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Common mechanisms for boundary formation in somitogenesis and brain development: shaping the 'chic' chick

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ABSTRACT When organs and tissues acquire their characteristic shapes and functions during early development, boundaries are established that distinguish between and delimit distinct areas. Such boundaries are not mere edges, but also play important roles as secondary signaling centers in subsequent morphogenesis. Following on pioneering findings provided by studies in *Drosophila*, the mechanisms underlying boundary formation in vertebrate embryogenesis have attracted the interest of an increasing number of researchers. Somitogenesis and brain development, in particular, serve as model systems for the study of the molecular and cellular events occurring at developing boundaries. Recent findings allow us to draw some general pictures concerning the shared mechanisms that participate in these processes of organogenesis, in which Notch, Eph/ephrin and cadherin-mediated signaling are among the main key regulators.

KEY WORDS: *somite, cell signaling, Notch, Eph, vertebrogenesis*

Boundary formation is a fundamental process during early body shaping

For an organism to exhibit discrete physiological functions in specific organs and tissues in the body, these sites need to be distinct from neighboring tissues with a functional boundary between them. A «boundary» during early development does not merely mean the edge of a shape but is an integral part of the mechanisms of body shaping and functions. Embryogenesis undergoes a variety of boundary formations. For instance, the somites, the entity giving rise to skeletal muscles and axial bones, appear reiteratedly along the anteroposterior (A-P) axis with an overt morphological fissure between each of them. An originally continuous digestive tract becomes distinct with a functional boundary between different regions, such as pancreas, liver and intestine. In the course of brain formation, where numerous sets of complex network of neuron circuits are constructed, a simple tube-like structure is successively carved to produce specified regions with distinct boundaries (Fig. 1).

Pioneering studies using molecular genetics in *Drosophila* provided valuable information and general concepts concerning the mechanisms underlying a boundary formation and this knowledge helped greatly to understand enigma of a boundary formation in vertebrates (Blair, 2003, Dahmann and Basler, 1999, Irvine and Rauskolb, 2001, Lawrence and Struhl, 1996, Mann and Morata, 2000, McNeill, 2000, Pasini and Wilkinson, 2002, Tepass *et al.*, 2002). During early ontogenesis a continuous tissue becomes regionalized by a morphogen gradient and this gradient is resolved into distinct subdivision by specific patterns of gene expression formed in response to the morphogen concentration. This process involves mutually exclusive regulation of gene expression, particularly of those genes encoding transcription factors. The interface between the neighboring tissues expressing different genes is subsequently specified as a functional boundary where border cells of either side are generated. These border cells then play central roles in cell communications between the neighboring regions. One remarkable action resulting from the border cell interactions is to produce a secondary signal (often a secreted morphogen) that subsequently organizes the flanking regions. In some phenomena, these interactions lead to repulsive signals between the two opposing regions. During segmentation of the body of Drosophila, region-specific activation of gap genes is ultimately resolved into fourteen stereotyped stripes where border cells demarcating two consecutive boundaries act as a signaling center by secreting Hedgehog (Hh) and Wingless proteins, which subsequently act as morphogens that organize each segment along the antero-posterior axis (Lawrence and Struhl, 1996, Mann and Morata, 2000, Sanson, 2001). In the case of the A-P subdivision of the wing imaginal disc, the posterior compartment

Abbreviations used in this paper: A-P, anteroposterior; Hh, hedgehog; PAPC, paraxial protocadherin; PSM, presomitic mesoderm.

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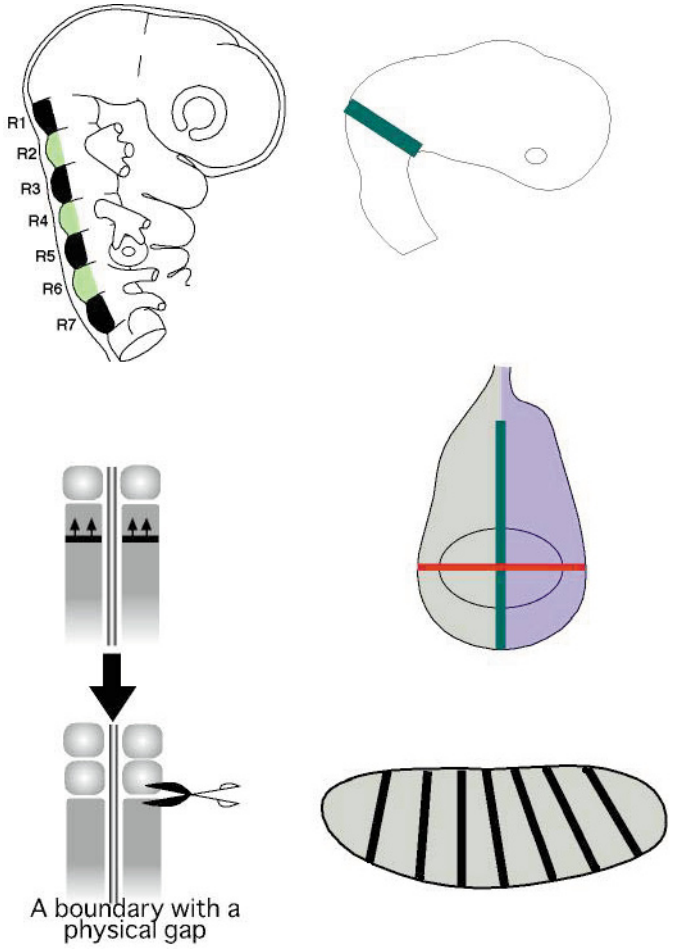

Fig. 1. Model systems which have extensively been studied for mechanisms of boundary formation, *including rhombomere boundaries, the midbrain/hindbrain boundary, somitic segmentation in vertebrates, anterior-posterior and dorsal-ventral boundaries in the wing disk and early body segmentation during early* Drosophila *development.*

expressing *engrailed* delineates its anterior border where Hh starts to be expressed. Hh subsequently induces the production of another morphogen Decapentaplegic (Dpp) in the anterior border cells, which acts as a long ranged morphogen that establishes an A-P gradient within each of anterior and posterior compartments. When the dorsal-ventral boundary forms in this organ (wing disc), Notch signaling takes the lead to define the site of Wg production along the boundary. Wg is a morphogen acting along the D-V axis in this organ (Blair, 2001, Blair, 2003, Irvine and Rauskolb, 2001, Lawrence and Struhl, 1996, Mann and Morata, 2000, Tepass *et al.*, 2002).

These mechanisms deciphered by *Drosophila* studies prompted researchers to learn about boundary formations in vertebrates. Some phenomena, indeed, employ similar mechanisms between flies and vertebrates. In this article I overview recent knowledge about the molecular and cellular mechanisms underlying a boundary formation and I will focus on somitogenesis, an area that is attracting an increasing number of investigators. I also will try to look for mechanisms of boundary establishment shared between somitogenesis and brain formation, the latter being a model system where border formation is also a central subject. It is emphasized in this article that many findings about novel morphological phenomena and cell behaviors responsible for boundary formation during vertebrate body patterning have been brought about by studies using chicken embryos in addition to those using mouse and zebrafish genetics. I finally discuss the significance of the somitic boundary and segmentation from evolutionary view points.

General picture of segmentation during somitogenesis

A prominent feature of the somites is their recurrent appearance along the A-P axis of the body and these segmented patterns are most evident in the vertebral column in adult. The periodic segmentation of somites also determines the reiterated patterning of the central and peripheral nervous systems. Somitic segmentation has long served as a model system to understand the mechanisms of border formation, since each somite undergoes complete morphological separation.

Segmentation proceeds regularly in time and space in an anterior-to-posterior order, each cycle taking about 90 min to complete in chicken embryos. At the end of a segmentation cycle, a regular amount of cell mass pinches off from the anterior end of the presomitic mesoderm (PSM), which lies paraxially, as a pair of longitudinal strips. A formed somite soon assumes a stereotyped spherical structure lined by epithelial cells. Recent studies have shown that even prior to the overt morphological segmentation, many molecular events are taking place in the PSM as simply diagrammed in Fig. 2. Posteriorly, in the «young» PSM a segmentation clock operates to determine the periodicity of the cycle. The model of the segmentation clock was proposed theoretically in 1976 (Cooke and Zeeman, 1976) and this was recently confirmed using chicken embryology by the remarkable finding that the expression pattern of *c hairy1* mRNA forms waves from posterior to anterior once each cycle (Palmeirim *et al.*, 1997). It appears that Hes (a transcription factor downstream of Notch signaling pathway) is a key player in generating this clock (Bessho *et al.*, 2003, Bessho and Kageyama, 2003). It is also remarkable that most of the molecules known to be involved in the segmentation clock are Notch signal-related (Pourquie, 2000, Pourquie, 2001, Saga and Takeda, 2001). Wnt signaling has also been recently shown to be a part of oscillation (Aulehla *et al.*, 2003).

As the segmentation wave reaches the anterior («mature») end of the PSM, this wave halts to determine the next forming boundary. Escaping from the influence of an Fgf8 signal, which is abundant in the caudal PSM, appears to be important for this step (Dubrulle *et al.*, 2001, Sawada *et al.*, 2001). Subsequently, MesP2, a bHLH transcription factor, starts to be expressed in a stripe corresponding to the presumptive somite and then becomes confined to the anterior half of that somite unit. In knock out mice for MesP2, the somitic segmentation is severely impaired and this phenotype is accompanied by downregulated expression of EphA4 and Lunatic fringe genes, which would normally be delineated at the next boundary (Saga and Takeda, 2001). MesP2 is in a loop of Notch signaling so that MesP2 is regulated by Notch and also regulates Notch ligand, Delta, resulting in the establishment of A-P compartments of a somite unit by a complex molecular cascade (Takahashi *et al.*, 2003). This A-P identity is critical for a subsequent formation of morphological boundary and also for providing a

discrete environment that neural crest cells encounter at later stages.

The somitic boundary is not formed by a lineage restriction mechanism

One of the conceptual mechanisms that has long been considered to account for a boundary formation is that of a «compartment» in a sense of cell lineage -that is, descendants of a cell(s) do not intermingle at the compartment boundary, whereas cells confined to each of opposing areas are free to intermingle within the area (Pasini and Wilkinson, 2002). The lineage compartment model was proposed for the A-P boundary in the wing imaginal disc in Drosophila (Blair, 2003, Dahmann and Basler, 1999, Lawrence and Struhl, 1996, Mann and Morata, 2000) and recently a lineage restriction is also shown to take place in some boundaries being formed during vertebrate brain development (Lumsden, 1999, Pasini and Wilkinson, 2002) (see also below). In the case of somite formation, in contrast, cell lineage restriction appears to be irrelevant. This was demonstrated by experiments in chickens in which a labeled single cell in the anterior PSM was shown to give rise to two consecutive somites (Stern *et al.*, 1988). The somitic boundary does not therefore form by the compartment lineage restriction mechanism and must proceed through some other processes.

Inductive events at the next forming somitic boundary

When a fissure forms at the prospective somitic boundary, a small gap perpendicular to the body axis appears within the mesenchymal population of the PSM and it is soon followed by cell epithelialization of the anterior border cells (the posterior edge of a forming somite) (Fig. 4). The site where the gap starts to appear is called the level 0 and the level -1 refers to the site of one somite unit posterior (Fig. 3). Thus, at -1, no morphological sign is yet recognizable. If there are any interactions between cells that lead to a fissure formation, they must take place at -1. Recent work indeed showed that this is the case (Sato *et al.*, 2002). When a small population of cells were taken from -1 of a donor (quail) and grafted into the level -1.5 of a chicken host embryo, the graft induced an ectopic boundary at the host position -1.5 (Fig. 3A). In some specimen the grafted cells were located in areas both anterior and posterior to the new boundary, but this was due to the PSM-intrinsic ability to segment. These experiments concluded that during normal somitogenesis the posterior border cells at -1 have an inductive activity (designated as a «segmenter») and the segmenter acts on the anterior cells to be separated and epithelialized (Sato *et al.*, 2002).

The inductive activity at the next forming somite boundary was further investigated to uncover the molecular mechanisms that control these interactions (Sato *et al.*, 2002). Among Notch-related molecules in the PSM, *L-fringe* displays a discrete pattern of expression with a sharp anterior boundary at -1, whereas mRNAs of *Notch* and *Delta* are broadly present in the PSM. To make an ectopic boundary of L-fringe in a non-segmentation site, overexpression by in ovo electroporation and cell transplantation techniques were combined (Fig. 3B). In these experiments, *L-fringe* was first broadly expressed in the PSM and a small piece of PSM from -1.5 was dissected and transplanted into -1.5 of a host embryo so that a boundary of L-fringe was ectopically created at -1.5 in a host. This manipulation resulted in a new fissure formation at the grafted site (Fig. 3B). Since L-fringe had been shown by biochemical studies to modify the Notch receptor with its glycosyltransferase activity, it was expected that the activity of L-fringe in fissure formation reflected a Notch action. This was confirmed to be the case by a molecular and embryonic manipulation similar to the above-mentioned experiment. Thus, Notch-active cells could induce an ectopic fissure at the anterior edge of the transplanted cells. Although L-fringe had already been shown to be required for somitic segmentation by knockout studies, it was not clear when, where or how this gene acts. Thus, knockout studies and embryonic manipulations in chickens have allowed us to learn about the roles of L-fringe in segmentation, where it acts (1) in the posterior PSM as a part of segmentation clock in harmony with *Hes* genes to establish a negative loop regulation (Bessho *et al.*, 2003, Dale *et al.*, 2003) and (2) acts in the anterior PSM as a basis of the segmenter at the final step of the segmentation cycle (Saga and Takeda, 2001, Sato *et al.*, 2002) (Fig. 3C).

Interestingly, Notch/L-fringe active cells never act posteriorly. When a piece of tissue overexpressing Notch/L-fringe was

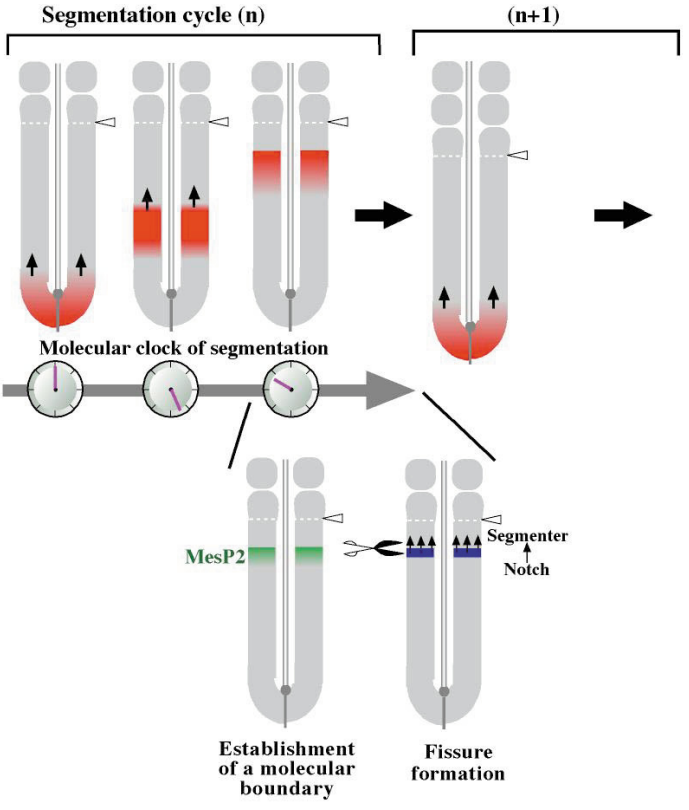

Fig. 2. A general picture of somitic segmentation. *During each cycle of segmentation, the segmentation clock operates in the posterior presomitic mesoderm, which is regulated by cyclic expression of Notch-related genes such as* Hes. *At the end of the cycle, MesP2 determines the next boundary border and also the anterior character in the prospective somitic unit. The segmentation process culminates in the formation of the morphological fissure, an action controlled by the inductive activity of the posterior cells (segmenter) that is also regulated by Notch signals.*

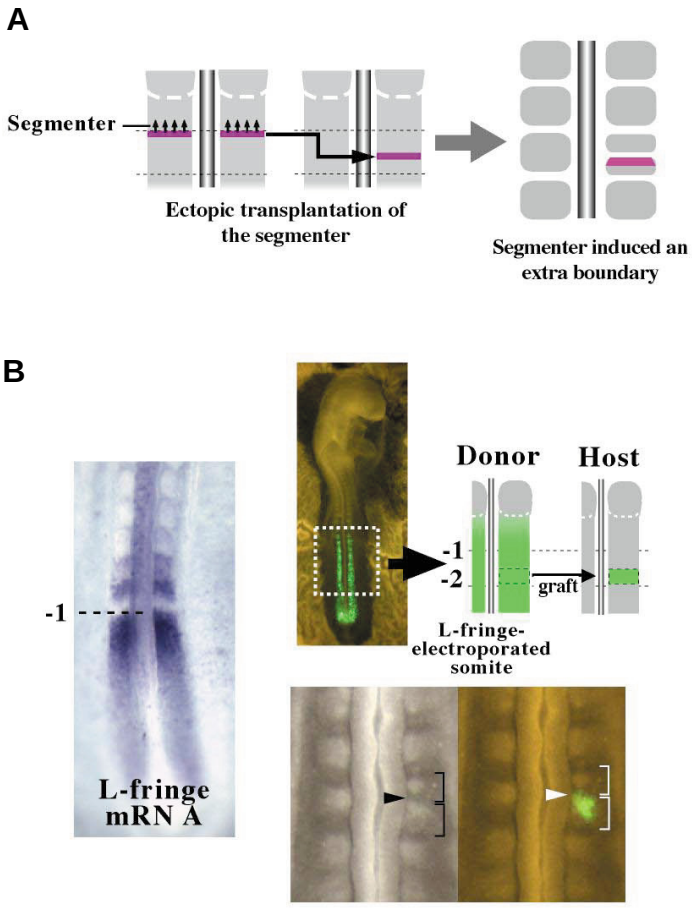

Fig. 3. Molecular events occurring at the next forming boundary. (A) *Donor cells taken from the next-forming border induce an ectopic border when transplanted into a non-border site of host.* **(B)** *L-fringe mRNA is expressed in a pattern with a sharp boundary at the next-forming boundary (-1). The L-fringe cDNA was overexpressed in the PSM by in ovo electroporation and then a small piece of tissue was taken from the electroporated donor to be implanted into a host embryo so that an ectopic boundary of L-fringe activity was made. This manipulation resulted in the formation of an ectopic fissure coinciding with the L-fringe boundary.* **(C)** *At the next-forming boundary, specific expression of L-fringe appears to restrict the Notch activity to this region, leading to the production of the segmenter activity that acts on the anterior cells.*

implanted into the anterior half of a presumptive somite, it never induced a new boundary at its posterior edge. This phenomenon cannot be accounted for by a simple morphogen gradient. It seems likely instead that each cell in the anterior PSM has an A-P polarity so that the cell can interpret from whence (either anterior or posterior) the segmenter signal comes. These possibilities are reminiscent of planar cell polarity, which has well been studied in Drosophila wing disk cells (Adler and Lee, 2001, Axelrod and McNeill, 2002, Strutt, 2003). It should be notified that Wnt 11, which is known to be involved in planar cell polarity by a non-canonical Wnt pathway, is expressed at -1 in the anterior PSM (Tonegawa *et al.*, 2003).

Although the molecular nature of the segmenter still remains undetermined, there are several candidates. Eph (receptor) and ephrin (ligand) comprise a large family, in which Eph A types interact with ephrin A types and so do Eph B types with ephrin B (Holder and Klein, 1999, Palmer and Klein, 2003, Wilkinson, 2001). As an exception, EphA4 binds to some members of ephrin B. When Eph and ephrin are expressed in neighboring cells, respectively, these cells repel each other. Eph/ephrin interactions are therefore considered as repulsive. During the somitic segmentation of chicken and mouse embryos, Eph A4 is specifically expressed in the anterior PSM with the anterior boundary at -1 whereas ephrin B2 is broadly present in PSM (unpublished data, Y.T.). In zebrafish, EphA4 is expressed in a presumptive somite unit and ephrin B2 expression alternates with the pattern of EphA4. When EphA4 is overexpressed or disrupted in a developing egg of zebrafish, resulting embryos show severely affected segmentation (Durbin *et al.*, 1998). More direct evidence was obtained by a mosaic analysis: when Eph-overexpressing cells were injected into a host *fused somite* embryo (Nikaido *et al.*, 2002), they produced a new boundary anterior to them in the PSM region (Durbin *et al.*, 2000). It is also consistent with the findings that the PSM requires the surrounding tissues, in particular, the surface ectoderm, to undergo morphological segmentation (Correia and Conlon, 2000, Sosic *et al.*, 1997) and the surface ectoderm is important to maintain the Eph expression in the PSM (Schmidt *et al.*, 2001).

Mesenchymal to epithelial transition at the forming somitic boundary

After a gap forms when the level -1 becomes the level 0, the anterior border cells undergo dynamic epithelialization, whereas the posterior border cells remain (for a while) mesenchymal (Fig. 4). This differential sequence of epithelialization between the anterior and posterior border cells is unambiguous in chicken embryos whereas in other vertebrates the posterior border cells seems to undergo epithelialization almost simultaneously with the anterior border cells. Whatever the sequence is, the important phenomena are the epithelialization of the anterior border cells so that a forming somite can be explicitly separated from the posterior tissue. In knock out mice for the *Paraxis* gene (encoding a bHLH transcriptional factor), a gap forms but subsequent epithelialization does not take place (Burgess *et al.*, 1996). In these embryos, cellular differentiation such as myotome formation proceeds almost normally, although the resultant patterning of the somites is severely disrupted. In the normal PSM, *paraxis* mRNA is expressed in a wide region (Sosic *et al.*, 1997), so it does not fully explain how this gene is involved in the epithelialization restricted to the forming boundary.

Mechanisms of cell polarization have extensively been investigated mostly using in vitro cell culture systems. Important and central players in these events are Rho family small GTPases, including Rho, Rac1 and Cdc42. Small GTPases are molecular switches that cycle between a GTP-bound (active) and a GDP-bound (inactive) form (Hall, 1998). It is the GTP-bound form that transmits signals inwardly, causing a cell to reorganize actin filaments. The roles the Rho family small GTPases play during

embryogenesis have recently begun to be studied. However, precocious lethality of null mutant embryos for these genes have precluded pinpointing the function of the proteins in a given cell polarization event. As an alternative, site-specific expression of constitutive active or negative forms of Rho family small GTPases must be useful to compensate the difficulties of knock out strategy. These approaches focusing on somitic epithelialization are currently in progress and specific roles for Rac 1 and Cdc42 start emerging (Nakaya and Takahashi, in preparation).

Another characteristic phenomenon observed during somitic segmentation/boundary formation is a specific augmentation of cell condensation in the forming somite (Christ and Ordahl, 1995). Coincidentally, *paraxial protocadherin* (PAPC) is specifically expressed in this region (Rhee *et al.*, 2003). Again, although knock out mice for PAPC did not show any defects in somitogenesis, overexpression experiments of PAPC into Xenopus or zebrafish embryos demonstrated that PAPC is important (Kim *et al.*, 1998, Yamamoto *et al.*, 1998). It has also been shown that adding a dominant negative form (soluble) of PAPC into a PSM explant culture hampered proper boundary formation (Rhee *et al.*, 2003). It is thus expected that PAPC plays a role in the somitic boundary formation by conferring high cell affinity on coalescing cells of a forming somite in the anterior PSM.

Dynamic cell movements at the somitic boundary

Recently, an elaborate technique of confocal time-lapse imaging of fluorescently labeled cells directly revealed that cells located at the forming boundary move more actively than previously thought, in that they violate the prospective boundary (Kulesa and Fraser, 2002). When viewed from a horizontal plane, the medial- and lateral-most cells of the forming somite that are originally located anterior to the presumptive boundary move posteriorly and end up with coalescing with cells of the segmental plate (the posterior somite unit to the one where these cells were originally localized). Likewise, intermediate cells along the medio-lateral axis at the forming boundary move anteriorly and eventually give rise to the posterior border cells of the forming somite. The allocation of these cells does not correspond to a segmental pattern of gene expression such as *EphA4,*—which has a sharp anterior boundary at -1. Thus, cells at the boundary are remarkably flexible in changing their position within a range of distance from one to several cells (Kulesa and Fraser, 2002). This study highlighted that a segmental pattern of gene expression is not inherited by all the cells participating in the boundary formation and raised the possibility that some specified cells (or tissue) initiate a coordination in the expression of segmental genes.

Integrating these recent findings, a possible scenario emerges for somitic morphological boundary formation (Fig. 4): After MesP2 determines the site of a next forming boundary, the segmenter emerges in the posterior border cells (= anterior edge of the *MesP2* -positive area) and acts on the anterior border cells. These activities include Eph/ephrin repulsive signaling to generate a gap within the mesenchymal cells. The gap formation is followed by two steps ensuring a complete separation between consecutive somites. One is cell epithelialization of the border cells, where Rho family small GTPases are involved. Another step confers different affinities to opposing regions, so that the posterior half of an anteriorly forming somite and the anterior half of a posteriorly forming somite coalesce, respectively. This process is regulated at least by PAPC. In addition, there seem to be some specified cells at -1 that take a leadership in coordinating these events so that the boundary takes place precisely along the level -1.

A somitic boundary acts as a signaling center

Is a somitic morphological fissure merely a mechanical separation of consecutive somites, or does the boundary cause any instructive influence on subsequent somitogenesis? The answer came from a series of segmenter experiments as shown earlier. When two miniature somites were induced to form, each of them displayed its own A-P identity, suggesting that the A-P identity was rearranged (Sato *et al.*, 2002). These results demonstrate that a confrontation of Notch-active and -inactive cells at the next-forming somite not only instructs a morphological fissure formation but also influences the A-P identity, in particular the posterior identity of the immediately

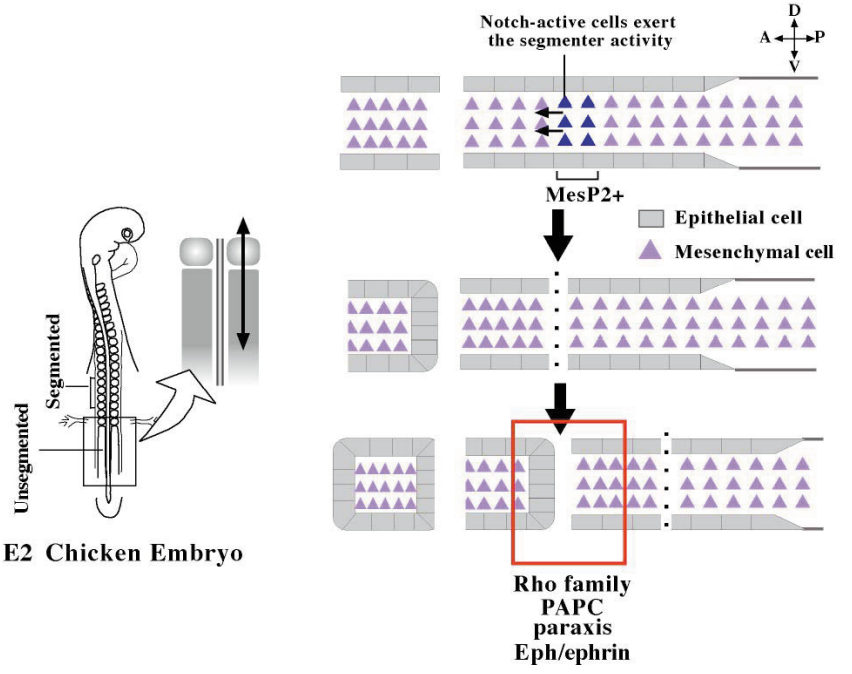

Fig. 4. A sagittal view showing cellular events during somitic boundary formation. *MesP2 endows PSM cells with the anterior character of a somite. Among these cells, Notch-active border cells (blue triangles) act on the anterior cells, leading to a gap formation between the anterior and posterior border cells. This is soon followed by a mesenchymal-epithelial transition undergone by the anterior border cells. Rho family small GTPases seem to play roles in this process (Nakaya and Takahashi, in preparation). Eph/ephrin-mediated repulsive signals are involved in the gap formation. A cell adhesion molecule, PAPC, might provide high affinity binding to mesenchymal cells of a forming somite. A role for the* paraxis *gene is implied in cell epithelialization of the anterior border cells.*

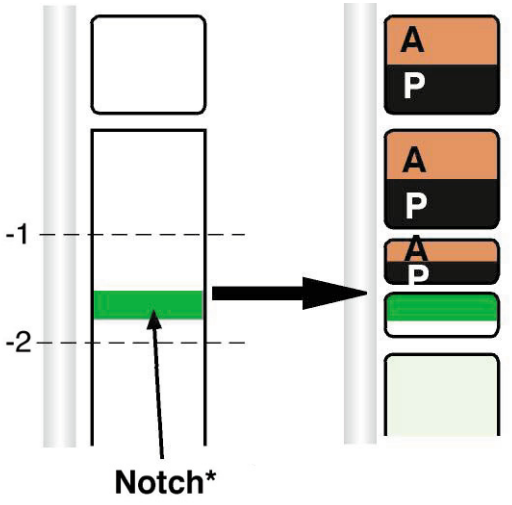

Fig. 5. A morphological boundary seems to act as a secondary signaling center. *An ectopic fissure that was caused by an experimentally localized Notch activity resulted in a rearrangement of the A-P character in the somite anterior to the new fissure.*

anterior somite (Fig. 5). It has been known that MesP2 specifies the A-P character (more precisely, MesP2 characterizes the anterior identity) within a presumptive somitic unit before a fissure forms, since dysfunction of MesP2 results in severely affected vertebrae with only posterior identity for each somite (thus the vertebrae are continuous) (Saga *et al.*, 1997). It is conceivable that one of the primary functions of MesP2 is to generate the segmenter activity in the anterior edge of the forming somite, which in turn makes a fissure. The fissure production subsequently ensures the A-P identity in the anteriorly located somite so that the posterior edge of this somite correctly displays posterior identity. Thus, the somitic boundary serves as a signaling center that ensures the pre-patterned A-P characters of a somite. Interestingly, a low activity of MesP2 is sufficient to *produce* a morphological boundary, but fails in *maintaining* the A-P identity in the formed somites, shown by a hypomorph mutant for the *MesP2* gene (Nomura-Kitabayashi *et al.*, 2002). Thus, the segmenter can be produced by a low activity of MesP2 at the level -1, but to ensure maintainance of the A-P identity, high MesP2 is required. It is also notable that boundary formation and persistent A-P character in the formed somites can be considered to be distinct steps. It is likely that epithelialization at the boundary is important for this secondary signaling step, since *paraxis* mutants, which cannot undergo epithelialization at the boundary, also displays disrupted A-P identity in formed somites (Johnson *et al.*, 2001). Molecular mechanisms for this secondary signaling are yet to be determined. It is worth investigating whether somite-specific signal molecules identified by a systematic signal sequence trap screening, including potential cell adhesion molecules and chemokine-related molecules, are involved in these cell communications (Tonegawa *et al.*, 2003).

The A-P identity of a somite leads to resegmentation for vertebrogenesis

The correct establishment of the A-P identity within a somite is further required for subsequent morphogenetic processes. The somitic A-P difference determines the environment that the neural crest cells encounter. The neural crest cells emigrating from the dorsal neural tube migrate only in the anterior half of the sclerotome, the ventro-medial component derived from each somite (Bronner-Fraser, 1986, Loring and Erickson, 1987, Rickmann *et al.*, 1985, Teillet *et al.*, 1987). Likewise, motor neurons extend their axons through the anterior sclerotome in each segment (Keynes and Stern, 1984). Thus, the segmental pattern of the somite is responsible for generating the reiterating pattern of the nervous system along the body axis. It is well known that ephrin B1 expressed in the posterior sclerotome in each segment exerts a repulsive signal on the immigrating neural crest cells, which express Eph B3, leading to selective migration of the neural crest cells in the anterior portion of each segment (review and references in Krull, 2001).

Another remarkable consequence of the A-P characterization within a somite is the resegmentation process, an essential step taking place in the sclerotomal cells for vertebrogenesis. After a somite forms, the dorsolateral portion remains an epithelial cell sheet (dermomyotome, precursors of skeletal muscles and dermis) whereas the ventro-medial cells deepithelialize to produce highly proliferative sclerotomes, the precursors of the vertebral cartilages. In chickens and mice, the anterior half sclerotome of a posteriorly adjacent somite meets with the posterior half sclerotome of the anteriorly adjacent somite (Aoyama and Asamoto, 2000, Stockdale *et al.*, 2000). Thus, the segmental unit of the resulting vertebra is out of register with the original pattern of segmentation, (which is retained in the pattern of the dermomyotome). This offset alignment of the segmentation between skeletal muscles and vertebrae enables the body to move laterally (i.e. lateral bending). Thus, the distinction between the anterior and posterior halves within an original somite is important for proper morphogenesis of the

TABLE 1

COMPARISON OF BOUNDARY FORMATION MECHANISMS IN SOMITOGENESIS AND BRAIN DEVELOPMENT

	Somites	Rhombomeres	M/H	zli
Lineage restriction	No	Yes	No	Yes
Selector genes defining a boundary	MesP2	Krox20 Kreisler Hox	GBX2/OTX2 (Mutual repression)	
Morphological gap by de-adhesion	Yes	Yes	No	No
Tissue intervening at the boundary	Intersegmental vessels	Early forming radial glia Axon-rich marginal zone		
Repulsion/adhesion/cell sorting	Eph/ephrin	Eph/ephrin		Yes
Secondary signal center	Yes		Fgf8/Wnt1	Shh
Notch signalling	Yes	(Yes)	(Yes)	Yes

(Yes): Personal communication; Rhombomeres by D. Wilkinson (Mill Hill), M/H by Y. Kageyama (Kyoto).

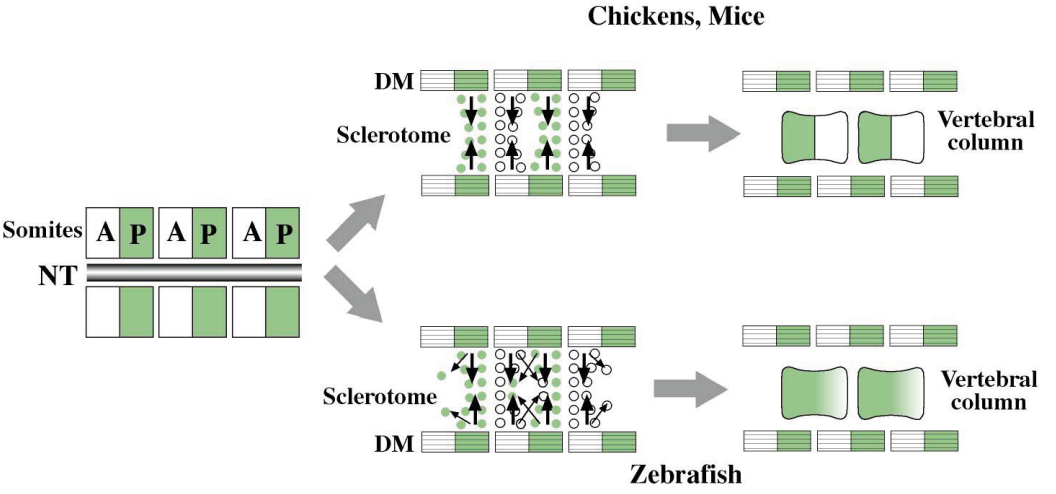

Fig. 6. Differences in behavior of sclerotomal cells in amniotes (studied in chickens/mice) and anamniotes (studied in fish) during resegmentation. *In amniotes, sclerotomal cells derived from the posterior half of an anterior somite meet with sclerotome cells coming from the anterior half of a posteriorly adjacent somite, constructing a vertebra in a lineage restricted manner. In fish, in contrast, a «leaky» resegmentation model has been proposed in which sclerotome cells from one somite contribute to two adjacent vertebrae in a manner that is not strictly dependent upon the A or P domain of origin in the somite (Morin-Kensicki* et al.*, 2002).*

vertebral column. Contribution by the sclerotome to vertebrogenesis is controlled by a lineage-restriction mechanism, contrasting with the case of somitic boundary formation as mentioned earlier.

Although mechanisms of resegmentation still remain unknown, phenotypes assumed by double-knockout mice for N-cadherin and Cadherin 11 are suggestive of different affinities between the anterior and posterior population of the sclerotome before resegmentation (Horikawa *et al.*, 1999). In these mice, primary segmentation takes place correctly, but soon after the somites form, the anterior and posterior halves become detached. It implies that each of these two cell populations has specific affinity in cell adhesion and N-cadherin and Cadherin 11 might play a role in associating the two populations together. It is possible that PAPC, which continues to be expressed in the anterior half of the somite after segmentation, is involved in coalescing the anterior population. At present no information is available as to what controls the reunion of the two sclerotomal halves during resegmentation.

Shared mechanisms between mesoderm segmentation and brain boundaries

Early brain patterning is also characterized by massive subdivision and the formation of functional boundaries. The anterior region of the neural tube undergoes partitioning into forebrain, midbrain and hindbrain. Within each partition, succeeding subdivisions are thought to determine discrete regions wherein specific neurons differentiate and also to define pathways which specific axons encounter. For the brain subdivisions, molecular mechanisms similar to those aforementioned for somitogenesis are also employed (see below). The readers are encouraged to see other reviews elegantly describing recent studies of brain boundaries for detailed information (Lumsden, 1999, Lumsden and Krumlauf, 1996, Pasini and Wilkinson, 2002). Here, I briefly summarize the outline of several types of boundary formation during brain development and try to compare their molecular and cellular events with those during somitogenesis with the aim of bringing out a general picture of boundary establishment during vertebrate ontogenesis.

The midbrain/hind brain boundary, which separates the midbrain and the cerebellum later in development, is first established by mutually exclusive regulation of transcriptional factors, Otx2 and Gbx 2. These interactions result in the generation of a secondary signaling center that secretes morphogens. Wnt 1 in the posterior end of the midbrain and Fgf8 in the anterior hindbrain, in turn, organize the respective regions through their gradients (Rhinn and Brand, 2001, Simeone, 2000). Cell lineage restriction is thought to be irrelevant to the boundary formation in these tissues. Among brain partitioning processes, the hindbrain displays the most peculiar patterning. After a series of subdivisions, eight metameric repetitions of rhombomeres (r1-r8) are aligned along the A-P axis (Lumsden, 1999, Lumsden and Krumlauf, 1996, Pasini and Wilkinson, 2002). Despite the metameric appearance of the rhombomeres, the overall process is distinct from that of somitic segmentation, in that the first subdivision produces large partitions, followed by further separations within each area, resulting in successively aligned rhombomeres. At the boundary, a subtle morphological gap is seen between neighboring rhombomeres. However, the gap is not the cause but the consequence of the separation. Wherever even and odd numbered rhombomere cells are experimentally juxtaposed, a boundary forms between them, whereas even numbered rhombomere cells intermingle as welll as odd numbered ones do(Jungbluth *et al.*, 2001, Lumsden, 1999, Lumsden and Krumlauf, 1996). Key players in rhombomere separations are Eph/ephrin molecules. The expression of Eph and ephrin alternates in each rhombomere in a complementary fashion. For example, Ephs (-A4, -B2 and -B3) are expressed in r3 and r5 whereas ephrins (-B1, -B2 and -B3) are expressed in r2, r4 and r6. Molecular mechanisms that account for these phenomena were elucidated by elegant studies using zebrafish embryos where Eph- or ephrin injected cells repel each

other, providing unambiguous evidence that Eph/ephrin-mediated repulsive events establish the rhombomere boundaries (Mellitzer *et al.*, 1999, Mellitzer *et al.*, 2000, Pasini and Wilkinson, 2002, Xu *et al.*, 1999). It has long been known that rhombomere boundaries result from cell lineage restriction and this is consistent with molecular mechanisms involving Eph/ephrin-mediated repulsive events. In the diencephalon, the discrete region, called zona limitans intrathalamica (zli), has recently been shown to act as a barrier that prohibits intermingling of neighboring cells (Larsen *et al.*, 2001). The presumptive zli region first appears as a relatively broad band, which is deprived of *L-fringe* expression, whereas flanking regions are positive for *L-fringe*. As the band narrows into a line, this region starts producing Shh, which is expected to act as a morphogen in subsequent morphogenesis. The zli boundary results from cell lineage restriction and L-fringe (probably by mediating Notch) directs cell sorting at the borders and thereby maintains compartmental integrity of the zli (Zeltser *et al.*, 2001). Cell sorting also plays an important role in compartmentalization of the telencephalon delineating the boundary between the presumptive lateral cerebral cortex and ganglionic eminence, mediated by cadherin 6 and R-cadherin (Inoue *et al.*, 2001).

It is worth comparing the molecular and cellular mechanisms that have so far been described between brain boundaries and a somitic fissure. Table 1 allows us to depict a general picture although the analyses are not thorough. It appears common that a selector gene(s) define the boundary, for instance, by mutual repressive regulation of transcription factors. In most cases, if not all, repulsion and/or cell sorting systems subsequently initiate a boundary at the cellular level. In many situations the border cells act as a secondary signaling center to determine further morphogenesis in flanking regions. Along this line, an assignment of different cell affinities, including cell sorting and repulsive signals, to the opposing regions seems to be a central issue in boundary formation (Tepass *et al.*, 2002). In the case of rhombomeres, soon after a few precursor cells are allocated to each region of rhombomeres, their descendants start exerting repulsive interactions between neighboring rhombomeres, resulting in a bounary formation. Thus, the lineage restriction phenomena appear to depend on a precocious assignment of differential cell affinity during a clonal expansion from a single cell. In contrast, in the paraxial mesoderm cells move relatively freely straddling the presumptive boundary and repulsive signals and differential affinity ultimately define the site of forming boundary (Kulesa and Fraser, 2002, Stern *et al.*, 1988). Another important issue is that border cells themselves are produced by interactions occurring at the interface of the opposing regions. Appearance of a morphological gap at a boundary is in concordance with subsequent morphogenesis: a gap between the somites and between rhombomeres is eventually exploited by intersegmental vessels and the axon-rich marginal zone, respectively (Lumsden, 1999, Stockdale *et al.*, 2000). Thus, some boundaries may not need to produce a morphological gap. It is of interest to know to what extent the Notch/fringe signals are involved in a boundary formation. And whether signal cascades triggered by Eph or cadherins are linked to the Notch signal in the morphological boundaries awaits further studies with embryological, biochemical and genetic approaches. In any case, there is no doubt that signals mediated by Eph/ephrin, cadherin and Notch are the most central key players during boundary morphogenesis (Tepass *et al.*, 2002). Beyond brain development and somitogenesis, an increasing number of studies also report the importance of Eph/ephrin in restricting specified cells to a specific field with a discrete boundary. Recently, one such study has elegantly shown a role for Eph and ephrin in positioning proliferative and differentiated cells in the crypt and villus regions of colon, respectively (Batlle *et al.*, 2002).

Significance of the somitic segmentation boundary: an evolutionary point of view

As aforementioned, resegmentation, which results in the out of register arrangement between dermomyotome and sclerotome, allows lateral movements of the body. Why was the vertebral column, the most characteristic trait of vertebrates, selected as the tissue that undergoes resegmentation? Why not the dermomyotome? No vertebrates employ resegmentation for myotomal arrangement. I think one answer is provided by a recent piece of work from Eisen's group using zebrafish embryos (Morin-Kensicki *et al.*, 2002).

In zebrafish, primary somitic segmentation proceeds through almost the same mechanisms as those implicated in chickens and mice (Saga and Takeda, 2001). Thus, zebrafish exhibit an explicit pattern of segmented myotomes. The sclerotome in these embryos, however, is less evident compared to chickens and mice (Morin-Kensicki and Eisen, 1997). The ratio between myotome and sclerotome within each somite is much different from that in higher vertebrates, the sclerotomal population being much smaller in zebrafish. Eisen's group investigated the contribution of the sclerotomal cells to the vertebral column by a single cell labeling technique. These investigators found that zebrafish sclerotome does undergo resegmentation, but in a different fashion. Whereas in chickens, as described earlier, the posterior half of an anteriorly-positioned somite meets with the anterior half of a posteriorly-positioned somite to make the vertebral column, in zebrafish such a rule is not faithfully obeyed: the posterior half of a somite can also participate in the posterior half of a resulting vertebra and likewise the anterior half of a somite can go into the anterior area of a formed vertebral column (Fig. 6). Thus, the resegmentation rule is «leaky» in fish (Morin-Kensicki *et al.*, 2002).

These interesting findings can be reconciled with the fact that while amniotes (i.e. chickens and mice) develop sclerotomes during early ontogenesis, in anamniotes (fish and frogs) the sclerotome emerges much later. This also reflects the fact that frog or fish tadpoles do not need the vertebral column to swim in water until late in development. In contrast, the amniotes need to resist gravity and also to move immediately after they are born/hatched. Thus, the amniotes should develop sclerotomes more precociously during ontogenesis. One can speculate that during vertebrate evolution the segmentation process has primarily evolved to make an elaborate pattern of muscle segmentation so that the animal (primitive fish) could acquire high locomotive activity in water, probably to escape from predators. And even when these primitive animals evolved vertebral bones, they were primarily used as a device for calcium storage reservoir rather than a locomotive or body-supporting purpose (Hall, 1999, Northcutt and Gans, 1983). Concomitantly, the development of a strong vertebral column helped animals enlarge their body size because a hard backbone could sustain a large body. Thus, the

mechanisms regulating the primary segmentation with common molecular cascades in vertebrates may not necessarily be linked to the resegmentation process directly. The amniotes might have developed additional devices to ensure vertebral formation, where stereotypically regulated mechanisms are employed including cell lineage restriction during resegmentation. This notion is also consistent with the fact that a low activity o the MesP2 gene is sufficient to make a boundary but a high activity is required to carry out the resegmentation process (Nomura-Kitabayashi *et al.*, 2002). Thus, unveiling the mechanisms that underlie the resegmentation and also comparing them between the amniotes and anamniotes would give insights into understanding how the amniotes evolved from amphibians.

Conclusion

Notch and its related molecules are key players both for establishing the segmentation periodicity and the somitic morphological boundaries in the paraxial mesoderm. In addition, the precise coordination of repulsive interactions mediated by Eph/ephrin, the augmentation of differential cell affinity in opposite regions and changes in cell polarity (mesenchymal-epithelial transition) lead to the separation of somitic tissues. By comparing boundary-forming mechanisms between somitogenesis and brain development, one can observe some shared features. These commonalities include interactions between neighboring cells mediated by Notch, Eph/ephrin and cadherin signaling, the dynamic rearrangement of cytoskeleton and the emergence of a secondary signaling center at the border. Importantly, molecular players involved in these events are recurrently used in a variety of developmental processes probably in combination with different partners or with different extent of contribution.

The Institut d'Embryologie of Nogent-sur-Marne allowed me to learn how to appreciate the *Beauty of Shape* in embryology, which can be largely attributed to boundary formation events and which can be elegantly investigated in the '*chic*' chick model. By manipulating the embryos, I can eavesdrop on the conversations within and between the developing cells. I have been and will continue enjoying listening to «what embryos are telling us».

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References

ADLER, P.N. and LEE, H. (2001). Frizzled signaling and cell-cell interactions in planar polarity. *Curr Opin Cell Biol* 13: 635-40.

AOYAMA, H. and ASAMOTO, K. (2000). The developmental fate of the rostral/caudal half of a somite for vertebra and rib formation: Experimental confirmation of the resegmentation theory using chick-quail chimeras. *Mech Dev* 99: 71-82.

AULEHLA, A., WEHRLE, C., BRAND-SABERI, B., KEMLER, R., GOSSLER, A., KANZLER, B. and HERRMANN, B.G. (2003). Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev Cell* 4: 395-406.

AXELROD, J.D. and MCNEILL, H. (2002). Coupling planar cell polarity signaling to morphogenesis. *ScientificWorldJournal* 2: 434-54.

BATLLE, E., HENDERSON, J.T., BEGHTEL, H., VAN DEN BORN, M.M., SANCHO, E., HULS, G., MEELDIJK, J., ROBERTSON, J., VAN DE WETERING, M., PAWSON, T. *et al.* (2002). Beta-catenin and tcf mediate cell positioning in the intestinal epithelium by controlling the expression of ephb/ephrinb. *Cell* 111: 251-63.

BESSHO, Y., HIRATA, H., MASAMIZU, Y. and KAGEYAMA, R. (2003). Periodic repression by the bhlh factor hes7 is an essential mechanism for the somite segmentation clock. *Genes Dev* 17: 1451-6.

BESSHO, Y. and KAGEYAMA, R. (2003). Oscillations, clocks and segmentation. *Curr Opin Genet Dev* 13: 379-84.

BLAIR, S.S. (2001). Cell lineage: Compartments and capricious. *Curr Biol* 11: R1017-21.

BLAIR, S.S. (2003). Lineage compartments in drosophila. *Curr Biol* 13: R548-51.

BRONNER-FRASER, M. (1986). Analysis of the early stages of trunk neural crest migration in avian embryos using monoclonal antibody hnk-1. *Dev Biol* 115: 44-55.

BURGESS, R., RAWLS, A., BROWN, D., BRADLEY, A. and OLSON, E.N. (1996). Requirement of the paraxis gene for somite formation and musculoskeletal patterning. *Nature* 384: 570-3.

CHRIST, B. and ORDAHL, C.P. (1995). Early stages of chick somite development. *Anat Embryol (Berl)* 191: 381-96.

COOKE, J. and ZEEMAN, E.C. (1976). A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *J Theor Biol* 58: 455-76.

CORREIA, K.M. and CONLON, R.A. (2000). Surface ectoderm is necessary for the morphogenesis of somites]. *Mech Dev* 91: 19-30.

DAHMANN, C. and BASLER, K. (1999). Compartment boundaries: At the edge of development. *Trends Genet* 15: 320-6.

DALE, J.K., MAROTO, M., DEQUEANT, M.L., MALAPERT, P., MCGREW, M. and POURQUIE, O. (2003). Periodic notch inhibition by lunatic fringe underlies the chick segmentation clock. *Nature* 421: 275-8.

DUBRULLE, J., MCGREW, M.J. and POURQUIÉ, O. (2001). Fgf signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal *hox* gene activation. *Cell* 106: 219-232.

DURBIN, L., BRENNAN, C., SHIOMI, K., COOKE, J., BARRIOS, A., SHANMUGALINGAM, S., GUTHRIE, B., LINDBERG, R. and HOLDER, N. (1998). Eph signaling is required for segmentation and differentiation of the somites. *Genes Dev* 12: 3096-109.

DURBIN, L., SORDINO, P., BARRIOS, A., GERING, M., THISSE, C., THISSE, B., BRENNAN, C., GREEN, A., WILSON, S. and HOLDER, N. (2000). Anteroposterior patterning is required within segments for somite boundary formation in developing zebrafish. *Development* 127: 1703-13.

HALL, A. (1998). Rho gtpases and the actin cytoskeleton. *Science* 279: 509-14.

HALL, B.K. (1999). The neural crest in development and evolution. *Springer Verlag, New York*.

HOLDER, N. and KLEIN, R. (1999). Eph receptors and ephrins: Effectors of morphogenesis. *Development* 126: 2033-44.

HORIKAWA, K., RADICE, G., TAKEICHI, M. and CHISAKA, O. (1999). Adhesive subdivisions intrinsic to the epithelial somites. *Dev Biol* 215: 182-9.

INOUE, T., TANAKA, T., TAKEICHI, M., CHISAKA, O., NAKAMURA, S. and OSUMI, N. (2001). Role of cadherins in maintaining the compartment boundary between the cortex and striatum during development. *Development* 128: 561-9.

IRVINE, K.D. and RAUSKOLB, C. (2001). Boundaries in development: Formation and function. *Annu Rev Cell Dev Biol* 17: 189-214.

JOHNSON, J., RHEE, J., PARSONS, S.M., BROWN, D., OLSON, E.N. and RAWLS, A. (2001). The anterior/posterior polarity of somites is disrupted in paraxis-deficient mice. *Dev Biol* 229: 176-87.

JUNGBLUTH, S., LARSEN, C., WIZENMANN, A. and LUMSDEN, A. (2001). Cell mixing between the embryonic midbrain and hindbrain. *Curr Biol* 11: 204-7.

KEYNES, R.J. and STERN, C.D. (1984). Segmentation in the vertebrate nervous system. *Nature* 310: 786-9.

KIM, S.H., YAMAMOTO, A., BOUWMEESTER, T., AGIUS, E. and ROBERTIS, E.M. (1998). The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during xenopus gastrulation. *Development* 125: 4681-90.

KRULL, C.E. (2001). Segmental organization of neural crest migration. *Mech Dev* 105: 37-45.

KULESA, P.M. and FRASER, S.E. (2002). Cell dynamics during somite boundary formation revealed by time-lapse analysis. *Science* 298: 991-5.

LARSEN, C.W., ZELTSER, L.M. and LUMSDEN, A. (2001). Boundary formation and compartition in the avian diencephalon. *J Neurosci* 21: 4699-711.

LAWRENCE, P.A. and STRUHL, G. (1996). Morphogens, compartments and pattern: Lessons from Drosophila? *Cell* 85: 951-61.

LORING, J.F. and ERICKSON, C.A. (1987). Neural crest cell migratory pathways in the trunk of the chick embryo. *Dev Biol* 121: 220-36.

LUMSDEN, A. (1999). Closing in on rhombomere boundaries. *Nat Cell Biol* 1: E83-5.

LUMSDEN, A. and KRUMLAUF, R. (1996). Patterning the vertebrate neuraxis. *Science* 274: 1109-15.

MANN, R.S. and MORATA, G. (2000). The developmental and molecular biology of genes that subdivide the body of drosophila. *Annu Rev Cell Dev Biol* 16: 243-71.

MCNEILL, H. (2000). Sticking together and sorting things out: Adhesion as a force in development. *Nat Rev Genet* 1: 100-8.

MELLITZER, G., XU, Q. and WILKINSON, D.G. (1999). Eph receptors and ephrins restrict cell intermingling and communication. *Nature* 400: 77-81.

MELLITZER, G., XU, Q. and WILKINSON, D.G. (2000). Control of cell behaviour by signalling through eph receptors and ephrins. *Curr Opin Neurobiol* 10: 400-8.

MORIN-KENSICKI, E.M. and EISEN, J.S. (1997). Sclerotome development and peripheral nervous system segmentation in embryonic zebrafish. *Development* 124: 159-67.

MORIN-KENSICKI, E.M., MELANCON, E. and EISEN, J.S. (2002). Segmental relationship between somites and vertebral column in zebrafish. *Development* 129: 3851-60.

NIKAIDO, M., KAWAKAMI, A., SAWADA, A., FURUTANI-SEIKI, M., TAKEDA, H. and ARAKI, K. (2002). Tbx24, encoding a t-box protein, is mutated in the zebrafish somite-segmentation mutant fused somites. *Nat Genet* 31: 195-9.

NOMURA-KITABAYASHI, A., TAKAHASHI, Y., KITAJIMA, S., INOUE, T., TAKEDA, H. and SAGA, Y. (2002). Hypomorphic mesp allele distinguishes establishment of rostrocaudal polarity and segment border formation in somitogenesis. *Development* 129: 2473-81.

NORTHCUTT, R.G. and GANS, C. (1983). The genesis of neural crest and epidermal placodes: A reinterpretation of vertebrate origins. *Q Rev Biol* 58: 1-28.

PALMEIRIM, I., HENRIQUE, D., ISH-HOROWICZ, D. and POURQUIE, O. (1997). Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* 91: 639-48.

PALMER, A. and KLEIN, R. (2003). Multiple roles of ephrins in morphogenesis, neuronal networking and brain function. *Genes Dev* 17: 1429-50.

PASINI, A. and WILKINSON, D.G. (2002). Stabilizing the regionalisation of the developing vertebrate central nervous system. *Bioessays* 24: 427-38.

POURQUIE, O. (2000). Segmentation of the paraxial mesoderm and vertebrate somitogenesis. *Curr Top Dev Biol* 47: 81-105.

POURQUIE, O. (2001). Vertebrate somitogenesis. *Annu Rev Cell Dev Biol* 17: 311-50.

RHEE, J., TAKAHASHI, Y., SAGA, Y., WILSON-RAWLS, J. and RAWLS, A. (2003). The protocadherin papc is involved in the organization of the epithelium along the segmental border during mouse somitogenesis. *Dev Biol* 254: 248-61.

RHINN, M. and BRAND, M. (2001). The midbrain—hindbrain boundary organizer. *Curr Opin Neurobiol* 11: 34-42.

RICKMANN, M., FAWCETT, J.W. and KEYNES, R.J. (1985). The migration of neural crest cells and the growth of motor axons through the rostral half of the chick somite. *J Embryol Exp Morphol* 90: 437-55.

SAGA, Y., HATA, N., KOSEKI, H. and TAKETO, M.M. (1997). Mesp2: A novel mouse gene expressed in the presegmented mesoderm and essential for segmentation initiation. *Genes Dev* 11: 1827-39.

SAGA, Y. and TAKEDA, H. (2001). The making of the somite: Molecular events in vertebrate segmentation. *Nat Rev Genet* 2: 835-45.

SANSON, B. (2001). Generating patterns from fields of cells: Examples from drosophila segmentation. *EMBO Rep* 2: 1083-8.

SATO, Y., YASUDA, K. and TAKAHASHI, Y. (2002). Morphological boundary forms by a novel inductive event mediated by lunatic fringe and notch during somitic segmentation. *Development* 129: 3633-44.

SAWADA, A., SHINYA, M., JIANG, Y.J., KAWAKAMI, A., KUROIWA, A. and TAKEDA, H. (2001). Fgf/mapk signalling is a crucial positional cue in somite boundary formation. *Development* 128: 4873-80.

SCHMIDT, C., CHRIST, B., MADEN, M., BRAND-SABERI, B. and PATEL, K. (2001). Regulation of epha4 expression in paraxial and lateral plate mesoderm by ectoderm-derived signals. *Dev Dyn* 220: 377-86.

SIMEONE, A. (2000). Positioning the isthmic organizer where otx2 and gbx2meet. *Trends Genet* 16: 237-40.

SOSIC, D., BRAND-SABERI, B., SCHMIDT, C., CHRIST, B. and OLSON, E.N. (1997). Regulation of paraxis expression and somite formation by ectoderm- and neural tube-derived signals. *Dev Biol* 185: 229-43.

STERN, C.D., FRASER, S.E., KEYNES, R.J. and PRIMMETT, D.R. (1988). A cell lineage analysis of segmentation in the chick embryo. *Development* 104 Suppl: 231-44.

STOCKDALE, F.E., NIKOVITS, W., JR. and CHRIST, B. (2000). Molecular and cellular biology of avian somite development. *Dev Dyn* 219: 304-21.

STRUTT, D. (2003). Frizzled signalling and cell polarisation in drosophila and vertebrates. *Development* 130: 4501-13.

TAKAHASHI, Y., INOUE, T., GOSSLER, A. and SAGA, Y. (2003). Feedback loops comprising dll1, dll3 and mesp2 and differential involvement of psen1 are essential for rostrocaudal patterning of somites. *Development* 130: 4259-4268.

TEILLET, M.A., KALCHEIM, C. and LE DOUARIN, N.M. (1987). Formation of the dorsal root ganglia in the avian embryo: Segmental origin and migratory behavior of neural crest progenitor cells. *Dev Biol* 120: 329-47.

TEPASS, U., GODT, D. and WINKLBAUER, R. (2002). Cell sorting in animal development: Signalling and adhesive mechanisms in the formation of tissue boundaries. *Curr Opin Genet Dev* 12: 572-82.

TONEGAWA, A., KASAI, T. and TAKAHASHI, Y. (2003). Systematic screening for signaling molecules expressed during somitogenesis by the signal sequence trap method. *Dev Biol* 262: 32-50.

WILKINSON, D.G. (2001). Multiple roles of eph receptors and ephrins in neural development. *Nat Rev Neurosci* 2: 155-64.

XU, Q., MELLITZER, G., ROBINSON, V. and WILKINSON, D.G. (1999). In vivo cell sorting in complementary segmental domains mediated by eph receptors and ephrins. *Nature* 399: 267-71.

YAMAMOTO, A., AMACHER, S.L., KIM, S.H., GEISSERT, D., KIMMEL, C.B. and DE ROBERTIS, E.M. (1998). Zebrafish paraxial protocadherin is a downstream target of spadetail involved in morphogenesis of gastrula mesoderm. *Development* 125: 3389-97.

ZELTSER, L.M., LARSEN, C.W. and LUMSDEN, A. (2001). A new developmental compartment in the forebrain regulated by lunatic fringe. *Nat Neurosci* 4: 683-4.