Coupling segmentation to axis formation

Julien Dubrulle* and Olivier Pourquié†

Stowers Institute for Medical Research, 1000E 50th street, Kansas City, MO 64110, USA
*Present address: Skirball Institute of Biomolecular Medicine, New York University School of Medicine, 540 First Avenue, New York, NY 10016-6497, USA
†Author for correspondence (e-mail: olp@stowers-institute.org)

Development 131, 5783-5793
Published by The Company of Biologists 2004
doi:10.1242/dev.01519

Summary

A characteristic feature of the vertebrate body is its segmentation along the anteroposterior axis, as illustrated by the repetition of vertebrae that form the vertebral column. The vertebrae and their associated muscles derive from metameric structures of mesodermal origin, the somites. The segmentation of the body is established by somitogenesis, during which somites form sequentially in a rhythmic fashion from the presomitic mesoderm. This review highlights recent findings that show how dynamic gradients of morphogens and retinoic acid, coupled to a molecular oscillator, drive the formation of somites and link somitogenesis to the elongation of the anteroposterior axis.

Introduction

The body axis of vertebrates and cephalochordates is subdivided along the anteroposterior (AP) axis into repeating segments. This segmental or metameric pattern is established early in embryogenesis by the process of somitogenesis. Somites are blocks of paraxial mesoderm cells that give rise to the vertebrae of the axial skeleton and their associated muscles and tendons, which retain a metameric pattern. They also yield all the skeletal muscles of the body wall and limbs, as well as of the dermis of the back (Brent and Tabin, 2002). During development, somitogenesis is tightly coupled to axis formation via a system involving dynamic gradients of morphogens, namely fibroblast growth factors (FGFs), Wnt proteins and retinoic acid (RA).

In the chick embryo, somitogenesis begins soon after ingression of cells from the epiblast into the mesodermal layer, through the anterior primitive streak (Garcia-Martinez and Schoenwolf, 1992; Psychoyos and Stern, 1996). The most anterior paraxial mesoderm cells form the head mesoderm, which does not display any overt metamerization (Freund et al., 1996; Jouve et al., 2002; Kuratani et al., 1999). The production of the trunk paraxial mesoderm then follows that of the head mesoderm without interruption. The first somitic boundary arises a couple of hours after the beginning of the ingression of the somitic mesoderm, and the first somite pair forms directly posterior to the otic vesicle region (Hinsch and Hamilton, 1956; Huang et al., 1997). From this moment, a new pair of somite boundaries forms sequentially, adding new segments along the AP axis. A striking feature of somitogenesis is that these boundaries form at a pace that is species dependent and takes 90 minutes in chick, 120 minutes in mouse and 30 minutes in zebrafish in optimal temperature conditions [although some variations in the pace of somitogenesis along the AP axis have been reported in the mouse embryo (Tam, 1981)].

Once formed, somitic cells progressively differentiate to give rise to five major cell types: the bone, cartilage and tendons of the trunk; skeletal muscles of the body; and the dermis of the back. This differentiation process is regulated by extrinsic signals that originate from the tissues surrounding the somites (for a review, see Marcelle et al., 2002), and different somitic compartments emerge along the dorsoventral and mediolateral axes of each segment. The dorsally located dermomyotome contains the precursors of dermal cells and skeletal muscles, whereas bone, cartilage and tendon precursors of the back arise from the ventrally located sclerotome (Brent et al., 2003; Brent and Tabin, 2002). Along the mediolateral axis, the medial part of the somite will contribute to the muscles of the back and the lateral one will contribute to the limbs and body wall musculature (Ordahl and Le Douarin, 1992)

Each somite is also subdivided into a rostral and caudal compartments (Stern and Keynes, 1987). This rostrocaudal compartmentalization has strong implications for vertebrae formation. The adult vertebrae directly arise from the sclerotome of somites; however, one vertebra is not produced from one sclerotome, but rather from the fusion of the caudal half of the sclerotome of one somite with the rostral half of the following somite in a process called resegmentation (Bagnall et al., 1988; Christ et al., 1998) (reviewed by Saga and Takeda, 2001). Therefore, the embryonic segments, the somites, and the adult segments, the vertebrae, are offset by a half-segment, much like the segments and parasegments in *Drosophila* (Lawrence, 1992). By contrast, each axial myotome derives from a single somite, which results in the muscles of the back being attached to two successive vertebrae, allowing the axial skeleton to bend. This rostrocaudal subdivision is already established in the anterior presomitic mesoderm (PSM) (Stern et al., 1991) and restricts the migration of neural crest cells and motor axons within the rostral region of the sclerotome, resulting in the segmentation of the peripheral nervous system (Bronner-Fraser, 2000).

Finally, the somitic mesoderm ultimately becomes patterned into cervical, thoracic, lumbar, sacral and caudal regions. This regionalization is established early on in the PSM and mostly relies on the activity of Hox genes (Kieny et al., 1972; Nowicki and Burke, 2000) (reviewed by Krumlauf, 1994). Although several links have been established between somitogenesis and

the nested expression domains of Hox genes in the paraxial mesoderm (Cordes et al., 2004; Dubrulle et al., 2001; Zakany et al., 2001), the coordination of these two patterning processes is poorly understood and will not be discussed further in this review.

Here, we compare the somitogenesis process between vertebrates and discuss the molecular mechanisms involved in the generation of the metameric pattern from an initial uniform field of cells, the PSM. Somitogenesis is also embedded into the global formation of the AP axis of the developing embryo, and the rate of axis elongation and of somite formation have to be finely balanced. Recent findings suggest that dynamic gradients of RA and FGF/Wnt link somitogenesis to axis elongation.

An overview of somitogenesis

During somitogenesis, at the body level, the paraxial mesoderm consists of the somitic region anteriorly and of the unsegmented PSM posteriorly. Because new somites are constantly added during somitogenesis, the ratio of segmented mesoderm over unsegmented mesoderm increases over time. While somite formation periodically removes unsegmented material from the PSM anteriorly, new mesodermal cells are added at the posterior extremity of the unsegmented tissue by the ongoing gastrulation process taking place in the primitive streak and later on in the tailbud. However, the net balance between the removal and addition of unsegmented tissue is not null and the length of the PSM varies during development. In mouse and chick embryos, PSM formation starts soon after the beginning of primitive streak regression. Its length progressively increases and peaks 1 day later, when it contains 12-14 presumptive somites in the chick embryo and around six somites in the mouse embryo (Packard and Meier, 1983; Tam and Beddington, 1986). The PSM length then gradually decreases until almost no unsegmented material remains, which coincides with the end of axis elongation. However, of these which ends first is unclear (see Bellairs, 1986). Somite formation lasts for 3 days in avian embryos, until the species-specific number of somites is reached (52 in the chick).

Two important properties of somitogenesis can be defined at this point. First of all, it is a sequential and directional process: the first formed (or eldest) somite is located at the anterior tip of the trunk paraxial mesoderm, and the last produced (youngest) somite is located more posteriorly. Second, this is a periodic process, where a new boundary is invariably formed after a given amount of time. Therefore, counting the number of somites is a very reliable way to stage an embryo. Somitogenesis also has two other striking properties. The vertebrate body shows bilateral symmetry; this is true for the paraxial mesoderm, which lies on both sides of the axial structures, the notochord and neural tube. This is also true for the position of somite boundaries, which are located at the same AP level for a given pair of somites. Finally, somite boundary formation is not only periodical but also synchronous, each somite of a pair being formed simultaneously, indicating that the development between the left and right side is tightly coordinated. These latter properties imply that the number of somites on each side of the embryo is absolutely the same at any given time point. The mechanisms that control this left/right symmetry are poorly understood. There are, however, some exceptions among chordates: in *Xenopus*, for example, even if somites are symmetrically arranged, their formation can be asynchronous (Li et al., 2003); in Amphioxus, somitogenesis is more advanced on the left embryonic side, i.e. it often contains one more somite than the right (Minguillon and Garcia-Fernandez, 2002; Schubert et al., 2001).

Each cell contributing to the paraxial mesoderm undergoes a series of stereotypical events, from its specification in the caudal part of the embryo to its incorporation into a somite (Fig. 1A). The first step in paraxial mesoderm formation is a change in the cellular adhesion properties. Cells contained in the primitive streak and fated to become paraxial mesoderm lose their epithelial characteristics and become free to migrate laterally to enter the nascent PSM. The forming caudal PSM thus resembles a loose mesenchymal tissue (Fig. 1B). In the rostral third of the PSM, the nuclei of cells occupying the dorsal and ventral aspects of the tissue begin to align close to the ectoderm and endoderm that, respectively, overlie and underlie this tissue (Duband et al., 1987). In the rostralmost PSM, epithelial characteristics can be easily detected in the cells that form the future somitic walls. By contrast, cells located in the middle of the anterior PSM retain a mesenchymal character and will eventually contribute to the somitocoele mesenchyme that fills the somite cavity (Fig. 1B). The final step requires the formation of the somitic cleft, leading to the individualization of the somite.

Similar maturation steps have been identified in different vertebrates based on molecular and cellular criteria (Griffin and Kimelman, 2002; Jen et al., 1999). There are, however, some species-specific differences in the cellular organization of the PSM. For example, somite formation in *Xenopus* is very different from that in the chick. PSM cells are oriented mediolaterally, their nuclei align towards the middle of the tissue and they show no epithelial characteristics. At the time of somite formation, frog cells undergo a 90° rotation to become distributed anteroposteriorly (Hamilton, 1969) and then rapidly differentiate into muscles. In cephalochordates, the unsegmented tissue is kept to a minimum, and somites form almost immediately after cells exit the growth zone (Schubert et al., 2001).

Genesis of the paraxial mesoderm

Because somitogenesis starts while the formation of the axis is far from being completed, the population of PSM cells has to be continuously renewed. The PSM is constantly supplied caudally with new cells by progenitors located in the primitive streak and tailbud. This section summarizes the molecular events that control the rate of PSM production, the regulation of which is crucial for somitogenesis.

Depending on their position along the embryonic axis, paraxial mesodermal cells originate from two related embryonic structures. In amniotes, the head mesoderm and the anterior somites (i.e. those in the occipital, cervical and thoracic regions) are formed by the ingression of the caudal epiblast through the anterior primitive streak (Garcia-Martinez and Schoenwolf, 1992). There is a direct correlation between the timing of the ingression of a cell and its future location along the AP axis: the later, the more posterior. After the completion of primitive streak regression around the 16-somite stage in chick, all the tissues contributing to the lumbar, sacral and caudal regions are produced by a particular embryonic

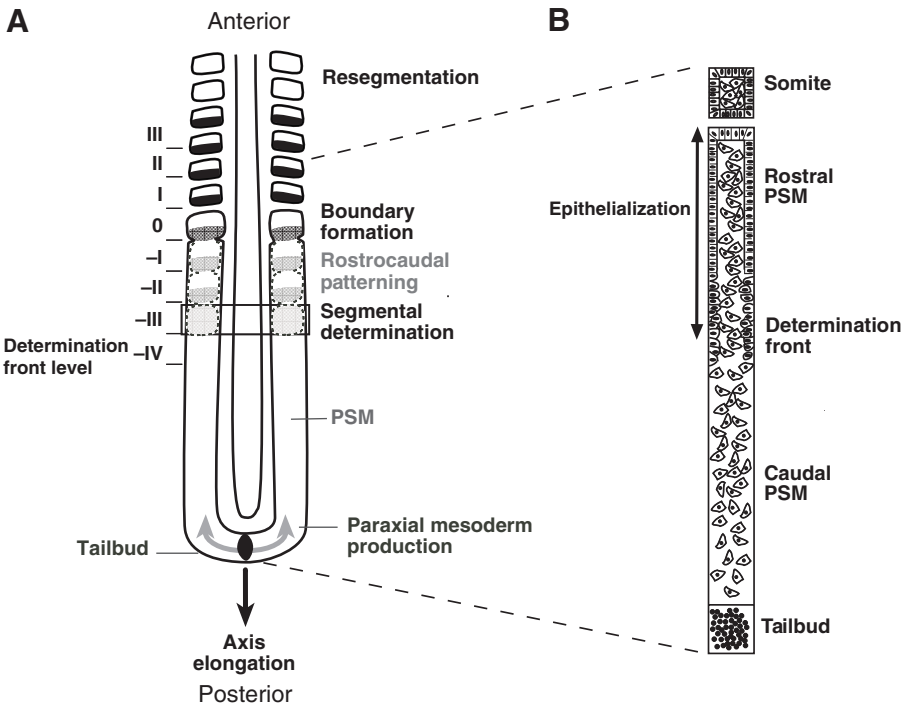

Fig. 1. The successive steps in somitogenesis. (A) The caudal part of a 2-day-old chicken embryo, representing the events taking place in the presomitic mesoderm (PSM) until somite formation. Somite nomenclature, as defined by Pourquie and Tam (Pourquie and Tam, 2001) is indicated on the left. Paraxial mesoderm production is indicated by gray arrows. (B) A parasagittal section through the PSM illustrating the cellular organization of cells along the AP axis. Caudal PSM cells are of a loose mesenchymal character and epithelialization begins anterior to the determination front level.

structure, the tailbud, which forms after the closure of the neuropore at the posterior tip of the embryo (Catala et al., 1995). The tailbud is a small mass of highly packed undifferentiated cells, which undergo complex stereotyped movements similar to gastrulation before contributing to the definitive layers. These features suggest that the tailbud corresponds to a functional remnant of the blastopore or of the primitive streak (Cambray and Wilson, 2002; Catala et al., 1995; Gont et al., 1993; Kanki and Ho, 1997; Knezevic et al., 1998).

From cell-labeling and lineage-tracing experiments, it has been proposed that the paraxial mesoderm derives from a population of resident paraxial mesoderm progenitors (PMP) that is located first in the primitive streak and then in the tailbud (Gardner and Beddington, 1988; Nicolas et al., 1996; Psychoyos and Stern, 1996; Selleck and Stern, 1991; Stern et al., 1992). There is a direct correlation between the localization of the PMPs along the AP axis of the primitive streak and the tailbud, and their final contribution to the mediolateral axis of the mesoderm: PMPs located anteriorly in the primitive streak or tailbud contribute to the medial aspect of the mesodermal layer, whereas lateral cells of the paraxial mesoderm derive from more posterior regions of the streak (Eloy-Trinquet and Nicolas, 2002; Freitas et al., 2001; Psychoyos and Stern, 1996; Schoenwolf et al., 1992; Selleck and Stern, 1991; Tam, 1988). Thus, the final position of paraxial mesodermal cells in the embryonic space depends both on the time they are produced, which specifies their AP position, and their location along the primitive streak/tailbud AP axis at the moment of their specification, which defines their position along the mediolateral axis.

Three main events take place in the rostral primitive streak and tailbud, which lead to the generation of the PSM: proliferation, specification and emigration. The multiple factors that play a role in each of these steps mainly belong to four pathways, the Wnt, FGF, RA and bone morphogenetic protein (BMP) pathways. Representative examples of gene mutations in each of these pathways are summarized in Table 1. The control of cell proliferation and survival in the growth zone is crucial to maintain the pool of PMPs during axis elongation. Inactivation of genes thought to regulate cell proliferation and survival in the tailbud and PSM, such as those of the Wnt and the RA pathway leads to a truncation of the axis (Abu-Abed et al., 2003; Greco et al., 1996; Lohnes et al., 1994; Marlow et al., 2004; Niederreither et al., 1999; Yamaguchi et al., 1999a) (Table 1). A second crucial step in paraxial mesoderm production from the pluripotent tailbud cells is their appropriate specification to the paraxial mesoderm lineage. Genes belonging to the T-box family of transcription factors, to the BMP4 and to the Wnt pathway have been shown to play an essential role in this process (Chapman et al., 1996; Galceran et al., 1999; Herrmann et al., 1990; Streit and Stern, 1999; Tonegawa and Takahashi, 1998; Yamaguchi et al., 1999b) (Table 1). Finally, the control of the emigration of cells from the primitive streak to form the PSM has been shown to largely rely on the FGF pathway (Ciruna and Rossant, 2001; Sun et al., 1999; Yamaguchi et al., 1994; Yang et al., 2002) (Table 1). However, all these pathways interact with each other, directly or indirectly, via positive or negative feedback loops, which makes it difficult to define a specific role for a given gene in one of these events.

Building a metameric pattern: clocks and gradients

Crucial events take place in the caudal PSM that irreversibly commit PSM cells to their definitive segmental fate. Once this segmental commitment has been achieved, the successive molecular steps that lead to somite formation are activated in a tissue-autonomous fashion in the anterior PSM, ultimately leading to the periodic formation of somites. The spatial and temporal information of segment specification acquired by posterior PSM cells relies on a maturation front and a biological clock that have been molecularly characterized in the past few years.

Generating periodicity within the PSM: the segmentation clock

The striking periodicity of somite distribution and production has led to the proposal of a series of theoretical models that

Table 1. Examples of mutations/genes that affect paraxial mesoderm production in the mouse*

Genes/genotype	Expression pattern	Mutant phenotype	Assigned functions	References
Wnt5a	Tailbud	Axis truncation (mouse); tail shortening (zebrafish)	Tailbud and PSM cell survival (mouse); regulation of tailbud cell movements (zebrafish)	Yamaguchi et al., 1999a; Marlow et al., 2004
vestigial tail (*Wnt3a* hypomorph)	Not applicable	Posterior axis truncation	Tailbud cell survival	Greco et al., 1996
Wnt3a	Tailbud	Posterior paraxial mesoderm replaced by ectopic neural tubes	Paraxial mesoderm fate specification	Yamaguchi et al., 1999b
Lef/Tcf double null	Not applicable	Posterior paraxial mesoderm replaced by ectopic neural tubes	Downstream effectors of Wnt in mesoderm fate specification	Galceran et al., 1999
Raldh2	Somites, anteriormost PSM	Severe axis truncation	Unclear, progenitor pool maintenance?	Niederreither et al., 1999
Cyp26	Tailbud	Severe axis truncation	Unclear, progenitor pool maintenance?	Abu-Abed et al., 2003
Compound RARs	Broadly expressed	Skeletal defects, axis agenesis	Unclear	Lohnes et al., 1994
brachyury (*T*), *no tail* (zebrafish)	Tailbud, notochord, posterior PSM	Tailbud hypertrophy, notochord replaced by ectopic neural tube (mouse); tail agenesis (zebrafish)	Notochord specification, tailbud elongation	Hermann et al., 1990; Yamaguchi et al., 1999b
Tbx6	Tailbud, PSM	Posterior paraxial mesoderm replaced by neural tubes	Paraxial mesoderm specification	Chapman and Papaioannou, 1998
Noggin	Node, anterior primitive streak	Loss of caudal vertebrae, shortened body axis (mouse); overexpression expands paraxial mesoderm (chick)	Antagonizes BMP4 to specify paraxial mesoderm versus lateral plate mesoderm	Streit and Stern, 1999; Tonegawa and Takahashi, 1998; McMahon et al., 1998
Fgf8	Primitive streak, tailbud, posterior PSM	Mesodermal cells fail to emigrate from the streak	Promotes gastrulation movements (mouse); chemorepulsant (chick)	Sun et al., 1999; Yang et al., 2002
Fgfr1	Primitive streak, tailbud, anterior PSM	Mesodermal cells fail to emigrate from the streak	Mediates FGF signaling	Ciruna and Rossant, 2001

*Unless otherwise specified.
Abbreviations: BMP, bone morphogenetic protein; FGF, fibroblast growth factor; PSM, presomitic mesoderm; RAR, retinoic acid receptors.

postulate the existence of an oscillator or clock that acts in the cells of the PSM (Cinquin, 2003; Cooke and Zeeman, 1976; Lewis, 2003; Meinhardt, 1986; Primmett et al., 1989) (for a review, see Dale and Pourquie, 2000). In these models, the oscillator sets the pace of the segmentation process by generating a periodic signal that is subsequently translated into the periodic array of somite boundaries. The first evidence for such a molecular oscillator, termed the segmentation clock, came from the observation of the periodic expression in PSM cells of chick *Hairy1*, a basic helix-loop-helix (bHLH) transcription factor belonging to the Hairy/enhancer of split family. This gene is expressed as a wave sweeping the unsegmented mesoderm in a posterior to anterior fashion, once during each somite formation (Fig. 2B) (Palmeirim et al., 1997). A growing number of genes that exhibit a seemingly dynamic expression pattern in the PSM, called 'cyclic genes', has now been characterized in fish, frog, birds and mammals, suggesting that the segmentation clock has been conserved in vertebrates. All identified cyclic genes thus far belong to the Notch and Wnt signaling pathways. These genes establish interacting feedback loops that all act downstream of Wnt signaling and generate oscillations in signaling activities (Aulehla et al., 2003; Bessho et al., 2003; Dale et al., 2003; Hirata et al., 2004). The newly identified receptor tyrosine phosphatase ψ is also involved in the control of the Notch-driven oscillator (Aerne and Ish-Horowicz, 2004). The role of the segmentation clock in somitogenesis still remains unclear. However, an important output of the oscillator is the periodic activation of the Notch signaling pathway in the PSM, which probably plays a crucial role in the initial definition of the segmental domain, as discussed later. As the molecular mechanisms of the segmentation clock machinery have been extensively reviewed elsewhere (Lewis, 2003; Pourquie, 2003; Rida et al., 2004), we do not discuss them further here.

Arranging repetitive patterns in embryonic space: FGF/Wnt and RA antagonistic gradients

The translation of the temporal periodicity of cyclic gene expression, as driven by the segmentation clock, into the spatial periodicity of somite distribution, is proposed to be mediated by a front of cell competence that travels along the AP axis of the embryo. The aim of such a front (called the determination front) is to position along the AP axis of the PSM the response of a cell to the periodic signaling (e.g. by defining an initial segmental domain of gene activation) at regular intervals within the PSM. Recent findings suggest that the mechanisms controlling the progression of this front involve two dynamic, antagonizing gradients of morphogens: a caudorostral Wnt/FGF gradient and a rostrocaudal RA gradient (Fig. 2A).

In the chick embryo, rotating a one-somite-length group of cells by 180° in the anterior PSM produces somites with a reversed rostrocaudal compartmentalization and/or generates segmentation defects (e.g. ectopic boundaries), suggesting that the position of somitic boundaries is already determined in this

region. By contrast, such rotations in the caudal PSM result in a normal segmentation pattern, suggesting that these cells are still naive with respect to their segmentation fate (Dubrulle et al., 2001). The interface between these two regions of the PSM has been called the determination front, which correlates with cellular and molecular changes and flags the beginning of the segmental determination of the PSM (Dubrulle et al., 2001). The relative position of this front in the PSM is constant, but because of the anterior-to-posterior progression of somitogenesis, its absolute position along the AP axis of the embryo is constantly shifted caudally at a velocity similar to that of somitogenesis.

The position of the determination front has been proposed to be defined by a threshold activity of FGF signaling. *Fgf8* transcripts are distributed along a caudorostral gradient in the posterior PSM (Figs 2 and 3), which is converted into graded FGF8 protein distribution (Dubrulle and Pourquie, 2004). Disrupting the FGF8 gradient by overexpressing *Fgf8* in the chick paraxial mesoderm blocks somitogenesis and causes cells to retain a mesenchymal character and to maintain the expression of caudal PSM markers, such as brachyury. Together with the graded distribution of FGF8 along the AP axis of the PSM, these results indicate that caudal PSM cells are maintained in an immature state by high levels of FGF signaling, and that they only activate their segmentation program when they reach a specific threshold of FGF activity (Dubrulle et al., 2001).

It is possible to alter locally the shape of the FGF gradient by implanting beads soaked in FGF8 in the caudal PSM. Such an experiment results in the formation of a series of smaller somites anterior to the bead, thus breaking the symmetrical arrangement of somites between the left and right sides of the embryo by shifting anteriorly boundaries of treated somites (Dubrulle et al., 2001; Sawada et al., 2001). This phenotype is dose dependent, and higher FGF doses result in even smaller somites (J.D. and O.P., unpublished). Interestingly, somites continue to form synchronously (the number of somites is the same on both sides of the embryo at any time), and the asymmetry of somite boundaries is rapidly compensated caudal to the bead by the formation of a larger somite. Expression of the cyclic genes remains synchronous between the two sides, indicating that the pace of the segmentation clock is not affected. Rather, cells exposed to FGF remain immature for slightly longer, resulting in an anterior shift of the determination front compared with the contralateral side. Integrated in time, this effect can be interpreted as a slowing down of the progression of the front across the PSM.

These data are consistent with a model in which the number of cells allocated to a given segment is defined by the number of PSM cells experiencing the passage of the front during one period of the segmentation clock. Based on this model, somite size can be altered by changing the speed of the regression of the front or the period of the clock. Although the involvement of these two parameters, a clock and a sweeping front to specify segments, was first postulated by Cooke and Zeeman (Cooke and Zeeman, 1976), the molecular nature of what generates a threshold most probably falls into a ‘clock and gradient’ model, as proposed by Meinhardt and Slack (Meinhardt, 1986; Slack, 1991). However, the essence of these models is the same. Interestingly, gain- or loss-of-function gene mutations in mice that affect the segmentation clock [such as those in lunatic fringe (*Lfng*) or Notch genes] result in a chaotic spacing of somite boundaries (Conlon et al., 1995; Dale et al., 2003; Serth et al., 2003). It is tempting to interpret these findings as a deregulation of the period of the clock, which leads to enlarged somites when the period is lengthened and to smaller ones when it is shortened.

RA signaling is the second known pathway that regulates the spatial arrangement of somite boundaries. The first evidence that RA signaling is involved in the positioning of somite boundaries came from the analysis of vitamin A-deficient (VAD) quail embryos, which do not synthesize RA. These embryos display significantly smaller somites than do control embryos, while the length of their PSM is increased: a phenotype similar to that seen in response to the grafting of a FGF8-soaked bead into chicken and zebrafish embryos (Diez del Corral et al., 2003; Maden et al., 2000). Conversely, treating *Xenopus* embryos with RA leads to the formation of enlarged somites, a phenotype similar to that seen in chicken and zebrafish embryos in which FGF signaling has been blocked by treating them with SU5402, an inhibitor of FGF signaling (Dubrulle et al., 2001; Moreno and Kintner, 2004). Although RA cannot be directly detected in the embryo, its distribution can be deduced by the expression of enzymes that are associated with its metabolism. *Raldh2* (*Aldh1a2* – Mouse Genome Informatics) an aldehyde dehydrogenase-like enzyme that converts retinaldehyde into RA, is expressed at high levels in the newly formed somites of vertebrate embryos and at a lower level in the anterior PSM (Figs 2 and 3), whereas *Cyp26* (*Cyp26a1* – Mouse Genome Informatics) an enzyme belonging to the p450 cytochrome family involved in the catabolism of RA, is strongly expressed in the tailbud region of the embryo (Blentic et al., 2003; Niederreither et al., 2003; Sakai et al., 2001). These expression patterns are expected to generate an anterior-to-posterior gradient of RA in the caudal part of the embryo (Fig. 3).

The RA gradient is thus in an opposite orientation to the FGF gradient, and it has been shown that they antagonize each other. In VAD quails, the *Fgf8* expression domain is extended anteriorly, and ectopic FGF8 inhibits *Raldh2* expression in the somitic region (Diez del Corral et al., 2003). In *Xenopus*, the formation of enlarged somites after RA treatment is preceded in the PSM by the precocious activation and the enlargement of *Thylacine1* stripes (the frog homolog of *Mesp2/Meso2,* see Fig. 2), which mark the earliest segmental domain in the PSM. These results suggest that the determination front has been shifted posteriorly in these experiments (Moreno and Kintner, 2004). They also indicate that RA controls the positioning of the determination front by antagonizing FGF signaling (Fig. 3). In frog, this antagonistic effect is not directly mediated by a downregulation of *Fgf8* expression, but by activating the mitogen-activated protein (MAP) kinase phosphatase 3 (MKP3), which negatively regulates the MAP kinase pathway (Moreno and Kintner, 2004). Mouse *Raldh2*-null mutants die early during development and exhibit axis truncation, and, in agreement with the VAD phenotype, these mutants display smaller somites (Niederreither et al., 1999). These results together suggest that the progression of the determination front is regulated by two mutually inhibitory, dynamic gradients: a caudorostral FGF8 gradient that prevents the initiation of the segmentation program; and a rostrocaudal RA gradient that relieves this inhibition by antagonizing FGF

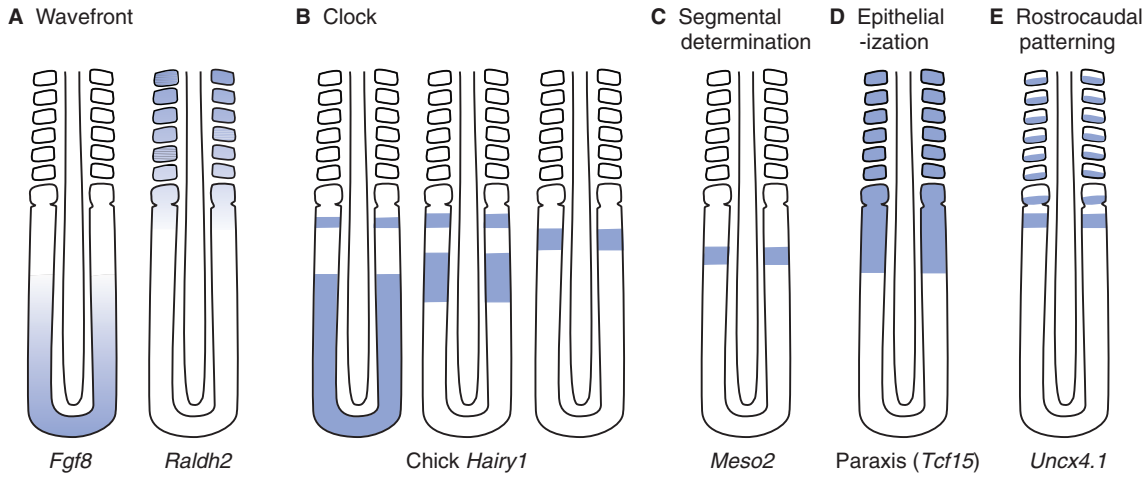

Fig. 2. Categories of gene expression patterns that are associated with paraxial mesoderm segmentation and maturation in the chick embryo. (A-E) In situ hybridization gene expression patterns in the presomitic mesoderm (PSM). (A) Genes that regulate the wavefront progression: the caudal-to-rostral *Fgf8* (fibroblast growth factor 8) gradient (left) and the rostral-to-caudal *Raldh2* (retinaldehyde dehydrogenase 2) gradient (right). (B) Expression of a cyclic gene, chick *Hairy1*, in three different phases that identify the segmentation clock. (C) Striped expression of genes involved in segment specification and rostrocaudal compartmentalization, such as these belonging to the Mesp/*Meso2*/*Thylacine1* bHLH transcription factor family. (D) Expression pattern of paraxis (*Tcf15*), which is involved in the epithelialization process during somite formation, in the anterior PSM and somites. (E) Genes highlighting the rostrocaudal compartmentalization of the formed somites, such as *Uncx4.1*, a paired homeobox transcription factor that has restricted expression in the caudal part of the somites.

activity and/or by directly activating genes involved in the segmentation process.

Finally, the last known pathway involved in boundary positioning is the Wnt pathway. Grafting clumps of cells that overexpress Wnt3a to the caudal part of the chick embryo leads to the formation of small somites in the vicinity of the overexpressing cells (Aulehla et al., 2003). In mouse, *Wnt3a* is strongly expressed in the growth zone of the primitive streak and tailbud, and it was proposed that, owing to its diffusion properties and to the elongation of the axis, the Wnt3a protein is distributed in a caudorostral gradient within the nascent PSM. In support of this idea, *Axin2*, a negative regulator of the Wnt/β-catenin cascade, which is a direct target of *Wnt3a*, is expressed in a gradient in the caudal PSM (Aulehla et al., 2003). A striking feature of *Axin2* is that its expression is not only graded in the posterior PSM but it also cycles out of phase with the cycling genes that belongs to the Notch pathway. It is proposed that the amount of Wnt signaling directly controls the amplitude of the oscillations of *Axin2*, until Wnt signaling drops below a given threshold where the *Axin2* oscillations stop, relieving the inhibitory effect of *Axin2* on the segmentation program. In the *vestigial tail* mutants (see Table 1), *Fgf8* expression is downregulated, suggesting that *Fgf8* acts downstream of *Wnt3a*. It has been proposed in this set of experiments that the segmentation clock and the morphogen gradients controlling the determination front are interegulated via Wnt signaling (Aulehla and Herrmann, 2004; Aulehla et al., 2003).

The fact that the oscillations driven by the segmentation clock are arrested by the passage of the determination front is consistent with the observation that the regulatory elements controlling *Lfng* expression are different in the anterior and posterior PSM. The promoter region of this gene has been studied in the mouse and has been found to have a complex enhancer organization (Cole et al., 2002; Morales et al., 2002). One regulatory block is strictly involved in the cyclic expression of *Lfng* in the caudal PSM, while another one is required for its expression in the anterior PSM.

Accomplishing the segmentation program: from the determination front to somite formation

The accomplishment of the segmentation program – the sequence of cellular and molecular events that lead to somite formation in a tissue-autonomous fashion – requires complex morphogenetic changes, which occur in the anterior PSM. The key events of this program include cellular reorganization leading to a progressive epithelialization of the tissue, specification of rostral and caudal compartments, and the formation of a somitic cleft between fully patterned blocks of cells (Fig. 1).

Output of the clock and gradients: the Mesp genes?

The immediate consequence of the interaction of the clock and the traveling wavefront is thought to be the definition of a discrete initial segmental domain anterior to the determination front level in the anterior PSM, which will be used as a template for subsequent somite formation (Fig. 1A). The first genes expressed in a segmental fashion in the PSM belong to the Mesp family of bHLH transcription factors (Fig. 2C), which have been characterized in fish, frog, birds and mammals (Buchberger et al., 1998; Jen et al., 1999; Saga et al., 1997; Sawada et al., 2000; Sparrow et al., 1998; Takahashi et al., 2000). In mouse, *Mesp2* is activated by periodic Notch signaling in the rostral PSM in a segment-wide domain, where it controls downregulation of Delta-like 1 (*Dll1*), a mouse homolog of the Notch ligand Delta, in a presenilin-independent fashion (Takahashi et al., 2000). The *Xenopus* homolog of *Mesp2*, *Thylacine1*, is also expressed in response to periodic Notch signaling in the anterior PSM, and its transcriptional activation requires direct RA signaling, thus explaining why it

can be activated only after the passage of the determination front (Jen et al., 1999; Moreno and Kintner, 2004). These observations have led to the idea that Mesp genes respond to the segmentation clock and thus participate in translating the periodic signal of the oscillator into a linear array of segmental domains.

In addition to their role in the initial definition of the segment, the Mesp genes are also involved in establishing the rostral and caudal compartment identities of the future somites (Saga et al., 1997; Takahashi et al., 2000). In the anterior PSM, Mesp genes expression become restricted to half-segment domains. In mouse, *Mesp2* becomes restricted to the future rostral somitic half and it acts downstream of Notch signaling to specify the rostral compartments of somites (Saga et al., 1997).

Maturation steps within the anterior PSM

Concomitantly to the rostrocaudal compartmentalization of the paraxial mesoderm, the anterior PSM undergoes a progressive maturation that ultimately leads to somite formation. Several factors control this maturation, and their loss of function usually leads to aberrant somite morphogenesis, or to the failure of somite formation, while segments are specified.

Foxc winged helix transcription factors have been identified as playing a crucial role in the control of somite prepatterning in the anterior region of the PSM, where they are strongly expressed. Mice null for both *Foxc1* and *Foxc2* display no segmentation of the paraxial mesoderm. In these mutants, some markers of rostrocaudal compartmentalization and of boundary formation such as *Notch1*, *Mesp1/2*, ephrin B2 (*Efnb2*) are absent, whereas others, such as *Lfng* or *Dll1*, are abnormally expressed (Kume et al., 2001). In zebrafish, the knock down of *foxc1a* leads to a similar phenotype (Topczewska et al., 2001). These results suggest that the Foxc factors may act as permissive signals for the segmentation program to proceed. These phenotypes are similar to those seen in the mutant fish *fused somites* (a *tbx24*-null mutant), in which no boundaries form along the entire AP axis of the embryo (Nikaido et al., 2002). *fused somites* fish ultimately form a segmented axial skeleton, suggesting that this mutation does not affect the specification of segments during somitogenesis (van Eeden et al., 1996). However, it has been shown that the later segmentation of the axial skeleton in *fused somites* is controlled by signals coming from the notochord (Fleming et al., 2004).

Another morphogenetic process that is likely to be initiated by the passage of the determination front is the epithelialization of the PSM (Fig. 1). The mesenchymo-epithelial transition that occurs concomitantly with the segmental patterning of the anterior PSM is not well understood, but several adhesion molecules such as integrins, fibronectins and cadherins are progressively accumulated in the anterior PSM (Duband et al., 1987). In the mouse, the inactivation of the paraxis gene (*Tcf15*), a bHLH transcription factor, results in a lack of epithelialization of the paraxial mesoderm (Burgess et al., 1996). *Tcf15* is expressed rostral to the determination front level in the anterior PSM and somites (Fig. 2D) (Burgess et al., 1995; Sosic et al., 1997). Despite the lack of epithelialization in *Tcf15* mutant mice, the paraxial mesoderm retains some degree of metameric organization, even though, at later stages, vertebrae and dorsal root ganglia are fused (Johnson et al., 2001). An outcome of *Tcf15* function might be to control the activity of Rho GTPases such as Rac1 and Cdc42, which have been recently shown to mediate the mesenchymo-epithelial transition during somite formation (Nakaya et al., 2004).

Once PSM cells have been allocated to a given metamer and have acquired their rostrocaudal identity, the last step in somitogenesis is to create an acellular somitic boundary. Several signaling pathways have been shown to play a key role in this process. During somite boundary formation in chick, the receptor *Notch1* is expressed in the forming somite, its ligands *Delta1* and *Serrate1* are expressed in the posterior compartment of the forming somite, whereas *Lfng*, a glycosyltransferase that modulates Notch signaling, is expressed in the anterior compartment (Sato et al., 2002). This situation is very reminiscent of the fly wing imaginal disc, where Notch signaling establishes the boundary between the dorsal and ventral compartments (Panin et al., 1997). Grafting cells from the region lying immediately posterior to the presumptive boundary of the next-to-be-formed somite (i.e. presumptive anterior compartment) can induce boundaries in ectopic position (i.e. in the middle of the somite) (Sato et al., 2002). This boundary induction can be mimicked by transplanting cells that do not normally induce boundaries but that overexpress *Lfng* or a constitutively active form of the Notch receptor, suggesting that modulation of Notch activity via *Lfng* triggers cleft formation (Sato et al., 2002).

The Eph receptor/ephrin pathway triggers one of the final step of boundary formation. This pathway controls cell mixing (Mellitzer et al., 1999) and is a bi-directional signaling pathway in which both the receptor (Eph) and the ligand (ephrin) direct downstream signaling events upon activation [called forward and reverse signaling, respectively (Murai and Pasquale, 2003)]. In zebrafish, the ligand and the receptor, in particular *ephrinB2* and *epha4* respectively, are expressed on each side of the forming boundary (Durbin et al., 1998). It has been shown in zebrafish that this pathway is directly responsible for the cellular change that is associated with boundary formation at the interface between ligand- and receptor-expressing cells: Eph receptor/ephrin interactions induce cell polarization, the basal localization of the nuclei, the apical distribution of β-catenin and columnar shape acquisition (Barrios et al., 2003). Despite the conservation of ephrin expression patterns between fish and amniotes, no somitic phenotype has been observed in the mouse *Epha4* (Helmbacher et al., 2000) or *Efnb2* (Wang et al., 1998) mutants, possibly owing to the redundant activities of the ephrins in this species. However, in Notch gene mutants, *Epha4* stripes are absent or severely disrupted, suggesting that Notch signaling acts upstream of Eph signaling during boundary formation (Barrantes et al., 1999).

Cell-adhesion proteins also play a crucial role in somite formation, by regulating the cellular organization of the somitic tissue. Interfering with the function of cadherins, such as N-cadherin, cadherin 11 or the protocadherin *Papc* (*Pcdh8* – Mouse Genome Informatics) results in the severe disruption of the epithelialization/somite formation process (Horikawa et al., 1999; Kim et al., 2000; Kimura et al., 1995; Linask et al., 1998; Rhee et al., 2003).

Finally, the cellular movements and behaviors that lead to cleft formation have been carefully monitored in chick and zebrafish embryos using time-lapse confocal microscopy. In

chick, the cleft between the forming somite and the unsegmented mesoderm is not a straight line perpendicular to the axial structure, but instead appears as a 'ball-and-socket': the ball being the forming somite and the socket the PSM (Kulesa and Fraser, 2002). Cells at the posterior edge of the forming somite, after loosing their adhesiveness with PSM cells, coalesce and move slightly anteriorly. In the meantime, cells of the dorsal, ventral, medial and lateral aspects of the PSM, which are in contact with the forming somite, retract and fold in, becoming the anterior border cells of the next-to-form somite. In zebrafish, presumptive cells of the anterior and posterior edge of the future border are first intermixed; they then progressively segregate and eventually face each other to form the intersomitic border (Henry et al., 2000).

Coupling somitogenesis to axis elongation

As discussed earlier, the segmentation of the paraxial mesoderm and the elongation of the AP axis are highly coordinated during early embryogenesis. The balance between the rate of somite formation and the production of new mesodermal cells from the growth zone has to be finely regulated to prevent the precocious depletion of unsegmented mesodermal material. Recent data suggest that FGF8 may provide a link between axis elongation and somitogenesis, via a mechanism relying on mRNA decay (Dubrulle and Pourquie, 2004).

Fgf8 mRNAs are distributed according to a caudorostral gradient in the posterior embryo. This mRNA gradient has been shown to be converted into a graded FGF signaling activity (Dubrulle and Pourquie, 2004; Sawada et al., 2001). The FGF gradient is dynamic, as it recedes in concert with axis formation. What is the link between the regulation of this gradient and the formation of the axis? This gradient does not rely upon extrinsic signals, because the ablation of the tailbud or the in vitro culture of isolated PSM does not affect the dynamics of the *Fgf8* mRNA gradient. The analysis of regions where *Fgf8* is actively transcribed in the embryo by in situ hybridization in chick and mouse using probes directed against intronic regions of *Fgf8* has revealed that its transcription is restricted to the tailbud (Dubrulle and Pourquie, 2004). Therefore, newly produced mesodermal cells stop transcribing *Fgf8* when they enter the caudal PSM. The pool of *Fgf8* mRNAs within these cells diminishes over time because of mRNA decay, and, because of the continuous production of PSM cells from the progenitor area, this patterning strategy leads to the establishment of a dynamic gradient of *Fgf8* mRNAs in the wake of axis elongation. In agreement with this model, *Fgf8* mature transcripts are very stable, as they can still be robustly detected in the PSM several hours after treatment with actinomycin D, a broad inhibitor of transcription. Therefore, the slope of the FGF8 gradient, which eventually regulates the progression of the determination front, is a direct function of the speed of axis elongation and of the rate of RNA degradation. The studies of RA previously described suggest that the extent of the *Fgf8* gradient is limited anteriorly by RA signaling. In contrast to *Fgf8,* the RA gradient progresses concomitantly to somitogenesis (and not axis elongation), and it may serve as a sensor of the rate of somite formation to further regulate FGF signaling. Whether RA acts directly on the stability of *Fgf8* transcripts or through another mechanism remains to be determined.

It is highly possible that the Wnt3a gradient is also tied to the elongation of the axis via a similar mechanism. However, in this case, the axis elongation will not define the slope of the mRNA gradient, but rather the slope of the protein gradient. In mouse, *Wnt3a* expression is restricted to the tailbud, and, although the protein distribution has not been reported yet, it has been proposed that the continuous production of the protein from the progenitor cells coupled to the elongation of the axis may generate a gradient of Wnt3a protein in the caudal part of the embryo (Aulehla et al., 2003). Wnt3a might also directly control the transcription rate of *Fgf8* in the progenitors and thus indirectly control the shape of FGF8 gradient, as, in this model, the extent of the *Fgf8* graded expression domain is directly dependent on the initial amount of *Fgf8* mRNAs.

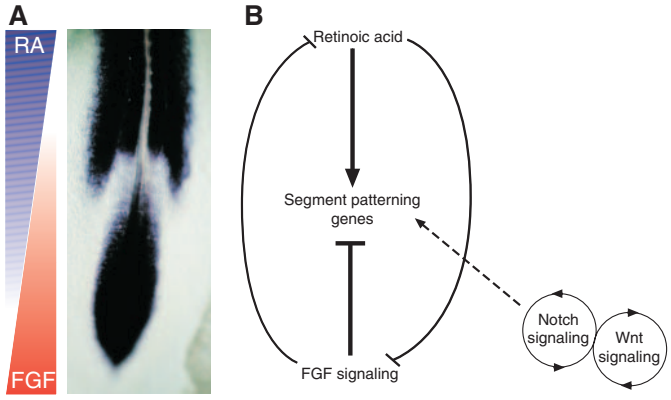

Fig. 3. A model for somitogenesis. (A) Double in situ hybridization of a 2-day-old chicken embryo with *Raldh2* (retinaldehyde dehydrogenase 2) and *Fgf8* (fibroblast growth factor 8) probes. Anterior is towards the top. These genes participate in the establishment of mutually inhibitory, antagonistic gradients of retinoic acid (RA) and fibroblast growth factor (FGF) signaling. (B) Molecular mechanisms leading to a segmental pattern. Segment patterning genes are periodically activated by the segmentation clock, whose main regulators are the Notch and Wnt signaling pathways. The spatial activation of the segment patterning genes is defined by the RA and FGF antagonistic gradients: RA positively regulates their transcription, whereas FGF signaling represses RA activity and inhibits presomitic mesoderm maturation.

Conclusions

Our current understanding of somitogenesis has greatly improved in the past couple of years. Segmentation appears to rely on two major components: an oscillator, the segmentation clock, which sets the periodicity of somite formation; and a traveling wavefront, which defines the level at which PSM cells respond to the clock, providing a mechanism that spaces the segment boundaries. We propose that the progression of the wavefront is controlled by antagonistic gradients of FGF/Wnt proteins and RA. This mechanism triggers the periodic initiation of the segmentation program in a spatially controlled fashion (Fig. 3). A complex genetic regulatory loop involving *Mesp2* and the Notch pathway then subdivides newly specified segments into rostral and caudal compartments. Finally, segments become epithelialized and ultimately separated by a boundary in the rostralmost PSM.

Although great advances have been made in the past decade in understanding aspects of the somitogenesis process, many things remain to be explained. First, the molecular machinery

that underlies the segmentation clock is far from understood. It clearly relies on negative feedback loops between different pathways, but the interactions between these pathways need to be investigated more deeply. In addition, whether the clock exclusively relies on the Notch and Wnt pathways or whether it involves a more complex molecular machinery remains to be established. Moreover, what defines the period of the clock, and what gives its species specificity is a fundamental question. Second, the recently discovered involvement of RA in positioning the determination front and its antagonizing effect on FGF signaling opens up a new perspective on this process. How this mutual inhibition affects both gradients is not clearly understood. These findings are especially exciting, as RA is directly involved in Hox gene regulation (Conlon, 1995). Finally, understanding the mechanisms that control the definitive numbers of segments and the forces that drive the elongation of the axis will provide invaluable insights into somitogenesis and into the evolution of vertebrate segmentation.

The authors thank Marie-Claire Delfini for critical reading of the manuscript. Work in O.P.'s laboratory is supported by the Stowers Institute for Medical Research, by the NIH and by the Muscular Dystrophy Association.

Note added in proof

Two recent papers published by Hofmann and colleagues and Galceran and collaborators show that LEF1-mediated Wnt signaling is involved in the regulation of *Delta-like1* in the mouse PSM, supporting the idea of close interactions between the Wnt and Notch pathways during somitogenesis (Hofmann et al., 2004; Galceran et al., 2004).

References

Abu-Abed, S., Dolle, P., Metzger, D., Wood, C., MacLean, G., Chambon, P. and Petkovich, M. (2003). Developing with lethal RA levels: genetic ablation of Rarg can restore the viability of mice lacking Cyp26a1. *Development* **130**, 1449-1459.

Aerne, B. and Ish-Horowicz, D. (2004). Receptor tyrosine phosphatase psi is required for Delta/Notch signalling and cyclic gene expression in the presomitic mesoderm. *Development* **131**, 3391-3399.

Aulehla, A. and Herrmann, B. G. (2004). Segmentation in vertebrates: clock and gradient finally joined. *Genes Dev.* **18**, 2060-2067.

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.

Bagnall, K. M., Higgins, S. J. and Sanders, E. J. (1988). The contribution made by a single somite to the vertebral column: experimental evidence in support of resegmentation using the chick-quail chimaera model. *Development* **103**, 69-85.

Barrantes, I. B., Elia, A. J., Wunsch, K., Hrabe de Angelis, M. H., Mak, T. W., Rossant, J., Conlon, R. A., Gossler, A. and de la Pompa, J. L. (1999). Interaction between Notch signalling and Lunatic fringe during somite boundary formation in the mouse. *Curr. Biol.* **9**, 470-480.

Barrios, A., Poole, R. J., Durbin, L., Brennan, C., Holder, N. and Wilson, S. W. (2003). Eph/Ephrin signaling regulates the mesenchymal-to-epithelial transition of the paraxial mesoderm during somite morphogenesis. *Curr. Biol.* **13**, 1571-1582.

Bellairs, R. (1986). The primitive streak. *Anat. Embryol.* **174**, 1-14.

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**, 1451-1456.

Blentic, A., Gale, E. and Maden, M. (2003). Retinoic acid signalling centres in the avian embryo identified by sites of expression of synthesising and catabolising enzymes. *Dev. Dyn.* **227**, 114-127.

Brent, A. E. and Tabin, C. J. (2002). Developmental regulation of somite derivatives: muscle, cartilage and tendon. *Curr. Opin. Genet. Dev.* **12**, 548-557.

Brent, A. E., Schweitzer, R. and Tabin, C. J. (2003). A somitic compartment of tendon progenitors. *Cell* **113**, 235-248.

Bronner-Fraser, M. (2000). Rostrocaudal differences within the somites confer segmental pattern to trunk neural crest migration. *Curr. Top. Dev. Biol.* **47**, 279-296.

Buchberger, A., Seidl, K., Klein, C., Eberhardt, H. and Arnold, H. H. (1998). cMeso-1, a novel bHLH transcription factor, is involved in somite formation in chicken embryos. *Dev. Biol.* **199**, 201-215.

Burgess, R., Cserjesi, P., Ligon, K. L. and Olson, E. N. (1995). Paraxis: a basic helix-loop-helix protein expressed in paraxial mesoderm and developing somites. *Dev. Biol.* **168**, 296-306.

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**, 570-573.

Cambray, N. and Wilson, V. (2002). Axial progenitors with extensive potency are localised to the mouse chordoneural hinge. *Development* **129**, 4855-4866.

Catala, M., Teillet, M. A. and le Douarin, N. M. (1995). Organization and development of the tail bud analyzed with the quail- chick chimaera system. *Mech. Dev.* **51**, 51-65.

Chapman, D. L., Agulnik, I., Hancock, S., Silver, L. M. and Papaioannou, V. E. (1996). Tbx6, a mouse T-Box gene implicated in paraxial mesoderm formation at gastrulation. *Dev. Biol.* **180**, 534-542.

Christ, B., Schmidt, C., Huang, R., Wilting, J. and Brand-Saberi, B. (1998). Segmentation of the vertebrate body. *Anat. Embryol.* **197**, 1-8.

Cinquin, O. (2003). Is the somitogenesis clock really cell-autonomous? A coupled-oscillator model of segmentation. *J. Theor. Biol.* **224**, 459-468.

Ciruna, B. and Rossant, J. (2001). FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. *Dev. Cell* **1**, 37-49.

Cole, S. E., Levorse, J. M., Tilghman, S. M. and Vogt, T. F. (2002). Clock regulatory elements control cyclic expression of lunatic fringe during somitogenesis. *Dev. Cell* **3**, 75-84.

Conlon, R. A. (1995). Retinoic acid and pattern formation in vertebrates. *Trends Genet.* **11**, 314-319.

Conlon, R. A., Reaume, A. G. and Rossant, J. (1995). Notch1 is required for the coordinate segmentation of somites. *Development* **121**, 1533-1545.

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**, 455-476.

Cordes, R., Schuster-Gossler, K., Serth, K. and Gossler, A. (2004). Specification of vertebral identity is coupled to Notch signalling and the segmentation clock. *Development* **131**, 1221-1233.

Dale, J. K. and Pourquie, O. (2000). A clock-work somite. *Bioessays* **22**, 72-83.

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**, 275-278.

Diez del Corral, R., Olivera-Martinez, I., Goriely, A., Gale, E., Maden, M. and Storey, K. (2003). Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. *Neuron* **40**, 65-79.

Duband, J. L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G. M. and Thiery, J. P. (1987). Adhesion molecules during somitogenesis in the avian embryo. *J. Cell Biol.* **104**, 1361-1374.

Dubrulle, J. and Pourquie, O. (2004). fgf8 mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. *Nature* **427**, 419-422.

Dubrulle, J., McGrew, M. J. and Pourquie, 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**, 3096-3109.

Eloy-Trinquet, S. and Nicolas, J. F. (2002). Clonal separation and regionalisation during formation of the medial and lateral myotomes in the mouse embryo. *Development* **129**, 111-122.

Fleming, A., Keynes, R. and Tannahill, D. (2004). A central role for the notochord in vertebral patterning. *Development* **131**, 873-880.

Freitas, C., Rodrigues, S., Charrier, J. B., Teillet, M. A. and Palmeirim, I.

(2001). Evidence for medial/lateral specification and positional information within the presomitic mesoderm. *Development* **128**, 5139-5147.
Freund, R., Dorfler, D., Popp, W. and Wachtler, F. (1996). The metameric pattern of the head mesoderm – does it exist? *Anat. Embryol.* **193**, 73-80.
Galceran, J., Farinas, I., Depew, M. J., Clevers, H. and Grosschedl, R. (1999). Wnt3a$^{-/-}$ like phenotype and limb deficiency in Lef1$^{-/-}$Tcf1$^{-/-}$ mice. *Genes Dev.* **13**, 709-717.
Galceran, J., Sustmann, C., Hsu, S.-C., Folberth, S. and Grosschedl, R. (2004). LEF1-mediated regulation of Delta-like1 links Wnt and Notch signaling in somitogenesis. *Genes Dev.* (in press).
Garcia-Martinez, V. and Schoenwolf, G. C. (1992). Positional control of mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dyn.* **193**, 249-256.
Gardner, R. L. and Beddington, R. S. (1988). Multi-lineage 'stem' cells in the mammalian embryo. *J. Cell Sci.* **10**, 11-27.
Gont, L. K., Steinbeisser, H., Blumberg, B. and de Robertis, E. M. (1993). Tail formation as a continuation of gastrulation: the multiple cell populations of the Xenopus tailbud derive from the late blastopore lip. *Development* **119**, 991-1004.
Greco, T. L., Takada, S., Newhouse, M. M., McMahon, J. A., McMahon, A. P. and Camper, S. A. (1996). Analysis of the vestigial tail mutation demonstrates that Wnt-3a gene dosage regulates mouse axial development. *Genes Dev.* **10**, 313-324.
Griffin, K. J. and Kimelman, D. (2002). One-Eyed Pinhead and Spadetail are essential for heart and somite formation. *Nat. Cell Biol.* **4**, 821-825.
Hamilton, L. (1969). The formation of somites in Xenopus. *J. Embryol. Exp. Morphol.* **22**, 253-264.
Helmbacher, F., Schneider-Maunoury, S., Topilko, P., Tiret, L. and Charnay, P. (2000). Targeting of the EphA4 tyrosine kinase receptor affects dorsal/ventral pathfinding of limb motor axons. *Development* **127**, 3313-3324.
Henry, C. A., Hall, L. A., Burr Hille, M., Solnica-Krezel, L. and Cooper, M. S. (2000). Somites in zebrafish doubly mutant for knypek and trilobite form without internal mesenchymal cells or compaction. *Curr. Biol.* **10**, 1063-1066.
Herrmann, B. G., Labeit, S., Poustka, A., King, T. R. and Lehrach, H. (1990). Cloning of the T gene required in mesoderm formation in the mouse. *Nature* **343**, 617-622.
Hinsch, C. W. and Hamilton, H. L. (1956). The developmental fate of the first somite in the chick embryo. *Anat. Rec.* **125**, 225-246.
Hirata, H., Bessho, Y., Kokubu, H., Masamizu, Y., Yamada, S., Lewis, J. and Kageyama, R. (2004). Instability of Hes7 protein is crucial for the somite segmentation clock. *Nat. Genet.* **36**, 750-754.
Hofmann, M., Schuster-Gossler, Watabe-Rudolph, M., Aulehla, A., Herrmann, B. G. and Gossler, A. (2004). WNT signaling, in synergy with T/TBX6, controls Notch signaling by regulating Dll1 expression in the presomitic mesoderm of mouse embryos. *Genes Dev.* (in press).
Horikawa, K., Radice, G., Takeichi, M. and Chisaka, O. (1999). Adhesive subdivisions intrinsic to the epithelial somites. *Dev. Biol.* **215**, 182-189.
Huang, R., Zhi, Q., Ordahl, C. P. and Christ, B. (1997). The fate of the first avian somite. *Anat. Embryol.* **195**, 435-449.
Jen, W. C., Gawantka, V., Pollet, N., Niehrs, C. and Kintner, C. (1999). Periodic repression of Notch pathway genes governs the segmentation of Xenopus embryos. *Genes Dev.* **13**, 1486-1499.
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**, 176-187.
Jouve, C., Iimura, T. and Pourquie, O. (2002). Onset of the segmentation clock in the chick embryo: evidence for oscillations in the somite precursors in the primitive streak. *Development* **129**, 1107-1117.
Kanki, J. P. and Ho, R. K. (1997). The development of the posterior body in zebrafish. *Development* **124**, 881-893.
Kieny, M., Mauger, A. and Sengel, P. (1972). Early regionalization of somitic mesoderm as studied by the development of axial skeleton of the chick embryo. *Dev. Biol.* **28**, 142-161.
Kim, S. H., Jen, W. C., de Robertis, E. M. and Kintner, C. (2000). The protocadherin PAPC establishes segmental boundaries during somitogenesis in xenopus embryos. *Curr. Biol.* **10**, 821-830.
Kimura, Y., Matsunami, H., Inoue, T., Shimamura, K., Uchida, N., Ueno, T., Miyazaki, T. and Takeichi, M. (1995). Cadherin-11 expressed in association with mesenchymal morphogenesis in the head, somite, and limb bud of early mouse embryos. *Dev. Biol.* **169**, 347-358.
Knezevic, V., de Santo, R. and Mackem, S. (1998). Continuing organizer function during chick tail development. *Development* **125**, 1791-1801.
Krumlauf, R. (1994). Hox genes in vertebrate development. *Cell* **78**, 191-201.
Kulesa, P. M. and Fraser, S. E. (2002). Cell dynamics during somite boundary formation revealed by time-lapse analysis. *Science* **298**, 991-995.
Kume, T., Jiang, H., Topczewska, J. M. and Hogan, B. L. (2001). The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and somitogenesis. *Genes Dev.* **15**, 2470-2482.
Kuratani, S., Horigome, N. and Hirano, S. (1999). Developmental morphology of the head mesoderm and reevaluation of segmental theories of the vertebrate head: evidence from embryos of an agnathan vertebrate, Lampetra japonica. *Dev. Biol.* **210**, 381-400.
Lawrence, P. A. (1992). *The Making of a Fly*. Oxford, UK: Blackwell Science.
Lewis, J. (2003). Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. *Curr. Biol.* **13**, 1398-1408.
Li, Y., Fenger, U., Niehrs, C. and Pollet, N. (2003). Cyclic expression of esr9 gene in Xenopus presomitic mesoderm. *Differentiation* **71**, 83-89.
Linask, K. K., Ludwig, C., Han, M. D., Liu, X., Radice, G. L. and Knudsen, K. A. (1998). N-cadherin/catenin-mediated morphoregulation of somite formation. *Dev. Biol.* **202**, 85-102.
Lohnes, D., Mark, M., Mendelsohn, C., Dolle, P., Dierich, A., Gorry, P., Gansmuller, A. and Chambon, P. (1994). Function of the retinoic acid receptors (RARs) during development (I). Craniofacial and skeletal abnormalities in RAR double mutants. *Development* **120**, 2723-2748.
Maden, M., Graham, A., Zile, M. and Gale, E. (2000). Abnormalities of somite development in the absence of retinoic acid. *Int. J. Dev. Biol.* **44**, 151-159.
Marcelle, C., Lesbros, C. and Linker, C. (2002). Somite patterning: a few more pieces of the puzzle. *Results Probl. Cell Differ.* **38**, 81-108.
Marlow, F., Gonzalez, E. M., Yin, C., Rojo, C. and Solnica-Krezel, L. (2004). No tail co-operates with non-canonical Wnt signaling to regulate posterior body morphogenesis in zebrafish. *Development* **131**, 203-216.
McMahon, J. A., Takada, S., Zimmerman, L. B., Fan, C. M., Harland, R. M. and McMahon, A. P. (1998). Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes Dev.* **12**, 1438-1452.
Meinhardt, H. (1986). Models of segmentation. In *Somites in Developing Embryos* (ed. R. Bellairs, D. A. Ede and J. W. Lash), pp. 179-191. London, UK: Plenum Press.
Mellitzer, G., Xu, Q. and Wilkinson, D. G. (1999). Eph receptors and ephrins restrict cell intermingling and communication. *Nature* **400**, 77-81.
Minguillon, C. and Garcia-Fernandez, J. (2002). The single amphioxus Mox gene: insights into the functional evolution of Mox genes, somites, and the asymmetry of amphioxus somitogenesis. *Dev. Biol.* **246**, 455-465.
Morales, A. V., Yasuda, Y. and Ish-Horowicz, D. (2002). Periodic lunatic fringe expression is controlled during segmentation by a cyclic transcriptional enhancer responsive to Notch signaling. *Dev. Cell* **3**, 63-74.
Moreno, T. A. and Kintner, C. (2004). Regulation of segmental patterning by retinoic acid signaling during Xenopus somitogenesis. *Dev. Cell* **6**, 205-218.
Murai, K. K. and Pasquale, E. B. (2003). 'Eph'ective signaling: forward, reverse and crosstalk. *J. Cell Sci.* **116**, 2823-2832.
Nakaya, Y., Kuroda, S., Katagiri, Y. T., Kaibuchi, K. and Takahashi, Y. (2004). Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1. *Dev. Cell* **7**, 425-438.
Nicolas, J. F., Mathis, L., Bonnerot, C. and Saurin, W. (1996). Evidence in the mouse for self-renewing stem cells in the formation of a segmented longitudinal structure, the myotome. *Development* **122**, 2933-2946.
Niederreither, K., Subbarayan, V., Dolle, P. and Chambon, P. (1999). Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. *Nat. Genet.* **21**, 444-448.
Niederreither, K., Vermot, J., le Roux, I., Schuhbaur, B., Chambon, P. and Dolle, P. (2003). The regional pattern of retinoic acid synthesis by RALDH2 is essential for the development of posterior pharyngeal arches and the enteric nervous system. *Development* **130**, 2525-2534.
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**, 195-199.
Nowicki, J. L. and Burke, A. C. (2000). Hox genes and morphological identity: axial versus lateral patterning in the vertebrate mesoderm. *Development* **127**, 4265-4275.
Ordahl, C. P. and le Douarin, N. M. (1992). Two myogenic lineages within the developing somite. *Development* **114**, 339-353.

Packard, D. S. J. and Meier, S. (1983). An experimental study of the somitomeric organization of the avian segmental plate. *Dev. Biol.* **97**, 191-202.

Palmeirim, I., Henrique, D., Ish-Horowicz, D. and Pourquié, O. (1997). Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesiss. *Cell* **91**, 639-648.

Panin, V. M., Papayannopoulos, V., Wilson, R. and Irvine, K. D. (1997). Fringe modulates Notch-ligand interactions. *Nature* **387**, 908-912.

Pourquie, O. (2003). The segmentation clock: converting embryonic time into spatial pattern. *Science* **301**, 328-330.

Pourquie, O. and Tam, P. P. (2001). A nomenclature for prospective somites and phases of cyclic gene expression in the presomitic mesoderm. *Dev. Cell* **1**, 619-620.

Primmett, D. R., Norris, W. E., Carlson, G. J., Keynes, R. J. and Stern, C. D. (1989). Periodic segmental anomalies induced by heat shock in the chick embryo are associated with the cell cycle. *Development* **105**, 119-130.

Psychoyos, D. and Stern, C. D. (1996). Fates and migratory routes of primitive streak cells in the chick embryo. *Development* **122**, 1523-1534.

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**, 248-261.

Rida, P. C., le Minh, N. and Jiang, Y. J. (2004). A Notch feeling of somite segmentation and beyond. *Dev. Biol.* **265**, 2-22.

Saga, Y. and Takeda, H. (2001). The making of the somite: molecular events in vertebrate segmentation. *Nat. Rev. Genet.* **2**, 835-845.

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**, 1827-1839.

Sakai, Y., Meno, C., Fujii, H., Nishino, J., Shiratori, H., Saijoh, Y., Rossant, J. and Hamada, H. (2001). The retinoic acid-inactivating enzyme CYP26 is essential for establishing an uneven distribution of retinoic acid along the anterio-posterior axis within the mouse embryo. *Genes Dev.* **15**, 213-225.

Sato, Y., Yasuda, K. and Takahashi, Y. (2002). Morphogical boundary forms by a novel inductive event mediated by Lunatic fringe and Notch during somitic segmentation. *Development* **129**, 3633-3644.

Sawada, A., Fritz, A., Jiang, Y., Yamamoto, A., Yamasu, K., Kuroiwa, A., Saga, Y. and Takeda, H. (2000). Zebrafish Mesp family genes, mesp-a and mesp-b are segmentally expressed in the presomitic mesoderm, and Mesp-b confers the anterior identity to the developing somites. *Development* **127**, 1691-1702.

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**, 4873-4880.

Schoenwolf, G. C., Garcia-Martinez, V. and Dias, M. S. (1992). Mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dyn.* **193**, 235-248.

Schubert, M., Holland, L. Z., Stokes, M. D. and Holland, N. D. (2001). Three amphioxus Wnt genes (AmphiWnt3, AmphiWnt5, and AmphiWnt6) associated with the tail bud: the evolution of somitogenesis in chordates. *Dev. Biol.* **240**, 262-273.

Selleck, M. A. and Stern, C. D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* **112**, 615-626.

Serth, K., Schuster-Gossler, K., Cordes, R. and Gossler, A. (2003). Transcriptional oscillation of Lunatic fringe is essential for somitogenesis. *Genes Dev.* **17**, 912-925.

Slack, J. M. (1991). *From egg to embryo.* Cambridge, UK: Cambridge University Press.

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**, 229-243.

Sparrow, D. B., Jen, W. C., Kotecha, S., Towers, N., Kintner, C. and Mohun, T. J. (1998). Thylacine 1 is expressed segmentally within the paraxial mesoderm of the Xenopus embryo and interacts with the Notch pathway. *Development* **125**, 2041-2051.

Stern, C. D. and Keynes, R. J. (1987). Interactions between somite cells: the formation and maintenance of segment boundaries in the chick embryo. *Development* **99**, 261-272.

Stern, C. D., Artinger, K. B. and Bronner-Fraser, M. (1991). Tissue interactions affecting the migration and differentiation of neural crest cells in the chick embryo. *Development* **113**, 207-216.

Stern, C. D., Hatada, Y., Selleck, M. A. and Storey, K. G. (1992). Relationships between mesoderm induction and the embryonic axes in chick and frog embryos. *Development Suppl.* 151-156.

Streit, A. and Stern, C. D. (1999). Mesoderm patterning and somite formation during node regression: differential effects of chordin and noggin. *Mech. Dev.* **85**, 85-96.

Sun, X., Meyers, E. N., Lewandoski, M. and Martin, G. R. (1999). Targeted disruption of Fgf8 causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev.* **13**, 1834-1846.

Takahashi, Y., Koizumi, K., Takagi, A., Kitajima, S., Inoue, T., Koseki, H. and Saga, Y. (2000). Mesp2 initiates somite segmentation through the Notch signalling pathway. *Nat. Genet.* **25**, 390-396.

Tam, P. P. (1981). The control of somitogenesis in mouse embryos. *J. Embryol. Exp. Morphol.* **65 Suppl**, 103-128.

Tam, P. P. (1988). The allocation of cells in the presomitic mesoderm during somite segmentation in the mouse embryo. *Development* **103**, 379-390.

Tam, P. P. and Beddington, R. S. P. (1986). The metameric organization of the presomitic mesoderm and somite specification in the mouse embryo. In *Somites in Developing Embryos* (ed. R. Bellairs, D. A. Ede and J. W. Lash), pp. 17-36. London, UK: Plenum Press.

Tonegawa, A. and Takahashi, Y. (1998). Somitogenesis controlled by Noggin. *Dev. Biol.* **202**, 172-182.

Topczewska, J. M., Topczewski, J., Shostak, A., Kume, T., Solnica-Krezel, L. and Hogan, B. L. (2001). The winged helix transcription factor Foxc1a is essential for somitogenesis in zebrafish. *Genes Dev.* **15**, 2483-2493.

van Eeden, F. J., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C. P., Jiang, Y. J., Kane, D. A. et al. (1996). Mutations affecting somite formation and patterning in the zebrafish, Danio rerio. *Development* **123**, 153-164.

Wang, H. U., Chen, Z. F. and Anderson, D. J. (1998). Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* **93**, 741-753.

Yamaguchi, T. P., Harpal, K., Henkemeyer, M. and Rossant, J. (1994). fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev.* **8**, 3032-3044.

Yamaguchi, T. P., Bradley, A., McMahon, A. P. and Jones, S. (1999a). A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* **126**, 1211-1223.

Yamaguchi, T. P., Takada, S., Yoshikawa, Y., Wu, N. and McMahon, A. P. (1999b). T (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev.* **13**, 3185-3190.

Yang, X., Dormann, D., Munsterberg, A. E. and Weijer, C. J. (2002). Cell movement patterns during gastrulation in the chick are controlled by positive and negative chemotaxis mediated by FGF4 and FGF8. *Dev. Cell* **3**, 425-437.

Zakany, J., Kmita, M., Alarcon, P., de la Pompa, J. L. and Duboule, D. (2001). Localized and transient transcription of Hox genes suggests a link between patterning and the segmentation clock. *Cell* **106**, 207-217.