of gastrula-stage zebrafish embryos in lateral view, dorsal to the right, illustrating the proposed distribution of RA, Fgf, and Wnt signaling in the neural ectoderm (Kudoh et al. 2002). All three signals are made at the margin of the gastrula, the future posterior, and are thought to diffuse anteriorly to promote posterior development. (B) Segmentally organized structures in vertebrates, including rhombomeres of the hindbrain, streams of migrating cranial neural-crest cells, and mesodermal somites in the trunk, are schematized in a zebrafish at the five-somite stage. (C) Role of RA in A-P patterning of the hindbrain and in Hox regulation (Schilling and Knight 2001). Diagrams illustrate embryonic morphology and patterns of gene expression in the embryonic zebrafish head, lateral views, anterior to the left. Major regions of the brain are indicated, including forebrain, midbrain and rhombomeres (r) 1–7 of the hindbrain. Shaded bars above each diagram indicate the A-P extent of mRNA expression for each gene. Open arrows indicate RA treatments (10^{-7} M all trans RA) or disruption of RA synthesis (fish mutants or mouse mutants lacking a functional Aldh1a2).

(Noden 1983; reviewed by Noden and Trainor, 2005). This has led to the notion that, unlike the trunk in which primary segmentation occurs in the mesodermal somites, head segments are established first in the ectoderm (e.g., hindbrain and neural crest) and secondarily imposed on surrounding mesoderm. Both require RA and Hox genes for proper segmentation and this is thought to coordinate the cranial nerves derived from individual rhombomeres with structures that they innervate within the arches. Because reducing RA signaling leads to expansion of anterior rhombomeres and arches and to loss of posterior ones (Fig. 1C; Niederreither 2000; Begemann et al. 2001; Dupe and Lumsden 2001), the simplest interpretation of these results is that an early gradient of RA coordinates head segments through its roles in patterning the early neural ectoderm.

RA and the morphogen model

Recently, however, the role of RA as a graded morphogen in this system has been under intense debate, particularly in relation to the hindbrain. RA has long been known to specify posterior rhombomere identities, and to regulate many segment-specific target genes directly, including Hox genes. RA is not produced in the neuroepithelium, but is generated by Raldh2 in paraxial mesoderm in the trunk, and degraded by the cytochrome p450 enzyme Cyp26 (retinoic acid-4-hydroxylase) in the anterior neural plate (Fig. 2A) (Niederreither et al. 1997; Swindell et al. 1999). This has led to a model in which RA forms a gradient, declining from its posterior source in mesoderm to a degradation sink anteriorly, thereby patterning rhombomere identities.

Support for this model came first from treatments of amphibian and avian embryos with exogenous RA, which causes posterior rhombomeres to expand, apparently at the expense of anterior segments, in a concentration-dependent manner (Durston et al. 1989; Sive et al. 1990; Marshall et al. 1992; Godsave et al. 1998). Conversely, reductions in RA signaling cause anterior rhombomeres to expand and replace posterior segments in a concentration-dependent manner (Dupe and Lumsden 2001; Begemann et al. 2004; Maves and Kimmel 2005).

Arguments against the morphogen model have centered around the time-dependent aspects of the formation of rhombomeres. Rhombomeres, like somites, form more or less in an anterior to posterior sequence. Length of exposure to the RA signal may be just as important as its concentration. Timing could be regulated by precise patterns of

synthesis and degradation. Consistent with this idea, three Cyp26 enzymes (Cyp26a1, Cyp26b1, and Cyp26c1) are dynamically expressed in the prospective hindbrain and are required for segmentation of
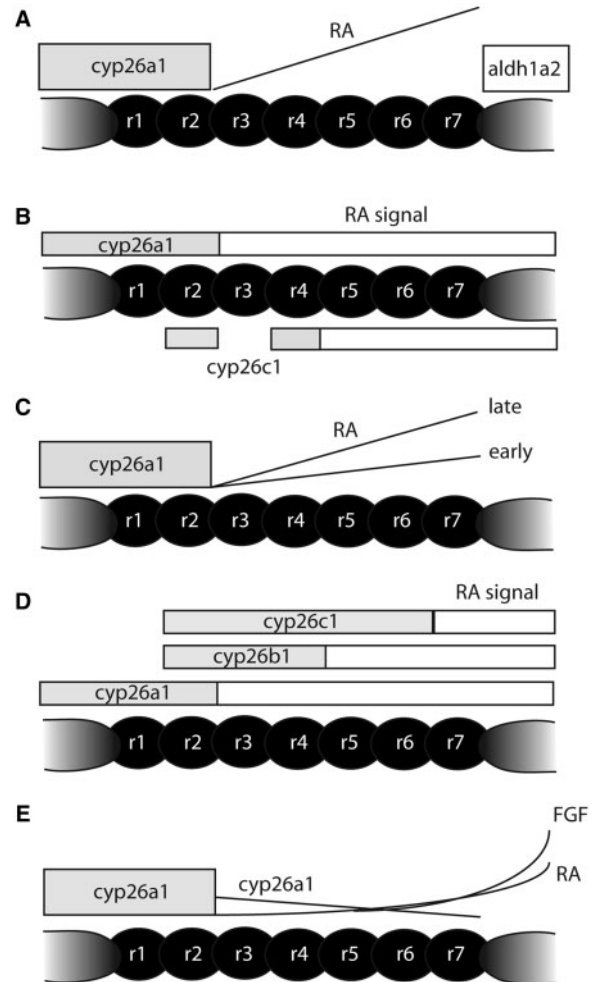
Fig. 2 Models for morphogen gradients in the hindbrain. Diagrams illustrating models emerging from recent studies. (A) The classic morphogen model. RA is synthesized posteriorly by Aldh1a2 and degraded anteriorly by Cyp26a1. Diffusion across the presumptive hindbrain creates a gradient that activates distinct target genes in a concentration-dependent manner. (B) A shifting boundaries model (Sirbu et al. 2005). Shifting domains of Cyp26-mediated degradation control the length of exposure to RA. In this model r5–7 are exposed longer than r3/4 because of cyp26c1 expression in the anterior hindbrain after gastrulation. (C) An increasing gradient model (Maves and Kimmel 2005). RA synthesis increases with time, activating posterior genes at higher concentrations and at later stages. (D) A modified shifting boundaries model (Hernandez et al. 2007). In this model, there are three distinct boundaries rather than two. (E) A modified morphogen model (White et al. 2007). Local degradation by Cyp26a1, under the positive control of RA and negative regulation by Fgf, shapes the RA signaling gradient. As the embryo grows, the RA gradient grows without increasing synthesis, integrating both spatial and temporal aspects of hindbrain patterning.

the hindbrain (Swindell et al. 1999; Abu-Abed et al. 2001; Sakai et al. 2001; Emoto et al. 2005; Sirbu et al. 2005; Hernandez et al. 2007). Recent studies have argued that ''shifting boundaries'' of degradation, rather than a gradient of RA, determine the pattern of rhombomeres (Fig. 2B and D). In these models, RA signaling initially extends up to the rhombomere 2/3 (r2/3) boundary, but later becomes restricted posteriorly to the r4/5 boundary due to degradation by Cyp26b1 and Cyp26c1. Posterior rhombomeres are specified by a longer exposure to RA than are more anterior segments, rather than by the concentration of RA.

None of these studies, however, can rule out a concentration-dependent role for RA. Concentration dependence is still the best explanation for the graded expansion or contraction of rhombomeres along the A-P axis with disruptions of RA signaling and for the responses of certain RA-dependent genes (i.e., hoxb1 and vHNF1; Sirbu et al. 2005). Zebrafish or mouse embryos lacking Cyp26c1 also do not show hindbrain defects, arguing against the later steps of degradation in these models (Uehara et al. 2006; Hernandez et al. 2007).

Simple timing of exposure to the RA signal also seems unlikely to play a major role in segmentation of the hindbrain. While Hox genes expressed in the posterior hindbrain are normally expressed later than are more anteriorly-expressed genes, their expression can be induced at earlier stages in zebrafish embryos with high concentrations of RA (Maves and Kimmel 2005). Thus, the thresholds needed to activate posteriorly-expressed genes may only be achieved at later stages, as the RA gradient grows with time, giving the appearance of a time-dependence where it does not exist (Fig. 2C).

If the spatial and temporal activities of RA are controlled by shifting boundaries of Cyp26-mediated degradation, some other A-P patterning information must control the expression of Cyp26 (Sirbu et al. 2005; Hernandez et al. 2007). Hernandez et al. (2007) argued that RA acts permissively, rather than instructively, in hindbrain segmentation and against a gradient model. They base this in part on the phenotypes of Cyp26-deficient zebrafish. Depletion of Cyp26b1 and Cyp26c1 in a $Cyp26a1^{-/-}$-mutant embryo leads to much more severe posteriorization than does loss of Cyp26a1 alone. However, the argument is also based on the curious fact that exogenous RA, applied uniformly to embryos (fish, frog, bird, or mammal) in an unlocalized manner, can rescue hindbrain defects in an RA-deficient animal. This argues strongly for a permissive role.

Two aspects of the RA signaling pathway, however, have not been taken into account in these models: (1) positive and negative feedback by various RA-induced components within the pathway and (2) their regulation by other signals, such as Fgf and Wnt. We have recently incorporated experiments addressing both of these components into a more computational, systems-level approach to RA signaling in the hindbrain of the zebrafish (Fig. 2E; White et al. 2007). This has led to yet another version of the gradient model, but one that we think can reconcile many features of the models discussed above.

We found that, in contrast to many previous studies, zebrafish cyp26a1 is expressed at low levels within the prospective hindbrain itself during gastrulation, rather than being restricted to presumptive forebrain and midbrain (Fig. 3; White et al. 2007). In addition, we showed that this low-level expression in the hindbrain is RA-dependent. RA induces Cyp26a1 expression, forming a negative feedback loop through inducible degradation that could play major roles in shaping the RA-signaling gradient. Thus, Cyp26 enzymes may not simply act as RA sinks wherever they are expressed, but rather act dynamically to modulate signaling gradients locally and compensate for fluctuations in RA levels. This makes sense for a signal derived from a dietary precursor (vitamin A) that may vary dramatically in its availability to the embryo.

In contrast, Cyp26a1 expression (and induction by RA) is inhibited by other posteriorizing signals, such as Fgfs (Kudoh et al. 2002). These form A-P gradients that parallel that of RA and yet appear to have the opposite effects on Cyp26a1. In our model, we propose that this creates an integrated signaling network that is extremely robust—i.e., able to compensate for changes in RA levels (Fig. 3; White et al. 2007). The model helps resolve questions surrounding both spatial/temporal and permissive/instructive aspects of RA signaling.

For instance, previous studies suggested that the RA gradient grows with time, possibly through a gradual increase in RA synthesis (Maves and Kimmel 2005). However, our model points instead to growth of the gradient through a gradual reduction in degradation as inhibition of Cyp26a1 by Fgf decreases during embryonic growth (Fig. 3A). This also seems preferable to a system dependent on precise control of RA synthesis from varying levels of dietary vitamin A. Instead, the RA gradient is tightly coupled to the less environmentally labile Fgf gradient. Our model also provides a very interesting resolution to the dilemma of why exogenous RA,
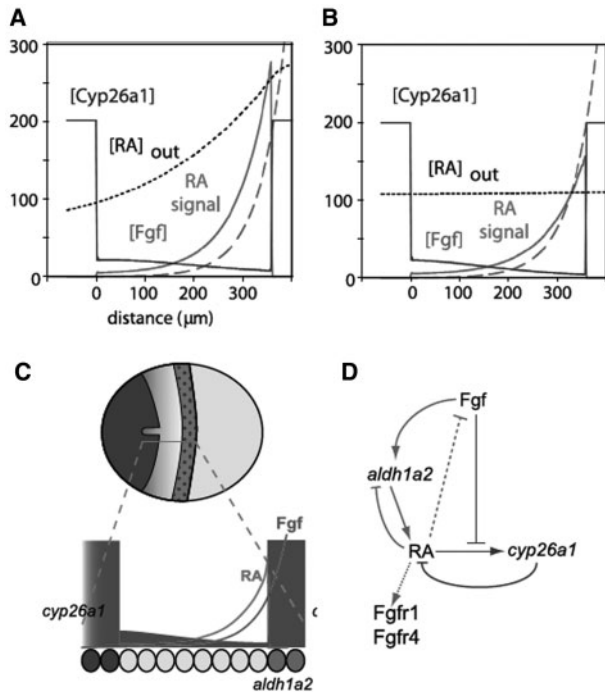
Fig. 3 The modified morphogen model. A one-dimensional mathematical model of the gastrula-stage zebrafish embryo (White et al. 2007). The model incorporates RA synthesis, diffusion, cell permeability, degradation and signaling, as well as a stable Fgf gradient, and expression of cyp26a1 under the control of RA and Fgf signals. (**A and B**) Zero on the abscissa denotes the posterior boundary of high cyp26a1 expression near the r1/2 border. Values on the ordinate are arbitrary. Fgf concentration is shown in green, cyp26a1 in blue, extracellular RA ([RA]out) in black, and RA signaling, which is a function of intracellular RA, in red. (**A**) Typical patterns generated by the model. Low-level cyp26a1 expression declines from anterior to posterior. (**B**) RA supplied in a delocalized manner can produce a relatively normal morphogen gradient. (**C**) A diagram illustrating a dorsal view of a gastrulating zebrafish embryo and corresponding gradients, anterior to the left. RA is produced by Aldh1a2 in somitic mesoderm (red) and diffuses through the neural ectoderm. Cyp26a1 (blue) degrades RA at differing rates to produce a gradient that specifies hindbrain fates. (**D**) Diagram of interactions between RA, Fgf, and Cyp26a1. Only those shown in blue are included in the mathematical model; dotted lines are extrapolated from results in other systems.

applied uniformly, rescues an RA-deficient embryo (Hernandez et al. 2007). The key is regulated degradation. By having RA induce and Fgf inhibit Cyp26a1 expression, uniform RA is rapidly converted into an intracellular concentration gradient (Fig. 3B).

It is important, however, to note that so far our model has only considered interrelationships between RA and Fgf signaling at early stages of gastrulation, when the synthesis of RA in trunk mesoderm and degradation by Cyp26a1 are only separated by a short distance (100–150 micrometers in zebrafish). Wnt signaling also inhibits Cyp26a1 expression at

these early stages and may act upstream to regulate competence to respond to RA and Fgf (Kudoh et al. 2002). In addition, a complex hierarchy of inter-rhombomeric interactions exists in the hindbrain by the end of gastrulation. For example, Fgfs (e.g., Fgf8) are expressed at the midbrain-hindbrain boundary and in r4, where in zebrafish they are required for patterning neighboring segments (Maves et al. 2002). Cyp26b1 and Cyp26c1 are expressed in different subsets of rhombomeres, in species-specific patterns, where they very likely protect cells from excess RA (Swindell et al. 1999; Sirbu et al. 2005; Hernandez et al. 2007). These may have important roles in modulating the later influences of RA on neurogenesis in the hindbrain, and this is an important topic for future research.

A reversal of the orientation of morphogen gradients in the head and trunk
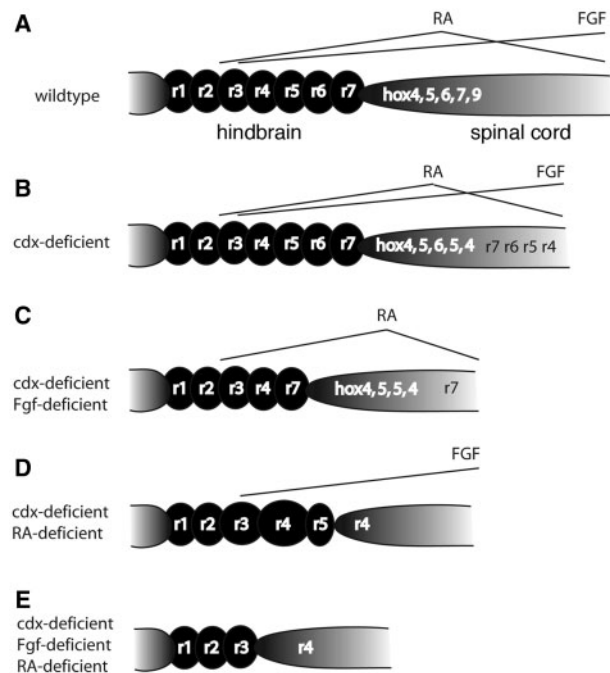
While the hindbrain subdivides into rhombomeres, the spinal cord remains unsegmented. However, both are patterned along the A-P axis by Hox genes. Fgf and RA also influence Hox gene expression and neurogenesis in the prospective spinal cord, as well as segmentation of the paraxial mesoderm into somites, suggesting that common signals control A-P patterning in head and trunk. Unlike the hindbrain, however, the gradients of RA and Fgf oppose one another in the trunk after gastrulation, with RA levels highest in the most anterior somites and Fgfs highest posteriorly (Fig. 4). This difference in the spatial deployment of at least one morphogen, RA, suggests a fundamental difference in the mechanisms by which these signals establish segmentation in the head and trunk.

Are apparent differences in A-P patterning between hindbrain and spinal cord due to some intrinsic difference in their ability to respond to the same signals? Two recent studies in zebrafish have shown that the spinal cord is competent to acquire a hindbrain identity.

Competence is under the control of the Cdx genes, homeobox-containing members of the ParaHox clusters, closely related to Drosophila *caudal*. Cdx genes suppress anterior development and promote posterior development both in vertebrates and invertebrates (Lohnes 2003; Deschamps and van Nes 2005). In mice, Cdx genes help integrate RA, Fgf and Wnt signals to regulate patterns of Hox expression in the paraxial mesoderm. Cdx proteins bind to cis-regulatory elements of posterior hox genes and directly activate their transcription (Subramanian et al. 1995; Pownall et al. 1996;

Charite et al. 1998; Isaacs et al. 1998). *cdx4*^{-/-}-mutant zebrafish have reductions in posterior *hox* gene expression (Davidson et al. 2003; Hammerschmidt et al. 1996) and combined depletion of cdx4 and cdx1a causes loss of *hoxb7a* and *hoxb9a* expression and severe posterior truncations (Davidson and Zon 2006; Shimizu et al. 2005).

Surprisingly, loss of Cdx function in these experiments on zebrafish also leads to ectopic hindbrain tissue in the spinal cord (Fig. 4; Shimizu et al. 2006; Skromne et al. 2007). Numerous hindbrain-specific *hox* genes, as well as differentiated branchiomotor neurons, are detected in the trunks and tails of Cdx-deficient embryos. Furthermore, genes marking different rhombomeres are spatially localized within this ectopic hindbrain tissue in a reversed orientation; anterior markers such as *hoxb4* are expressed further posteriorly than is *hoxb6* (Fig. 4B). The authors interpret this to mean that the Cdx genes control the responsiveness of spinal-cord cells to RA, and that RA gradients are reversed between the hindbrain and spinal cord, with the pivot point occurring somewhere around the level of the third somite. These studies also demonstrate that,
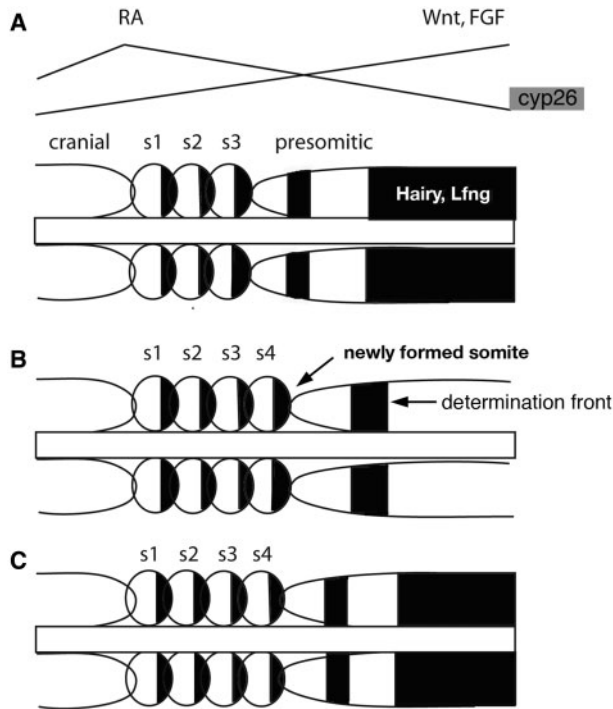
at least within the neural ectoderm, cells are competent to acquire hindbrain fates and possibly to become organized into rhombomeres all along the A-P axis.

Consistent with the hypothesis that Cdx genes modulate the responses of cells to posteriorizing signals, ectopic hindbrain tissue in Cdx-deficient embryos is suppressed by inhibiting RA or Fgf (Shimizu et al. 2006; Skromne et al. 2007) (Fig. 4C–E). Embryos deficient for both Cdx1a/4 and Fgf signaling still form mirror-image duplicates of hindbrain tissue in the spinal cord, but these duplicates only retain markers of more posterior rhombomeres and appear to lack r4-6 (Fig. 4C). Likewise, Cdx-deficient embryos with severely reduced RA signaling only show duplications of r4 and r5 (Fig. 4D). Without either RA or Fgf signaling, Cdx-deficient embryos fail to form any duplicate tissue and lack posterior rhombomeres altogether (Fig. 4E).

Cdx-Hox functions also permit both RA and Fgf signaling pathways to operate. They are required autonomously (within the cells that express them) for Hox expression, and restoration of Hox expression in Cdx-deficient zebrafish embryos can at least partially rescue Krox20 expression (Shimizu et al. 2006). Like Fgfs, Cdx proteins are also negative regulators of Cyp26a1 (Wingert et al. 2007). In addition, Cdx transcription factors regulate, to some extent, the formation of the mesodermal sources of RA and Fgfs. For example, Raldh2 expression in the paraxial mesoderm shifts posteriorly in Cdx-deficient embryos, possibly altering the spatial relationships between RA and Fgf gradients such that both are high posteriorly.

Mesodermal segmentation

Strikingly, the same mirror-image reversal in the orientation of the RA gradient between the head and trunk is also a crucial feature of recent models of somite formation and segmentation of the paraxial mesoderm in the trunk and tail (Fig. 5). Somites and their derivatives (e.g., vertebrae, axial muscles) are the most clearly segmented portions of the vertebrate body. Somite formation depends on a ''clock and wavefront'' mechanism involving oscillating patterns of gene expression that move progressively toward the posterior end. An oscillating signal combines with a moving wavefront from anterior to posterior that sets the boundaries of somites within the presomitic mesoderm. A working model at the molecular level suggests that the clock involves Notch signaling, the wavefront involves RA and Fgf

Fig. 4 Distinct gradients in the head and trunk and the roles of Cdx genes. Diagrams illustrating neural patterning in Cdx-deficient embryos in the presence or absence of Fgf and RA signaling (Shimizu et al. 2006; Skromne et al. 2007). Anterior to the left, Rhombomere-specific domains (r1-7) are indicated. The A-P order of *hox* gene expression in the spinal cord is also indicated. (**A**) Wild-type. (**B**) Cdx-deficient. (**C**) Cdx-deficient and Fgf-deficient. (**D**) Cdx-deficient and RA-deficient. (**E**) Cd-deficient, Fgf-deficient, and RA-deficient.

Fig. 5 Gene oscillations and mesodermal segmentation. Diagrams illustrating gene oscillations involved in mesodermal patterning and in the clock and wavefront model for somite formation. (**B**) Cranial mesoderm shows no such gene oscillations or molecular domains corresponding to early head segments, but becomes secondarily segmented within the pharyngeal arches through influences from surrounding neural crest and endoderm.

signaling, and the two are interconnected through cycles of Wnt signaling (Palmeirim et al. 1997; Dequeante et al. 2006; reviewed by Andrade et al. 2005; Dequeante and Pourquie 2008). The discovery of this precise control of the timing of gene expression has revolutionized our understanding of the mechanisms underlying mesodermal segmentation. Can similar rules be applied to head segments?

At first glance, many of the signals involved are the same. However, unlike the hindbrain, RA and Fgf in the presomitic mesoderm are thought to form opposing gradients, with RA highest anteriorly and Fgf posteriorly (Fig. 5; Goldbeter et al. 2007). This parallels their orientations in the spinal cord, as revealed by the studies of Cdx genes (Fig. 4), and reflects the fact that after gastrulation most of the RA in the embryo is synthesized in anterior somites near the head-trunk boundary. An elegant computational model incorporating these components of the wavefront argues that a delicate balance between levels of these signals, where their morphogen gradients intersect, determines the boundaries of somites (Fig. 5; Goldbeter et al. 2007). Mutually inhibitory gradients, in this model, generate sharp

morphogen thresholds through bistability at two steady states, and in this way synchronize activation of segmentation genes along a determination front. Interestingly, a second domain of Cyp26a1 expression at the posterior end of the embryo forms a mirror-image sink to complement the anterior source of RA in the somites, and future studies are needed to determine the roles of degradation in the context of this system.

All of this new information on the mechanisms of mesodermal segmentation in the trunk also has important implications for understanding the relationship between head and trunk segmentation. Comparative embryologists have long wondered if the equivalent of mesodermal somites and their segmental derivatives extend into the cranial region (Goethe 1820; Balfour 1876; Kuratani and Schilling 2008). We can now ask if similar cycles of gene expression extend into the cranial mesoderm. For the best-studied elements of this system, such as Hairy, Lunatic fringe, and other components of Notch signaling, the answer appears to be "no" (Fig. 5). If any cycling occurs in presumptive head mesoderm, it occurs during gastrulation, prior to formation of the first somite. Only two pulses of Hairy or Lfng expression have been observed in avian embryos at these early stages, which are insufficient to account for the full set of head segments (Jouve et al. 2002). Head segments may form independently of the segmentation clock or a similar clock may act through a distinct set of cycling genes.

Cranial mesoderm also shows no obvious morphological signs of segmentation. As discussed extensively at our symposium, a lack of any molecular evidence for reiterated patterning within this tissue has led most researchers to the conclusion that cranial "somitomeres" do not exist (Kuratani and Schilling 2008). Instead, cranial mesoderm appears to become secondarily segmented within the pharyngeal arches through influences from surrounding neural crest and endoderm (Noden and Trainor 2005). Thus, consistent with the "new head" hypothesis (Northcutt 2008), the cranial mesoderm does not appear to be built simply through modifications of the same processes that pattern segments in the trunk (i.e., somites).

Conclusions and perspectives—evolution of systems of A-P patterning

Given all of this new information, how do we envisage the link between A-P patterning and head segmentation and how do these mechanisms differ between germ layers and body regions? One major

take-home message of this work, and of the entire Head Segmentation symposium at the SICB Meeting in January 2008, is that it seems unlikely that a single global patterning system controls all types of segments. Ectoderm differs in fundamental ways from mesoderm, and both germ layers become segmented quite differently in the head, compared with more posterior body regions. Part of this difference appears to lie in the links between early A-P patterning and the formation of segments.

In the cranial region, most of the evidence points to a primary role for the ectoderm, including the hindbrain and neural crest, in establishing segments. These seem to impart patterning on an otherwise unsegmented mesoderm during formation of the pharyngeal arches. Pharyngeal endoderm also segments into a series of pouches, which form the boundaries between adjacent arches, and less is known about the mechanisms that underlie pouch formation. Pouches do form in the absence of cranial neural crest (Veitch et al. 1999) but also require interactions with adjacent mesoderm and with both RA and Fgf signaling. Cdx genes also specify a posterior domain within the endoderm that alters its responsiveness to RA signaling, similar to their roles in the nervous system (Kinkel et al. 2008). Future studies are needed to determine how segmentation in the endodermal germ layer is coordinated with that of other germ layers, and whether or not the endoderm is subjected to similar morphogen gradients during its early development.

Much of the distinction between mechanisms of A-P patterning in the head and trunk centers around the role of RA. This is interesting from a comparative perspective, since RA seems to have acquired roles in A-P patterning in deuterostomes (Schilling and Knight 2001). Protostomes (e.g., nematodes and arthropods) lack both RARs and the enzymes that synthesize RA (Marletaz et al. 2006). In contrast, Retinoid X receptors (RXRs) and cytochrome p450 enzymes similar to Cyp26s are found throughout the animal kingdom (Niwa et al. 2004; Szanto et al. 2004; Bogwitz et al. 2005; King-Jones et al. 2006). Our model for regulation of the degradation of RA in the hindbrain suggests that an RA gradient system could have evolved simply by bringing one of these degrading enzymes under the control of an ancestral A-P patterning system, so that its expression in embryos became graded from posterior to anterior. This would automatically form a gradient of any substrate creating a primitive RA-like gradient system that could later be refined by localizing the storage and synthesis of precursors. Gradients of nuclear hormone–receptor ligands, together with the

feedback loops discussed here, may have distinct advantages for forming sharp boundaries such as those between hindbrain rhombomeres or mesodermal somites (Kerszberg 1996). Likewise, the ability to evolve gradients readily through changes in localized degradation could help explain many of the perceived differences between the head and trunk segments of vertebrates.

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