ANNUAL REVIEWS **Further**

Click here for quick links to Annual Reviews content online, including:

- Other articles in this volume
- Top cited articles
- Top downloaded articles
- Our comprehensive search

Formation and Segmentation of the Vertebrate Body Axis

Bertrand Bénazéraf and Olivier Pourquié

Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS (UMR 7104), Université de Strasbourg, Illkirch F-67400, France; email: Pourquie@igbmc.fr

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Annu. Rev. Cell Dev. Biol. 2013. 29:1–26

First published online as a Review in Advance on June 26, 2013

The *Annual Review of Cell and Developmental Biology* is online at http://cellbio.annualreviews.org

This article's doi:
10.1146/annurev-cellbio-101011-155703

Copyright © 2013 by Annual Reviews.
All rights reserved

Keywords

somitogenesis, chicken embryo, axis elongation, morphogenesis

Abstract

Body axis elongation and segmentation are major morphogenetic events that take place concomitantly during vertebrate embryonic development. Establishment of the final body plan requires tight coordination between these two key processes. In this review, we detail the cellular and molecular as well as the physical processes underlying body axis formation and patterning. We discuss how formation of the anterior region of the body axis differs from that of the posterior region. We describe the developmental mechanism of segmentation and the regulation of body length and segment numbers. We focus mainly on the chicken embryo as a model system. Its accessibility and relatively flat structure allow high-quality time-lapse imaging experiments, which makes it one of the reference models used to study morphogenesis. Additionally, we illustrate conservation and divergence of specific developmental mechanisms by discussing findings in other major embryonic model systems, such as mice, frogs, and zebrafish.

Contents

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

GASTRULATION AND FORMATION OF THE ANTERIOR PART OF THE BODY AXIS

Primitive Streak Formation and Extension

Primitive streak: elongated ridge of cells located at the midline of the early developing amniote embryo, from which cells gastrulate to form the endoderm and the mesoderm; the formation of the streak is the first sign of both gastrulation and anteroposterior axis formation; the streak extends first and then regresses at later developmental stages

Hensen's Node: embryonic region located at the tip of the primitive streak that forms the most axial structures of the body axis, such as the prechordal plate mesoderm and the notochord; avian homolog of the Spemann organizer located in the dorsal lip of the blastopore in amphibians, the Node regresses posteriorly as the embryo develops

Vertebrate embryos develop in a head-to-tail sequence; in other words, the production of the anteriormost structures (e.g., the head) is followed by the progressive formation of more posterior ones (the neck, trunk, and tail). The three embryonic layers (ectoderm, endoderm, and mesoderm) are formed during the gastrulation process, which begins with the formation of the primitive streak (the amniote equivalent of the blastopore). In the primitive streak, cells from the epiblast undergo an epithelial-to-mesenchymal transition (EMT) and ingress to form the mesoderm and the endoderm (Nakaya & Sheng 2008, Nakaya et al. 2008, Nieto 2011). During the early stages of gastrulation, the presumptive territories of the endoderm, the heart, the prechordal plate, and the notochord ingress at the level of the primitive streak, which expands anteriorly (Hatada & Stern 1994, Tam & Beddington 1992). At Hamburger-Hamilton stage 4 (4HH) (Hamburger 1992), the primitive streak reaches its maximal extension (Spratt 1946). Although the anteroposterior axis of the primitive streak correlates with the future anteroposterior axis of the body axis, after stage 4HH the distribution of the presumptive territories in the primitive streak in fact reflects the mediolateral organization of the mesoderm (Garcia-Martinez & Schoenwolf 1992, Psychoyos & Stern 1996a). The tip of the primitive streak forms the Hensen's Node, which corresponds to the amniote organizer and which will form the most axial tissue, the notochord. The paraxial mesoderm precursors, and then the intermediate mesoderm, the lateral plate, and the extraembryonic/blood precursors, are found posterior to this territory. The anterior neural plate, which will form the brain and the cranial neural crest, is found anterior to the Node at stage 4HH.

The cellular mechanisms involved in the formation of the primitive streak are still debated, and different models have been proposed (Chuai & Weijer 2009). Primitive streak formation starts at the posterior pole of the blastoderm next to the posterior margin of the area pellucida. During this process, the epiblast tissue located laterally to the streak moves circularly in a counterrotative motion, forming cell vortices on each side of the forming primitive streak (**Figure 1*a***). These collective circular movements, which take place concomitantly with the anterior expansion of the primitive streak, have been named polonaise movements (Cui et al. 2005, Gräper 1929,

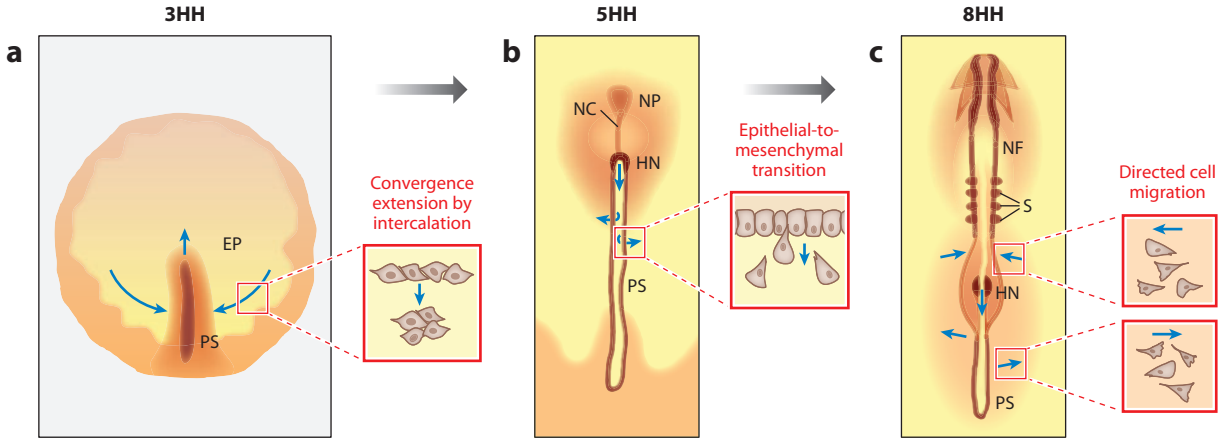


Figure 1

Cellular mechanism involved in axis extension during the formation of the anterior part of the body axis. Schematic representations of chicken embryos at Hamburger-Hamilton (HH) stage 3, 5, and 8 (dorsal view). Blue arrows represent major tissue movements linked to axis extension at a given stage. Details of the cell behaviors underlying these global movements are shown in the red squares. (*a*) At stage 3HH, cells intercalate in the epiblast (EP). This behavior leads to the extension of the primitive streak (PS). (*b*) At stage 5HH, cells ingress from the EP to the mesoderm simultaneously with Node and streak regression. (*c*) Ingression and directed cell migration lead to mesodermal reorganization at stage 8HH. At this stage, there is a convergent movement of the paraxial mesoderm toward the midline. Abbreviations: HN, Hensen's Node; NC, notochord; NF, neural folds; NP, neural plate; NT, neural tube; S, somites.

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Wetzel 1929). The movement of cells when examined in time-lapse movies is composed of the intrinsic (active) movement as well as a passive movement that results from tissue deformation. This deformation can either be controlled intrinsically and result from the collective behavior of cells or be imposed extrinsically, by external forces, for instance. Active and passive movements have been investigated by determination of cells versus extracellular matrix (ECM) motion during primitive streak extension (Zamir et al. 2008). This work, based on the tracking of fibronectin fibers and cellular nuclei, shows that ECM movement is essentially the same as cell movement during the polonaise movements of the epiblast cells. These results suggest that the movements that define streak formation are not solely a result of the crawling or migration of the epiblast cells on their matrix, which would otherwise result in a differential movement between the two entities. They point to the importance of large-scale bulk tissue movements in morphogenetic processes, a notion that has been underestimated in the field of morphogenesis.

Wei & Mikawa (2000) proposed that oriented cell division localized in the posterior part of the streak drives the elongation of the structure. However, inhibiting cell proliferation did not block streak extension, and analysis of mitosis by time-lapse imaging did not reveal any prominent orientation, which thus questions the central role of cell division in driving the morphogenesis of the streak (Chuai et al. 2006, Voiculescu et al. 2007). Chemotactic attraction/repulsion-based mechanisms have been proposed as another mechanism for streak morphogenesis (Chuai & Weijer 2007). Here, directional cellular movements would be controlled by a chemoattractant signal located at one extremity of the streak and a chemorepulsive signal at the other extremity. This appealing hypothesis can explain both the streak and the epiblast motions and can be modeled by mathematical simulation (Sandersius et al. 2011, Vasiev et al. 2010). Interestingly, recent experiments suggest that nodal signaling could be implicated in driving polonaise movements in the epiblast, and therefore in contributing to streak formation (Yanagawa et al. 2011).

Convergence extension: process by which embryonic tissue narrows in one axis and extends in the perpendicular dimension

Somite: block of mesodermal cells located on both sides of the neural tube; the somites will give rise to the dermis, skeletal muscles, and vertebrae

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

The other main cellular behavior involved in streak morphogenesis is convergence extension resulting from mediolateral cell intercalation. Lawson & Schoenwolf (2001) first observed the existence of these movements in the chicken embryo by using DiI staining of the epiblast during streak formation (**Figure 1*a***). These convergence-extension movements of the epiblast have been subsequently analyzed using high-resolution time-lapse imaging. This technique, combined with chick embryo culture *ex ovo*, allowed the detailed analysis of the epiblast convergent behavior at cellular resolution (Voiculescu et al. 2007). Epiblast cells become polarized and move toward the embryonic midline, where they intercalate; this intercalation behavior therefore leads to primitive streak lengthening. Interestingly, Voiculescu et al. (2007) also showed that these movements rely on Wnt/planar cell polarity (PCP) signaling, which is also involved in frog, fish, and mouse convergence-extension and intercalation movements (Gray et al. 2011, Skoglund & Keller 2010), suggesting a conserved role for this pathway.

The convergence-extension movements in the epiblast of chicken embryos take place much earlier than the mediolateral intercalation of the mesoderm and the neural ectoderm in frogs and fish. These movements, which are linked to a later phase of axis extension and are observed after cell internalization during gastrulation, are discussed in the section on Hensen's Node and Primitive Streak Regression. Epithelial-cell intercalation has also been shown to drive germ-band extension (axis elongation) in the *Drosophila* embryo (Bertet et al. 2004). This epithelial intercalation in flies requires active reorganization of cell junctions involving myosin2-dependent contractions of the cortical actin cytoskeleton and cadherin-mediated adhesion, and it also requires the PCP pathway. Nishimura et al. (2012) recently reported that a similar mechanism is also active during neural tube closure of vertebrates. Novel culture and imaging protocols developed for mammalian embryos have recently begun to shed light on the conservation of the mechanisms involved in primitive streak formation. In rabbit embryos, streak extension relies on oriented cell division and specific planar movements (Halacheva et al. 2011). Imaging studies of murine streak formation suggest that this process works differently than in chicken and does not require large-scale or convergence-extension movements. The primitive streak arises in situ by progressive initiation of EMT beginning in the posterior epiblast (Williams et al. 2012). Indeed, an obvious difference between the two species lies in the number of cells at the beginning of gastrulation, which is approximately 500 in the mouse compared with approximately 50,000 in the chicken embryo. Therefore, whereas formation of a primitive streak marks the earliest stage of formation of the embryonic axis in amniotes, there exists a significant plasticity in the cellular mechanisms deployed in different species.

Hensen's Node and Primitive Streak Regression

Stage 4HH marks the beginning of the regression of the Hensen's Node and the primitive streak, which progressively lays the embryonic body in its wake (Spratt 1947). The size of the body axis increases during the early phases of primitive streak regression, although the total size of the embryonic area does not change (**Figure 1*b***). This stage marks the transition between the ingression of the head mesoderm, which forms the muscles of the head, and the beginning of the ingression of the somitic cells, which will contribute to the axial skeleton, the skeletal muscles of the body, and the dermis of the back (Jouve et al. 2002). The first morphological somite forms soon after at stage 7HH, and it lies at the level of the future otic vesicle (Hinsch & Hamilton 1956). Gastrulation continues at the primitive streak level, the Node regresses, and the streak progressively diminishes in length. Ingression of mesodermal precursors from the epiblast continues at the level of the primitive streak, which produces streams of mesoderm that become progressively patterned, leading to the formation of the body axis. After stages 13–14HH (19–22 somites), the

primitive streak no longer exists and the posterior body is formed by the tail bud, which continues to produce the embryonic tissues (Schoenwolf 1979a,b).

Fate-mapping studies using quail-chick chimeras or DiI staining have allowed identification of the Node, streak, and epiblast derivatives (Hatada & Stern 1994, Nicolet 1971, Psychoyos & Stern 1996a, Schoenwolf et al. 1992). The Hensen's Node provides the precursors of the axial mesoderm structures, the roof of the endoderm, and the floor plate of the neural tube (Catala et al. 1996, Charrier et al. 1999, Kirby et al. 2003, Selleck & Stern 1991). Anterior primitive streak cells located in the territory caudally and adjacent to the Node are of particular interest. This specific region contains long-term progenitors endowed with self-renewal properties, which will be retained in the streak as the Node regresses (Cambray & Wilson 2002, 2007; Iimura et al. 2007; McGrew et al. 2008; Psychoyos & Stern 1996a; Selleck & Stern 1991). They will give rise to the medial portion of the paraxial mesoderm (Iimura et al. 2007, Selleck & Stern 1992). Posterior to this region, the primitive streak and adjacent epiblast will give rise to the lateral part of the paraxial mesoderm (Iimura et al. 2007). The more posterior regions of the streak will give rise to the intermediate mesoderm, the lateral plate, and the extraembryonic mesoderm (Garcia-Martinez & Schoenwolf 1992, Psychoyos & Stern 1996a).

Tail bud: mass of undifferentiated embryonic tissue, located at the caudal extremity of the developing embryo, in which gastrulation is still ongoing; the tail bud derives from the primitive streak, Node, and ectodermal tissue and will give rise to different embryonic tissues in the trunk and posterior parts of the body

The exact mechanisms underlying the regression of the Hensen's Node are poorly understood. Early deletion of the Node in mouse and chicken does not block elongation of the embryo, which suggests that Node regression is not intrinsically controlled (Charrier et al. 1999, Davidson et al. 1999, Joubin & Stern 1999). Thus, this also suggests that the Node movements could be controlled by extrinsic forces generated by adjacent tissues. The axial mesoderm (head process and notochord), which derives from the regressing Hensen's Node, elongates by accretion at the posterior end of the embryo as well as by convergence and oriented cell division (Sausedo & Schoenwolf 1993, 1994; Yamanaka et al. 2007). The axial mesoderm does not seem to play a key role in axis extension, because embryos in which the notochord is absent still retain the capacity to form an elongated body axis (Ang & Rossant 1994, Charrier et al. 1999, Davidson et al. 1999). The neuroectoderm undergoes massive morphogenetic events during Node regression. The neural plate elongates and bends, and at the spinal cord level, it progressively closes in an anteroposterior sequence to form the neural tube (Colas & Schoenwolf 2001, Nishimura et al. 2012). Convergence-extension movements and oriented cell divisions have been observed in the neuroectoderm of elongating embryos in different species (Concha & Adams 1998; Elul et al. 1997; Keller et al. 1992; Nishimura et al. 2012; Schoenwolf & Alvarez 1989, 1992). As a result, the neuroectoderm extends posteriorly and may push the Node, which is located at its caudal end (Mathis et al. 2001). However, the influence of these movements on the global embryonic axis extension still has to be determined.

The flux of mesodermal cells generated by gastrulation is necessary for axis extension (**Figure 1*b***). Inhibition of cell movements from the epiblast to the mesoderm through the primitive streak leads to severe truncations of the embryonic body axis. For instance, inhibition of FGF and Wnt signaling pathways, which prevents gastrulation movements, blocks axis elongation. Mouse mutants for the FGF ligands FGF4 or FGF8 or for the receptor FGFR1 show severe gastrulation defects and axis truncation, because cells cannot leave the streak structure to form the mesoderm (Boulet & Capecchi 2012, Deng et al. 1994, Naiche et al. 2011, Sun et al. 1999). Genetic deletion of *Wnt3* in mice leads to absence of the primitive streak and gastrulation defects (Liu et al. 1999), whereas mutation of *Wnt3a* leads to axis-elongation defects (Greco et al. 1996, Takada et al. 1994). Inactivation of the Wnt coreceptors Lrp5 and Lrp6, or of the Lef1 and TCF1 effectors of the Wnt pathway, gives a similar phenotype (Galceran et al. 1999, Hsieh et al. 2003, Kelly et al. 2004). In chicken embryos, blockage of FGF and noncanonical Wnt signaling also interferes with gastrulation movements linked to streak formation and ingression (Chuai et al. 2006, Hardy et al. 2008).

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

The movements of the newly ingressed mesoderm cells, which lead to their allocation in their future mesodermal compartment (e.g., axial, paraxial, lateral), also play an important role in the control of body axis extension. Directed migration guided by chemotaxis could play a role in this process (**Figure 1*c***). Yang et al. (2002) proposed that migration of the mesoderm away from the streak was mediated by chemorepulsion guided by FGF8. The same cells, once away from the streak, converge back toward the medial part of the embryo in response to FGF4 chemoattraction mediated by the notochord (Yang et al. 2002). Other signals, such as PDGF, PTEN, and WNT signals, are also implicated in the movement of the ingressed mesoderm cells in chicken embryos (Leslie et al. 2007, Sweetman et al. 2008, Yang et al. 2008, Yue et al. 2008).

A key mechanism involved in the control of elongation of the anterior portion of the body axis is convergence extension. Convergence-extension movements were first documented in frog embryo gastrulation (R. Keller et al. 2008, Solnica-Krezel & Sepich 2012, Tada & Heisenberg 2012). During the formation of the body axis in the frog, cells of the mesoderm and neural plate converge toward the midline, where they intercalate, which leads to the elongation of the neural tube, the notochord, and the paraxial mesoderm (Shih & Keller 1992a,b). These movements, which play a key role in the elongation of the anterior portion of the body axis (head and neck) in lower vertebrates, are mostly observed until blastopore closure. At the cellular level, converging cells orient their axis perpendicular to the anteroposterior axis to slide in between each other. These convergence movements, which are mediated by mediolateral intercalation and require the PCP pathway, have been well documented in frog and fish embryos (Keller & Danilchik 1988; Shih & Keller 1992a,b; Trinkaus et al. 1992; Yin et al. 2008). Similar movements have been reported in mouse and chicken embryos at these stages of development (Yang et al. 2002, Yen et al. 2009), when ectoderm, endoderm, and mesoderm converge toward the midline and elongate simultaneously during Node regression. Mediolateral intercalation in the neural tube or epiblast of chicken embryos requires the function of the noncanonical Wnt PCP pathway (Gray et al. 2011, R. Keller et al. 2008, Nishimura et al. 2012, Roszko et al. 2009, Voiculescu et al. 2007). Besides its function in polarizing mesenchymal cells, the PCP signaling pathway is involved in the deposition of the ECM, which is necessary for cell rearrangements (Dzamba et al. 2009, Skoglund & Keller 2010). Therefore, mesodermal cell rearrangements, and in particular convergence-extension movements, play a crucial role for extension of the anterior body axis in different species. These cellular rearrangements might have an active role in pushing the Node posteriorly, leading to its regression. Interestingly, and contrary to the situation encountered earlier during streak formation, avian mesoderm cells located close to the regressing Node migrate with a high degree of independence compared with the ECM movements (Zamir et al. 2006). This region could be a zone in which cells efficiently pull on the ECM and therefore actively contribute to tissue deformation and axis elongation.

Recent advances in imaging techniques now allow high-accuracy tracking of the movement in different embryonic layers (P.J. Keller et al. 2008, Olivier et al. 2010, Truong et al. 2011). Combining these techniques with quantitative measurements will help to accurately describe the tissue and cell motions associated with axis extension. However, owing to the complex interactions between embryonic layers, it is very difficult to distinguish active and passive movements (Zamir et al. 2008). The forces generated, as well as the tissue resistance to deformation, are an essential element of morphogenetic processes. The study of physical properties, such as stiffness or adhesion of the different layers and embryonic regions, is therefore a crucial step toward a profound understanding of the physical basis of embryonic elongation. Recently, studies done in *Xenopus*, but also in chicken, allowed researchers to access some biophysical properties of embryonic tissues (Agero et al. 2010, Damon et al. 2008, Davidson 2011, von Dassow et al. 2010, Zhou et al. 2009). Interestingly, in frog embryo, the paraxial mesoderm was found to be twice as stiff as the

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

notochord or the neural tube, consistent with a role for this tissue in the control of axis elongation (Zhou et al. 2009). The analysis of the mechanical properties of the cells and tissues during axis elongation constitutes a major challenge for the future.

Presomitic mesoderm (PSM): part of the paraxial mesoderm (mesoderm flanking the neural tube) located in the posterior region of the embryo, the PSM will progressively segment to give rise to the somites throughout development

AXIS EXTENSION AND FORMATION OF THE POSTERIOR BODY

As described above, formation and elongation of the most anterior part of the axis are largely the result of a massive convergence of embryonic tissue toward the embryonic midline. In birds and mammals, the primitive streak has essentially fully regressed at the time of posterior neuropore closure at the 16- to 22-somite stage (Schoenwolf 1979b). After this stage, axis growth becomes restricted mostly to the tail bud in the posterior part of the embryonic body, a process often referred to as secondary body formation (Catala et al. 1995, Holmdahl 1925, Pasteels 1937). Gastrulation and neurulation continue to provide new mesodermic and neuroectodermic cellular material, but the converging movement of these newly formed tissues becomes progressively less prominent (Gont et al. 1993). A good illustration of this phenomenon is reflected in the shape of the presomitic mesoderm (PSM) in the chicken embryo, which essentially keeps the same width after closure of the posterior neuropore (**Figure 2**). This suggests that the formation of the trunk and the caudal region of the body relies on morphogenetic mechanisms other than convergence. The streak and Node structures persist until they are progressively transformed into the tail bud (Schoenwolf 1979a). Gastrulation movements continue in the tail bud, which appears as a mass of undifferentiated cells and gives rise to the three embryonic derivatives (Catala et al. 1995, Davis & Kirschner 2000, Holmdahl 1925, Kanki & Ho 1997, Knezevic et al. 1998, Pasteels 1937, Schoenwolf 1977, Schoenwolf et al. 1985). It also forms the secondary body axis, from the lumbosacral region to the tip of the tail (Catala et al. 1995). Formation of the secondary body axis is characterized by a different mode of neurulation called secondary neurulation, which occurs by cavitation (Catala et al. 1995, Schoenwolf 1979a).

Ingression and Proliferation During Tail Bud Regression

During the first phase of axis elongation, the primitive streak continuously shrinks, and when the tail fold starts to form (during the formation of the hindgut), it eventually becomes located ventrally to become the ventral ectodermal ridge (VER) (Ohta et al. 2007) (**Figure 2**). Whereas mesoderm ingression still continues at the level of the VER up to stage 16HH (approximately 25 somites), ingression has stopped at stage 20HH (approximately 40 somites) (Ohta et al. 2007). The fate of the cells ingressing at the level of the VER has, however, not been established by precise fate mapping (Knezevic et al. 1998, Liu et al. 2004, Ohta et al. 2007, Schoenwolf 1979b). The derivatives of the most anterior part of the streak and the Hensen's Node become incorporated into a region called the chordoneural hinge (CNH), which contains progenitors able to give rise to axial and paraxial mesoderm and neuroectoderm (Cambray & Wilson 2002, Catala et al. 1995, Davis & Kirschner 2000, McGrew et al. 2008, Wilson et al. 2009). The CNH produces the precursors of the notochord and the floor plate, and its posterior wall produces paraxial mesoderm in the caudal part of the embryo (Cambray & Wilson 2007, Catala et al. 1995, Gont et al. 1993, McGrew et al. 2008). Retrospective lineage analysis and serial transplants in mouse and chicken embryos suggest that cells that reside in this region can produce progenitors that self-renew and produce progeny over long distances along the trunk and tail (Cambray & Wilson 2002, Eloy-Trinquet & Nicolas 2002, McGrew et al. 2008). The bulk of the paraxial mesoderm produced by the tail bud derives from the CNH and from the so-called posterior tail bud mesenchyme, which lies posterior to the CNH. Whether the VER contributes to these mesodermal precursors through

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

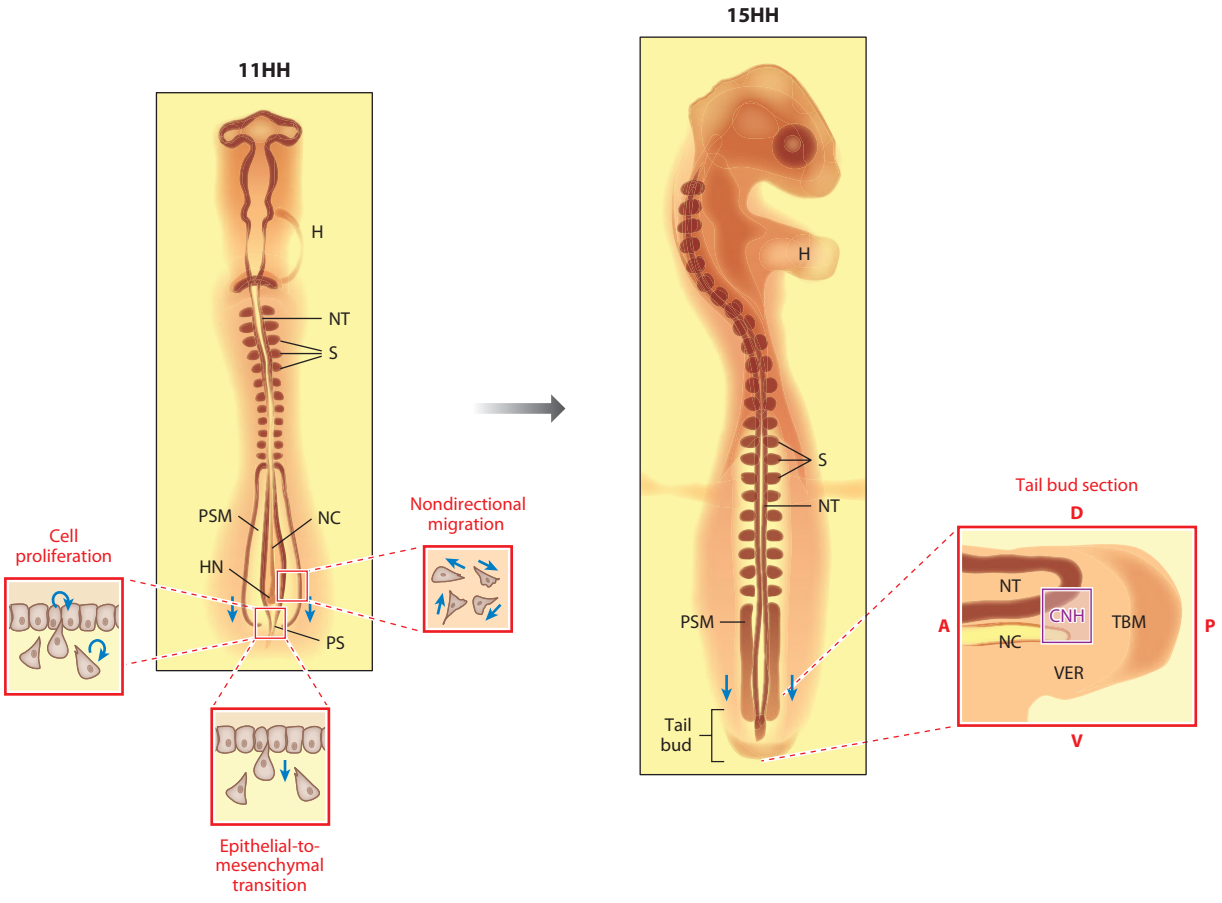


Figure 2

Cellular mechanisms involved in axis extension during the formation of the posterior part of the body axis. Scheme of chicken embryos at Hamburger-Hamilton (HH) stages 11 and 15 (dorsal view). Blue arrows represent major tissue movements linked to axis extension at a given stage. Details of the cell behaviors underlying these global movements are shown in the red squares. At stage 11HH, cells proliferate and ingress in the caudal zone before entering the presomitic mesoderm (PSM). While in the PSM, they undergo a gradient of nondirectional movement that leads to the caudal expansion of the tissue. These cellular behaviors continue at stage 15HH and until the end of axis extension. A schematic representation of a tail bud sagittal section at stage 15HH is presented (*red square*). Abbreviations: A, anterior; CNH, chordoneural hinge; D, dorsal; H, heart; HN, Hensen's Node; NC, notochord; NT, neural tube; P, posterior; PS, primitive streak; S, somites; TBM, tail bud mesoderm; V, ventral; VER, ventral ectodermal ridge.

ingression is unclear. Mutations in the FGF or the Wnt pathway lead to posterior-axis truncations and formation of ectopic neural-tube tissue at the expense of mesoderm (Kondoh & Takemoto 2012). Fate-mapping studies demonstrated the existence of a pool of bipotential cells able to generate the paraxial mesoderm and neural tube (Lawson & Pedersen 1992, Tzouanacou et al. 2009). However, the precise location and identification of these cells remains to be established.

In frog, formation of the tail bud results from interaction of different territories of the posterior neural plate and mesoderm (Tucker & Slack 1995), and it has been shown to require Notch and BMP4 signaling (Beck & Slack 1998, 1999; Beck et al. 2001). In fish, formation of the posterior body involves a tail organizer that controls activation of BMP, nodal, and Wnt signaling pathways (Agathon et al. 2003, Szeto & Kimelman 2006). In mouse and fish, mutants for WNT pathway

genes or for the T-box family Brachyury (T) transcription factor (or its fish ortholog, *Notail*) exhibit specific truncations of the posterior part of the embryonic body axis (Amacher et al. 2002, Galceran et al. 1999, Herrmann et al. 1990, Takada et al. 1994). *Brachyury* is important for gastrulation movements because cells mutant for this gene cannot leave the streak (Rashbass et al. 1991; Wilson & Beddington 1997; Wilson et al. 1993, 1995). In zebrafish, the autoregulatory loop between Brachyury and Wnt3a is involved in the maintenance of the axial progenitors (Martin & Kimelman 2010), and *Msgn1* has recently been implicated in positively regulating the cellular movements from the tail bud to the PSM (Fior et al. 2012). Mouse mutants for Cdx genes, which are targets of Wnt and FGF signaling, exhibit posterior truncation of the axis (Chawengsaksophak et al. 2004, Young et al. 2009). These defects are rescued by Wnt overactivation (Yamaguchi et al. 1999, Young et al. 2009). Elongation is also highly sensitive to retinoic acid (RA), a signaling pathway active in the anterior PSM (Diez del Corral et al. 2003, Kessel & Gruss 1991, Rossant et al. 1991). Cyp26, an enzyme that can degrade RA, is expressed in the tail bud downstream of FGF signaling. Interestingly, Cyp26-null mutant mice exhibit truncations of the axis (Abu-Abed et al. 2001, Fujii et al. 1997, Sakai et al. 2001). Treatment of embryos with RA leads to inhibition of Wnt signaling and arrest of elongation, resulting in truncation (Iulianella et al. 1999, Kessel 1992, Shum et al. 1999). Thus, FGF and Wnt signaling are required for body axis elongation, and their effect is antagonized by RA.

In amniotes, *Hox* genes are a family of 39 transcription factors that are organized in four clusters that arose by serial duplication of an ancestral cluster (Duboule 2007). *Hox* genes are organized in a collinear fashion on the chromosome, meaning that the distribution of *Hox* expression domains along the anteroposterior axis of the body reflects their position along the cluster (Duboule & Dollé 1989, Graham et al. 1989). *Hox* genes control the anatomical identity of the different regions of the body axis. *Hox* genes were implicated recently in the control of axis extension (Mallo et al. 2010, Young et al. 2009). Inactivation of the *HoxB13* gene leads to a small increase in segment number, whereas overexpression of posterior genes, such as *HoxC13*, leads to premature axis truncation (Economides et al. 2003, Young et al. 2009). These truncations can be rescued by overexpressing central *Hox* genes, such as *HoxB8* or *HoxA5*, in these mutants, which suggests that these genes play an important role in the control of elongation in the trunk region (Young et al. 2009). *Hox* genes were also involved in the control of ingression of the mesodermal precursors from the epiblast to the primitive streak (Iimura & Pourquié 2006). *HoxB* genes are expressed in a collinear sequence in the epiblast adjacent to and overlying the anterior primitive streak prior to the ingression of the cells in the mesoderm. Overexpression of *Hox* genes in the epiblast showed that posterior *Hox* genes can delay epiblast cell ingression. Furthermore, the posteriormost *Hox* genes can suppress the function of more anterior ones, a property that has been termed posterior prevalence (Duboule & Morata 1994). The progressive collinear activation of *HoxB* genes in the epiblast cells thus regulates the flux of gastrulating cells and ensures the correct positioning of the future *Hox*-expressing domain, and hence the anatomical domains along the AP axis (Iimura et al. 2009). How the control of cell ingression downstream of *Hox* genes relates to elongation remains to be investigated. The addition of new cellular material and tissue movement away from the tail bud progenitor zone are important aspects of body axis elongation. Lawton et al. (2013) recently used 3D time-lapse imaging to describe that, within the zebrafish tail bud, different tissue fluidities coexist during axis elongation. Based on mathematical modeling data, they propose that tissue coherence and flow rate are key parameters of axis elongation.

The Posterior Presomitic Mesoderm Controls Axis Elongation

Ingression and proliferation in the tail bud are required to produce the cellular material necessary for axis extension. However, the posterior movement of the tail bud necessitates force generation

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Nondirectional migration: process by which cells migrate actively without any particular direction

and cell reorganization to produce the elongation movements involved in shaping the body axis. Which tissues are mechanically active in posterior-axis extension? Bénazéraf et al. (2010) addressed this question in the chicken embryo by deleting caudal structures and completing time-lapse imaging analysis to identify the regions controlling axis elongation. Morphogenetic movements of axis extension are not impaired, at least for a short period of time, when axial structures are ablated. As reported in younger embryos, the organizer region can regenerate after deletion to continue to give rise to trunk progenitors (Joubin & Stern 1999, Psychoyos & Stern 1996b). The fact that elongation does not stop after the microsurgery suggests that morphogenetic mechanisms driving extension are taking place in other tissues (Schoenwolf 1978). Ablation of the neural tube, including in the most caudal part of the embryo, does not prevent elongation of the embryo, which indicates that it does not act as the motor of axis elongation (Rong et al. 1992). In contrast, deletion of the caudal part of the paraxial mesoderm leads to an abrupt slowing down of axis extension, which suggests that this tissue plays a key role in axis extension. In line with these observations, time-lapse imaging shows extensive movements of cells in the caudal part of the PSM (Bénazéraf et al. 2010, Delfini et al. 2005, Kulesa & Fraser 2002). Analysis of these cellular movements in comparison with the posterior tissue movement (i.e., the ECM movement) showed that the posteriorly oriented movement of the cells of the PSM is essentially a result of the posterior deformation of the tissue and that the local movement of the cells is essentially random (Bénazéraf et al. 2010). Accordingly, cells in the caudal PSM do not show any preferential polarity with respect to the embryonic axis (**Figure 2**). The PSM is rich in ECM proteins such as fibronectin or fibrillin 2 (Czirok et al. 2004, Duband et al. 1987, Martins et al. 2009, Rifes et al. 2007, Rifes & Thorsteinsdóttir 2012). Thus, cells in the posterior PSM can crawl on the matrix fiber network to generate the forces necessary for tissue expansion. This hypothesis is reinforced by the fact that mouse embryos mutant for fibronectin or its receptor integrin α5 display posterior truncations (George et al. 1993; Georges-Labouesse et al. 1996; Yang et al. 1993,1999). Mouse embryos in which the binding site of fibronectin to integrin, the motif RGD, has been mutated show truncation as well as PSM patterning and migration defects (Girós et al. 2011). These results suggest that the forces generated by the nondirectional cellular movements within the PSM rely on the interaction between cells and their ECM. High cellular density in the anterior part of the PSM and in neighboring tissues also participates in the control of elongation by creating the necessary tissue resistances and thus biasing the tissue movement posteriorly. Gain- and loss-of-function experiments showed that the gradient of random cellular movement in the posterior PSM is driven by the gradient of FGF signaling in this tissue. The global posterior movement of PSM cells that drive elongation is thus an emergent property of the gradient of random motility imposed on individual cells (**Figure 2**) (Bénazéraf et al. 2010). Limited convergent movements are nevertheless observed in the rostral PSM, but they likely result from the elongation taking place at the posterior end. The behavior of the PSM cells is conceptually similar to what is observed for diffusion in physics: Molecules exhibiting random motion controlled by a gradient of temperature will move in the direction of the gradient. Such a mechanism is likely taking part in other morphogenetic processes involving the formation of an outgrowth. In the limb bud, for instance, FGF signaling also regulates the random movement of mesenchymal cells, which could drive the proximo-distal elongation of the bud (Gros et al. 2010).

Recent progress in the analysis of collective cell migration allowed the direct measurement of force distribution in a group of cells through traction microscopy techniques. It showed that forces in a migrating tissue are transmitted through several cell diameters and that these forces are distributed among several directions, even though the tissue is moving in one direction (Serra-Picamal et al. 2012, Trepat & Fredberg 2011, Trepat et al. 2009). These studies are informative of the physical constraints that define the mechanics of a dense cellular environment and might be

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

generalized to embryonic tissue mechanics as well. However, these studies have mainly been carried out on epithelial cells, and how they will translate to mesenchymal cells, such as the posterior PSM, remains to be established. In several embryonic locations, such as the PSM, cell migration is not directional at the individual level, whereas the tissue appears to be collectively flowing in one direction. This is also the case in neural crest migration, where individual movements are driven by a contact inhibition mechanism, whereas collective motion is unidirectional (Carmona-Fontaine et al. 2008). In the future, direct force measurement in the embryo will be key to understanding how individual cell movements can be integrated to produce deformation on the tissue scale. The growth of posterior structures likely relies on a variety of different cellular strategies. Integrative studies based on live imaging and biophysical modeling will help to define the mechanics of posterior-axis extension.

Segmentation clock: molecular oscillator active in the cells in the posterior part of the PSM; defined by a subset of genes that undergo an oscillatory transcriptional behavior; the period of oscillation (e.g., the time for a gene to go from an ON phase to an OFF phase to another ON phase) matches the period of somite formation; different models explain how this oscillator can encode positional information to allocate PSM cells to a given segment

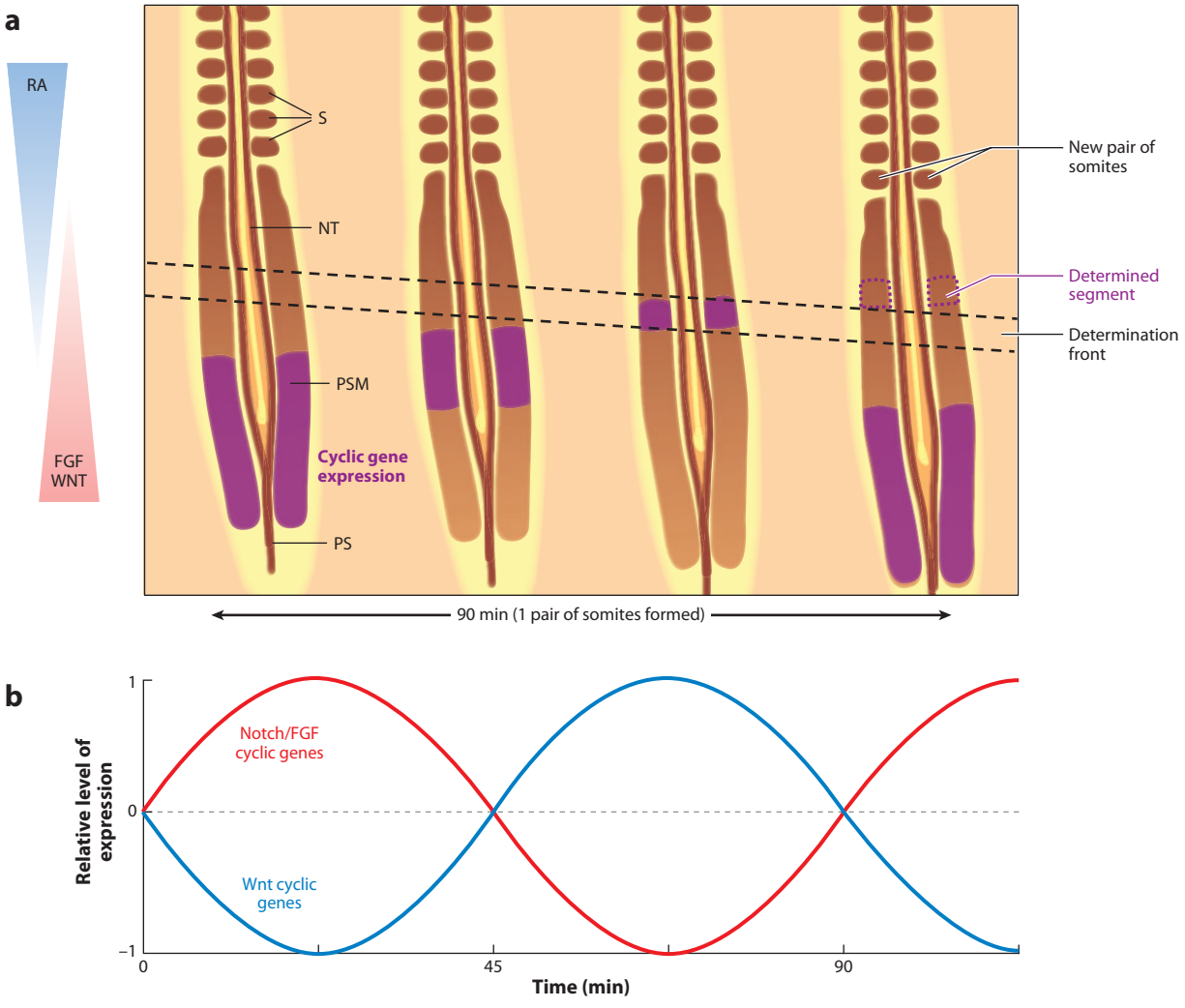
REGULATION OF SEGMENT NUMBER AND AXIS LENGTH

The vertebrate embryonic body is segmented along its anteroposterior axis. Segmentation takes place by progressively subdividing the PSM into somites during axis formation. Somitogenesis progresses in a rostro-caudal manner in concert with axis elongation. Somites are epithelial structures that contain precursors of vertebrae, ribs, skeletal muscles, and the dermis of the back (Chal & Pourquié 2009). Somites form rhythmically in a synchronous fashion, as pairs, by pinching off from the anterior tip of the PSM on each side of the neural tube. The period of this process is species dependent: 120 minutes in mouse embryos, 90 minutes in chicken embryos, and ~30 minutes in zebrafish embryos. As PSM cells are incorporated into newly formed somites anteriorly, new tissue is constantly supplied caudally by gastrulation and cell division occurring within the PSM tissue itself. In the chicken, segmentation starts during the phase of Node regression at stage 7HH. Axis extension and somitogenesis terminate at stage 26HH. The number of somites can vary tremendously between vertebrate species but is usually invariant between individuals of one given species. The coordination of somitogenesis and axis extension defines segment numbers and body length (Gomez & Pourquié 2009).

Mechanisms of Segment Formation

Somitogenesis relies on a molecular oscillator, which controls the rhythmic activation of the Wnt, FGF, and Notch signaling pathways in the PSM (**Figure 3*a*,*b***) (Pourquié 2011). Existence of such an oscillator had been predicted in a theoretical model called the clock and wavefront model, which posited that segmentation results from the displacement of an oscillator in space (Cooke & Zeeman 1976). Such an oscillator has been identified and called the segmentation clock (Palmeirim et al. 1997). It delivers periodic signaling pulses that are involved in the definition of the segmental prepattern in the PSM. These signaling pulses manifest as bilateral traveling waves of mRNA expression of targets of these pathways that sweep across the PSM each time a new somite forms (**Figure 3*a***) (Aulehla et al. 2008, Dequeant et al. 2006, Palmeirim et al. 1997). A cross-species analysis of the segmentation clock mechanism using a microarray approach in fish, chicken, and mouse PSM demonstrated that, although the same three pathways are activated periodically, the individual cyclic genes differ between species (Krol et al. 2011). These differences suggest a certain degree of evolutionary flexibility, at least at the molecular level, in the segmentation mechanism. Evidence for a similar oscillator associated to periodic segment production was also recently described in short-germ-band insects, in which elongation of the body axis follows a mode similar to that of vertebrates (Sarrazin et al. 2012). This supports the idea that the use of an oscillator to pattern embryonic segments might represent an ancestral strategy in bilateria. Furthermore,

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.



Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Figure 3

Molecular mechanism involved in somite formation. (*a*) Schematic representation of the clock and wavefront model. Dynamic patterns of a cyclic (clock) gene (*purple*) are represented in the presomitic mesoderm (PSM) at different stages in the somite formation cycle (90 min). The cyclic gene is expressed in the caudal part of the PSM, then in the intermediate part, and finally in a more restricted area of the anterior PSM; this cyclic pattern of expression is then repeated. As the embryo elongates, the wavefront or determination front [set up by gradients of FGF/WNT and retinoic acid (RA) signaling] regresses (*dashed black lines*). The position of the future somite is set up once the clock genes are expressed in the window of competence delimited by the determination front. (*b*) The different cyclic genes oscillate with different phase patterns. Genes belonging to Notch/FGF cyclic genes oscillate in antiphase with the cluster of cyclic genes from Wnt signaling. Abbreviations: NT, neural tube; PS, primitive streak; S, somites.

in *Arabidopsis*, Moreno-Risueno et al. (2010) also proposed an oscillator to control the periodic formation of the rootlets. Thus, this provides an interesting example of convergent evolution and suggests that the displacement of an oscillator in space might represent a basic strategy for periodic pattern generation in eukaryotes.

The relative and apparent simplicity of the zebrafish clock led to several models aiming to explain the mechanisms driving the transcriptional oscillation. Central to these models is a delayed

negative feedback loop that regulates expression of the Her family of transcription repressors (Henry et al. 2002, Lewis 2003, Morelli et al. 2009, Oates & Ho 2002, Schroter et al. 2012). In amniotes, however, we do not yet fully understand the interplay between the different cyclic gene products and their role in the segmentation clock. Particularly, whether the cyclic genes act as the pacemaker of the oscillator remains controversial. It has recently been demonstrated in mice that removing two introns of the *Hes7* gene leads to more rapid oscillations. This confirms that *Hes* genes are a central element in setting up the tempo of the segmentation clock (Harima et al. 2013). However, the mouse and chick segmentation clocks seem to include far more players than members of Hes family genes. Several modeling efforts have been made to understand how these different players could regulate the clock (Dequeant et al. 2006; Goldbeter & Pourquié 2008; Krol et al. 2011; Niwa et al. 2007, 2011). Most of the cyclic genes code for negative-feedback inhibitors of the Notch, Wnt, and FGF signaling pathways, which suggests a possible mechanism for their periodic control. In this hypothesis, the pathways that are constitutively activated in the PSM trigger the activation of these negative-feedback inhibitors, which in turn shut down their expression. The inhibitors then become degraded, and activation of the pathways resumes once the inhibitors have disappeared. Such a mechanism could explain the control of the oscillations of the clock and can be modeled mathematically (Dequeant et al. 2006, Dequeant & Pourquié 2008, Goldbeter & Pourquié 2008). However, such a model is hard to reconcile with the results of experiments in which the Notch or the Wnt pathways were constitutively activated in mouse and in which oscillations were still observed (Aulehla et al. 2008, Dunty et al. 2008, Feller et al. 2008, Ozbudak & Pourquié 2008). Such genetic experiments in mouse seem to suggest that the clock pacemaker cannot be explained only by feedback-loop mechanisms of the three major signaling pathways. If this is true, it will be very interesting to identify the signals that can trigger the clock signal. The possibility of another oscillator entraining the cyclic gene network cannot be excluded at this point.

The use of real-time reporters in vivo helped to decipher many aspects of the dynamics of segmentation (Aulehla et al. 2008, Masamizu et al. 2006). Time-lapse imaging of a clock reporter for the cyclic gene *Lunatic fringe* in mice allowed researchers to monitor oscillation properties and their link to signaling gradients in the PSM. For example, graded WNT/β-catenin signals regulate the size of the oscillatory domain of the cyclic genes within the PSM (Aulehla et al. 2008). Within this oscillatory domain, a gradual shift in *Lunatic fringe* oscillation phases (phase gradient) exists between cells. Using time-lapse imaging in PSM explanted tissue, Lauschke et al. (2013) recently observed that this phase gradient varies in tissue-size dependence—a mechanism that explains how somite size scales with embryonic size.

The recent advances in understanding cell movements in the PSM, which implicate convergence at early stages and graded random movements at later stages, raised the question of the coordination of such movements with the highly coordinated clock oscillations that take place in this tissue. Based on theoretical grounds, Uriu et al. (2010) proposed that random movements participate in the regulation of clock oscillation by improving the synchronization of the oscillations among cells. Until now, most of the dynamic analysis of the clock signal in vivo has been done at the tissue level. In a recent report, Delaune et al. (2012) used the zebrafish embryo as a model to study the segmentation clock at the single-cell level using time-lapse imaging. They provide direct evidence that Notch signaling is crucial to synchronize the clock phases between PSM cells. By tracking individual cells, they were also able to demonstrate that PSM cells divide preferentially in a certain clock phase.

A second system (the wavefront) is required to translate the rhythmic signal delivered by the clock into the periodic series of somites. This system involves traveling posterior gradients of the Wnt and FGF signaling pathways. These gradients define a particular threshold of signaling along

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

the PSM, called the determination front, at which cells first become competent to respond to the clock signal (Aulehla et al. 2003, Dubrulle et al. 2001, Sawada et al. 2001). Thus, when the clock signal reaches the cells that have passed the determination front during the previous cycle, segmentation genes (such as the transcription factor Mesp2) are activated in these cells, thus forming a striped domain that prefigures the future segment (**Figure 3*a***) (Saga 2007, Saga et al. 1997). A dynamic analysis using an FGF and a Delta/Notch bioluminescent cyclic reporter allowed Niwa et al. (2011) to propose a new model in which the wavefront window would be defined by the dynamics of FGF oscillations instead of being a linear readout of graded signaling. The Mesp2 expression regulation is a key element integrating both the cyclic and the determination front signals (Morimoto et al. 2007; Oginuma et al. 2008, 2010). Mesp2 subsequently triggers a molecular cascade that leads to the specification of segment boundaries (Saga 2012). An opposing gradient of RA activity coming from the formed somites and the anterior PSM was also implicated in positioning the determination front by counteracting the posterior gradients (Diez del Corral et al. 2003, Goldbeter et al. 2007, Moreno & Kintner 2004). The rapid gene activation in the segmental stripe seen at the determination-front level may result from a bistable transition controlled by the system of mutually opposing gradients of FGF and RA (Goldbeter et al. 2007).

Determination front: window of competence within the PSM tissue in which cells can become determined to form a somite; the position and size of this window is determined by signaling gradients (FGF or WNT), and this window moves toward the posterior part of the embryo in parallel with somite formation and Node/tail bud regression

Another challenge in the field of segmentation is to understand the dynamics of signaling gradients in the PSM. The FGF gradient is established by regulation of the *Fgf8* mRNA decay (Dubrulle & Pourquié 2004). Transcription of the *Fgf8* mRNA is restricted to the paraxial mesoderm precursors in the tail bud, and it stops when their descendants enter the posterior PSM. Thus, the *Fgf8* gradient observed in the posterior PSM simply reflects the progressive decay of the mRNA. Such a mechanism also likely controls the establishment of dynamic gradients of other Fgfs and Wnt ligands in the posterior PSM. Remarkably, the FGF gradient controls both the random motility gradient involved in axis elongation and the segmentation process, which ensures a tight coordination between these two fundamental patterning processes.

Mutations in genes involved in the mechanism of segment formation, and particularly genes involved in the segmentation clock, are linked to vertebral malformations, such as congenital scoliosis in humans (Pourquié 2011). Therefore, basic discoveries in the field of segmentation can have a tremendous impact on our understanding of vertebral malformations, currently a poorly studied area of medicine. Studies in mouse embryos show that the combination of heterozygote mutations in genes involved in the control of segmentation, coupled to an environmental injury such as hypoxia, can lead to segmental malformations reminiscent of congenital scoliosis (Sparrow et al. 2012). This study shows that the effect of oxygen level on the mechanism of segmentation involves a downregulation of the FGF pathway. These results illustrate the importance of both the genetic and the epigenetic environment in the etiology of such pathologies.

Regulation of the Segment Number

The total number of somites varies tremendously between species: There are 32 in zebrafish, 55 in chicken, 65 in mice, and up to 344 in snakes. But within a given species, the number of segments is remarkably constant. The ratio between the speed of axis elongation and of somitogenesis was proposed to play a critical role in controlling the size of the PSM and thus in defining the number of body segments (**Figure 4*a***) (Gomez et al. 2008, Gomez & Pourquié 2009). The number of cell generations required to generate the embryonic axis was calculated for fish (2.8), chicken (13), mouse (15), and snake (21) embryos. This suggests that posterior growth continues for a longer time in snakes. Interestingly, in contrast to the several posterior *Hox* genes expressed in the lizard tail, only two posterior *Hox* genes (*HoxC12* and *C13*) are expressed in the snake embryo tail region (Di-Poï et al. 2010). It has been hypothesized that posterior *Hox* genes participate in the

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

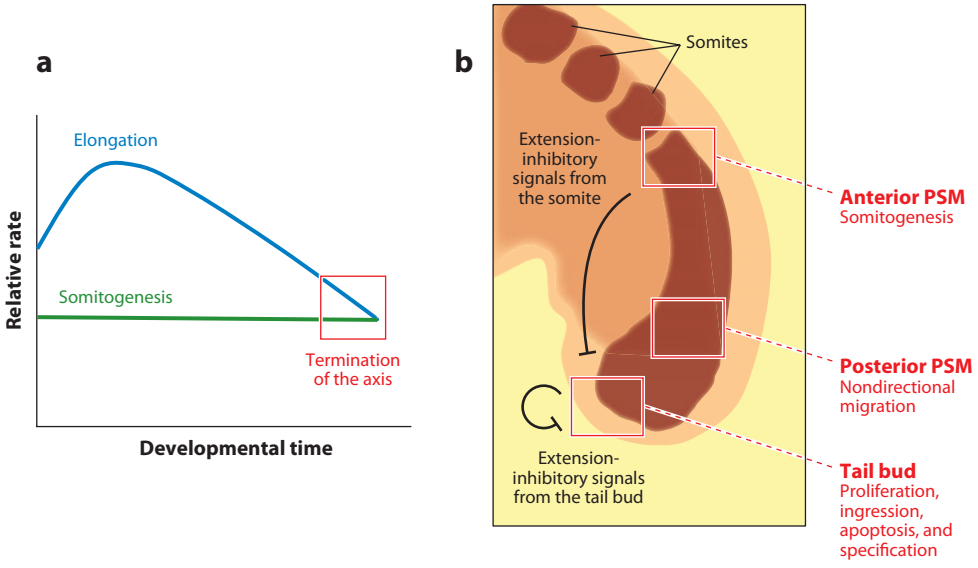


Figure 4

Mechanisms that are potentially involved in the regulation of axis extension termination. (*a*) The termination of the axis and the regulation of species-specific somite number can be viewed as a balance between the rate of axis elongation and the rate of somitogenesis. The rate of somitogenesis is relatively constant throughout development for a given species, and the diminution of the elongation rate is a key factor in regulating axis length and therefore segment number. (*b*) Schematic representation of the posterior part of the axis at the end of elongation (Hamburger-Hamilton stage 24, side view). In the anterior presomitic mesoderm (PSM), somitogenesis still occurs. Extension of the axis is sustained by nondirectional migration in the caudal PSM and proliferation and ingression from the tail bud. Diminution of ingression, specification, migration, proliferation, or apoptosis can lead to the slowing down of elongation. Signals from the anterior PSM and/or from the tail bud may cause the cessation of axis growth.

cessation of axis elongation (Young et al. 2009). Therefore, the reduced number of posterior *Hox* genes in snake might explain the extended period of posterior growth. These differences cannot, however, explain the differences in somite numbers observed between these species. Analysis of the segmentation clock in snake showed that, relative to the speed of development, the snake clock ticks much faster than does the chicken or the mouse clock. The analysis of cyclic gene-expression patterns further supports a specific increase in the clock pace in snakes (Gomez et al. 2008). As a result, snakes generate more segments for a similar amount of axis growth compared with other species. These results argue that both the number of cell generations required to form the body axis and the clock pace control the number of segments for each species. This model is now supported by genetic evidence in zebrafish. Analysis of the clock period in *hes6* fish mutants indicates that the clock period is slowed compared with that in wild-type animals (Schroter & Oates 2010). Mutant embryos have a reduced segment number, whereas elongation rate and body length are unaltered. Interestingly, the relative anteroposterior patterning of the axis remains unchanged, which suggests that it scales with the body length and not with the segment number. Harima et al. (2013) conducted the complementary experiment in mouse embryos by reducing the number of introns in the *Hes7* gene. They observed that when the tempo of oscillations is accelerated, there is an increase in the numbers of somites and vertebrae. Although experimental alterations in clock speed clearly result in changes of segment number, the molecular basis of such regulation among different species remains to be determined.

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Termination of Body-Axis Elongation

The chicken embryonic axis stops elongating around stage 24–25HH, when the tail bud progressively stops its posterior movement (Sanders et al. 1986, Tenin et al. 2010). The causes of the arrest of elongation have been much debated, and several models have been proposed. Proliferation and cell rearrangement are key drivers of axis elongation, and altering their regulation would be a logical solution to end embryonic axis growth (**Figure 4*b***). Another possibility would be to stop elongation by depleting stem cells or progenitors from the tail bud. The fact that a burst of apoptosis indeed arises in the tail bud at the end of elongation supports this hypothesis (Sanders et al. 1986). Depletion of cells from the tail bud owing to their precipitated ingression, or by blockage of their ingression into the mesoderm, might also be a way to terminate axis extension. Interestingly, major signaling pathways involved in gastrulation are downregulated in the tail bud at the end of the elongation process (Cambray & Wilson 2007, Tenin et al. 2010). For example, transcription of Wnt and FGF ligands is reduced in late tail bud compared with earlier stages. As these pathways are also implicated in cell proliferation, their reduced activity at late–tail bud stage may be linked to a reduced proliferation of tail bud progenitor cells. Whereas elongation slows down at the end of axis extension, the pace of somitogenesis is relatively constant, except for the very last somites, which are formed at a slower pace (Tam 1981, Tenin et al. 2010). This situation leads to the progressive shrinking of the PSM, which brings the tail bud in close proximity to the last segmented somites. The somites produce RA, which induces axis truncation, and at these late stages, the expression of Cyp26, which catabolizes RA, is downregulated (Iulianella et al. 1999, Kessel & Gruss 1991, Tenin et al. 2010). Furthermore, Raldh2, an enzyme involved in RA synthesis and expressed in the somites, is expressed in the chick tail bud at late stages of development (Tenin et al. 2010). In line with this observation is the finding that exposure of cultured tail bud to RA leads to an increase in apoptosis (Shum et al. 1999, Tenin et al. 2010). Thus, RA signaling likely participates in the signaling that terminates axis elongation. In a recent study, Olivera-Martinez et al. (2012) integrated several aspects of the studies mentioned in this paragraph. Their experimental evidence suggests that the rise of RA signaling in the late tail bud not only induces cell death but also promotes the decline of FGF signaling, which leads to a loss of mesoderm identity. This suggests that both loss of specification and induced cell death participate in the cessation of axis extension.

CONCLUSION

Our understanding of the morphogenetic and patterning events that control vertebrate axis formation has greatly improved over the past decade. In particular, technological advances in microscopy have allowed us to observe the details of the cellular behaviors that underlie axis formation and segmentation over different stages of development and within different animal models. In parallel, functional experiments, such as genetic approaches, have favored the identification of most of the molecular players involved in these cellular behaviors. However, several questions remain open and will probably give rise to exciting research in the future. Concerning posterior axis extension, the studies conducted until now have been focused mainly on one particular tissue, the paraxial mesoderm, and its role during elongation. Different layers and tissues interact in the extending embryo, and the comprehension of how tissues move compared with each other and how they mechanically interact will be important to establish the physical principles underlying axis formation. We still know little about the forces that shape the embryo, the viscoelastic properties of the tissues, and the mechanically active and passive embryonic regions. The design of sensors that allow us to measure forces in vivo will be a necessary step toward integrating physics into the field

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

of morphogenesis. Finally, a quantitative analysis of somitogenesis will be required to understand the exact mechanism that underlies the robust oscillatory pattern that takes place during somite formation.

SUMMARY POINTS

- Axis extension during the early phases of development (formation of the anterior part of the body) implies oriented cell behaviors, such as intercalation and directed cell migration; it also involves convergence-extension movements at the tissue level.
- Axis extension during the late phases of development (formation of the posterior part of the body) requires a gradient of nondirectional migration to take place in the PSM.
- Segmentation (the formation of somites) relies on the coordination of dynamic patterning signals that take place in the PSM, including the segmentation clock (a molecular oscillator) and the wavefront (a spatial determination window that is defined by signaling gradients).
- Axis extension and somitogenesis rates and speed are tightly regulated and coordinated to produce the species-specific number of segments and axis length.
- The cessation of axis extension can be controlled by a combination of several cellular behaviors, including apoptosis, loss of specification, proliferation, ingression, and cellular migration.

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank Daniela Roellig and the Pourquié lab members for critical reading of the manuscript as well as Sandra Bour for her help with graphical design. We apologize to the many authors we could not cite because of the space limit.

LITERATURE CITED

Abu-Abed S, Dolle P, Metzger D, Beckett B, Chambon P, Petkovich M. 2001. The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity, and development of posterior structures. *Genes Dev.* 15:226–40

Agathon A, Thisse C, Thisse B. 2003. The molecular nature of the zebrafish tail organizer. *Nature* 424:448–52

Agero U, Glazier JA, Hosek M. 2010. Bulk elastic properties of chicken embryos during somitogenesis. *Biomed. Eng. Online* 9:19

Amacher SL, Draper BW, Summers BR, Kimmel CB. 2002. The zebrafish T-box genes *no tail* and *spadetail* are required for development of trunk and tail mesoderm and medial floor plate. *Development* 129:3311–23

Ang S-L, Rossant J. 1994. *HNF-3β* is essential for node and notochord formation in mouse development. *Cell* 78:561–74

Aulehla A, Wehrle C, Brand-Saberi B, Kemler R, Gossler A, et al. 2003. *Wnt3a* plays a major role in the segmentation clock controlling somitogenesis. *Dev. Cell* 4:395–406

Aulehla A, Wiegraebe W, Baubet V, Wahl MB, Deng C, et al. 2008. A β-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. *Nat. Cell Biol.* 10:186–93

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Beck CW, Slack JMW. 1998. Analysis of the developing *Xenopus* tail bud reveals separate phases of gene expression during determination and outgrowth. *Mech. Dev.* 72:41–52

Beck CW, Slack JMW. 1999. A developmental pathway controlling outgrowth of the *Xenopus* tail bud. *Development* 126:1611–20

Beck CW, Whitman M, Slack JM. 2001. The role of BMP signaling in outgrowth and patterning of the *Xenopus* tail bud. *Dev. Biol.* 238:303–14

Bénazéraf B, Francois P, Baker RE, Denans N, Little CD, Pourquié O. 2010. A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature* 466:248–52

Bertet C, Sulak L, Lecuit T. 2004. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* 429:667–71

Boulet AM, Capecchi MR. 2012. Signaling by FGF4 and FGF8 is required for axial elongation of the mouse embryo. *Dev. Biol.* 371:235–45

Cambray N, Wilson V. 2002. Axial progenitors with extensive potency are localised to the mouse chordoneural hinge. *Development* 129:4855–66

Cambray N, Wilson V. 2007. Two distinct sources for a population of maturing axial progenitors. *Development* 134:2829–40

Carmona-Fontaine C, Matthews HK, Kuriyama S, Moreno M, Dunn GA, et al. 2008. Contact inhibition of locomotion *in vivo* controls neural crest directional migration. *Nature* 456:957–61

Catala M, Teillet MA, De Robertis EM, Le Douarin NM. 1996. A spinal cord fate map in the avian embryo: while regressing, Hensen's node lays down the notochord and floor plate thus joining the spinal cord lateral walls. *Development* 122:2599–610

Catala M, Teillet MA, Le Douarin NM. 1995. Organization and development of the tail bud analyzed with the quail-chick chimaera system. *Mech. Dev.* 51:51–65

Chal J, Pourquié O. 2009. Patterning and differentiation of the vertebrate spine. In *The Skeletal System*, ed. O Pourquié, pp. 41–116. Woodbury, NY: Cold Spring Harb. Lab.

Charrier JB, Teillet MA, Lapointe F, Le Douarin NM. 1999. Defining subregions of Hensen's node essential for caudalward movement, midline development and cell survival. *Development* 126:4771–83

Chawengsaksophak K, de Graaff W, Rossant J, Deschamps J, Beck F. 2004. *Cdx2* is essential for axial elongation in mouse development. *Proc. Natl. Acad. Sci. USA* 101:7641–45

Chuai M, Weijer CJ. 2007. The mechanisms underlying primitive streak formation in the chick embryo. *Curr. Top. Dev. Biol.* 81:135–56

Chuai M, Weijer CJ. 2009. Who moves whom during primitive streak formation in the chick embryo. *HFSP J.* 3:71–76

Chuai M, Zeng W, Yang X, Boychenko V, Glazier JA, Weijer CJ. 2006. Cell movement during chick primitive streak formation. *Dev. Biol.* 296:137–49

Colas JF, Schoenwolf GC. 2001. Towards a cellular and molecular understanding of neurulation. *Dev. Dyn.* 221:117–45

Concha ML, Adams RJ. 1998. Oriented cell divisions and cellular morphogenesis in the zebrafish gastrula and neurula: a time-lapse analysis. *Development* 125:983–94

Cooke J, Zeeman EC. 1976. A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *J. Theor. Biol.* 58:455–76

Cui C, Yang X, Chuai M, Glazier JA, Weijer CJ. 2005. Analysis of tissue flow patterns during primitive streak formation in the chick embryo. *Dev. Biol.* 284:37–47

Czirok A, Rongish BJ, Little CD. 2004. Extracellular matrix dynamics during vertebrate axis formation. *Dev. Biol.* 268:111–22

Damon BJ, Mezentseva NV, Kumaratilake JS, Forgacs G, Newman SA. 2008. Limb bud and flank mesoderm have distinct "physical phenotypes" that may contribute to limb budding. *Dev. Biol.* 321:319–30

Davidson BP, Kinder SJ, Steiner K, Schoenwolf GC, Tam PP. 1999. Impact of node ablation on the morphogenesis of the body axis and the lateral asymmetry of the mouse embryo during early organogenesis. *Dev. Biol.* 211:11–26

Davidson LA. 2011. Embryo mechanics: balancing force production with elastic resistance during morphogenesis. *Curr. Top. Dev. Biol.* 95:215–41

Davis RL, Kirschner MW. 2000. The fate of cells in the tailbud of *Xenopus laevis*. *Development* 127:255–67
Delaune EA, François P, Shih NP, Amacher SL. 2012. Single-cell-resolution imaging of the impact of Notch signaling and mitosis on segmentation clock dynamics. *Dev. Cell* 23:995–1005
Delfini MC, Dubrulle J, Malapert P, Chal J, Pourquié O. 2005. Control of the segmentation process by graded MAPK/ERK activation in the chick embryo. *Proc. Natl. Acad. Sci. USA* 102:11343–48
Deng C-X, Wynshaw-Boris A, Shen MM, Daugherty C, Ornitz DM, Leder P. 1994. Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev.* 8:3045–57
Dequeant ML, Glynn E, Gaudenz K, Wahl M, Chen J, et al. 2006. A complex oscillating network of signaling genes underlies the mouse segmentation clock. *Science* 314:1595–98
Dequeant ML, Pourquié O. 2008. Segmental patterning of the vertebrate embryonic axis. *Nat. Rev. Genet.* 9:370–82
Di-Poï N, Montoya-Burgos JI, Miller H, Pourquié O, Milinkovitch MC, Duboule D. 2010. Changes in *Hox* genes' structure and function during the evolution of the squamate body plan. *Nature* 464:99–103
Diez del Corral R, Olivera-Martinez I, Goriely A, Gale E, Maden M, 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 JL, Dufour S, Hatta K, Takeichi M, Edelman GM, Thiery JP. 1987. Adhesion molecules during somitogenesis in the avian embryo. *J. Cell Biol.* 104:1361–74
Duboule D. 2007. The rise and fall of Hox gene clusters. *Development* 134:2549–60
Duboule D, Dollé P. 1989. The structural and functional organization of the murine HOX gene family resembles that of *Drosophila* homeotic genes. *EMBO J.* 8:1497–505
Duboule D, Morata G. 1994. Colinearity and functional hierarchy among genes of the homeotic complexes. *Trends Genet.* 10:358–64
Dubrulle J, McGrew MJ, Pourquié O. 2001. FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal *Hox* gene activation. *Cell* 106:219–32
Dubrulle J, Pourquié O. 2004. *fgf8* mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. *Nature* 427:419–22
Dunty WC Jr, Biris KK, Chalamalasetty RB, Taketo MM, Lewandoski M, Yamaguchi TP. 2008. Wnt3a/β-catenin signaling controls posterior body development by coordinating mesoderm formation and segmentation. *Development* 135:85–94
Dzamba BJ, Jakab KR, Marsden M, Schwartz MA, DeSimone DW. 2009. Cadherin adhesion, tissue tension, and noncanonical Wnt signaling regulate fibronectin matrix organization. *Dev. Cell* 16:421–32
Economides KD, Zeltser L, Capecchi MR. 2003. *Hoxb13* mutations cause overgrowth of caudal spinal cord and tail vertebrae. *Dev. Biol.* 256:317–30
Eloy-Trinquet S, Nicolas JF. 2002. Cell coherence during production of the presomitic mesoderm and somitogenesis in the mouse embryo. *Development* 129:3609–19
Elul T, Koehl MA, Keller R. 1997. Cellular mechanism underlying neural convergent extension in *Xenopus laevis* embryos. *Dev. Biol.* 191:243–58
Feller J, Schneider A, Schuster-Gossler K, Gossler A. 2008. Noncyclic Notch activity in the presomitic mesoderm demonstrates uncoupling of somite compartmentalization and boundary formation. *Genes Dev.* 22:2166–71
Fior R, Maxwell AA, Ma TP, Vezarro A, Moens CB, et al. 2012. The differentiation and movement of presomitic mesoderm progenitor cells are controlled by Mesogenin 1. *Development* 139:4656–65
Fujii H, Sato T, Kaneko S, Gotoh O, Fujii-Kuriyama Y, et al. 1997. Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. *EMBO J.* 16:4163–73
Galceran J, Fariñas I, Depew MJ, Clevers H, Grosschedl R. 1999. Wnt3a$^{-/-}$-like phenotype and limb deficiency in *Lef1*$^{-/-}$*Tcf1*$^{-/-}$ mice. *Genes Dev.* 13:709–17
Garcia-Martinez V, Schoenwolf GC. 1992. Positional control of mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dyn.* 193:249–56
George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO. 1993. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development* 119:1079–91
Georges-Labouesse EN, George EL, Rayburn H, Hynes RO. 1996. Mesodermal development in mouse embryos mutant for fibronectin. *Dev. Dyn.* 207:145–56

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Girós A, Grgur K, Gossler A, Costell M. 2011. α5β1 integrin-mediated adhesion to fibronectin is required for axis elongation and somitogenesis in mice. *PLoS ONE* 6:e22002

Goldbeter A, Gonze D, Pourquié O. 2007. Sharp developmental thresholds defined through bistability by antagonistic gradients of retinoic acid and FGF signaling. *Dev. Dyn.* 236:1495–508

Goldbeter A, Pourquié O. 2008. Modeling the segmentation clock as a network of coupled oscillations in the Notch, Wnt and FGF signaling pathways. *J. Theor. Biol.* 252:574–85

Gomez C, Ozbudak EM, Wunderlich J, Baumann D, Lewis J, Pourquié O. 2008. Control of segment number in vertebrate embryos. *Nature* 454:335–39

Gomez C, Pourquié O. 2009. Developmental control of segment numbers in vertebrates. *J. Exp. Zool. B Mol. Dev. Evol.* 312:533–44

Gont LK, Steinbeisser H, Blumberg B, De Robertis EM. 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

Graham A, Papalopulu N, Krumlauf R. 1989. The murine and *Drosophila* homeobox gene complexes have common features of organization and expression. *Cell* 57:367–78

Gräper L. 1929. Die Primitiventwicklung des Hünchens nach stereokinematographischen Untersuchungen, kontrolliert durch vitale Farbmarkierung und verglichen mit der Entwicklung anderer Wierbeltiere. *Arch. EntwMech. Org.* 116:382–429

Gray RS, Roszko I, Solnica-Krezel L. 2011. Planar cell polarity: coordinating morphogenetic cell behaviors with embryonic polarity. *Dev. Cell* 21:120–33

Greco TL, Takada S, Newhouse MM, McMahon JA, McMahon AP, Camper SA. 1996. Analysis of the *vestigial tail* mutation demonstrates that *Wnt-3a* gene dosage regulates mouse axial development. *Genes Dev.* 10:313–24

Gros J, Hu JK-H, Vinegoni C, Feruglio PF, Weissleder R, Tabin CJ. 2010. WNT5A/JNK and FGF/MAPK pathways regulate the cellular events shaping the vertebrate limb bud. *Curr. Biol.* 20:1993–2002

Halacheva V, Fuchs M, Donitz J, Reupke T, Puschel B, Viebahn C. 2011. Planar cell movements and oriented cell division during early primitive streak formation in the mammalian embryo. *Dev. Dyn.* 240:1905–16

Hamburger V. 1992. The stage series of the chick embryo. *Dev. Dyn.* 195:273–75

Hardy KM, Garriock RJ, Yatskievych TA, D'Agostino SL, Antin PB, Krieg PA. 2008. Non-canonical Wnt signaling through Wnt5a/b and a novel Wnt11 gene, Wnt11b, regulates cell migration during avian gastrulation. *Dev. Biol.* 320:391–401

Harima Y, Takashima Y, Ueda Y, Ohtsuka T, Kageyama, R. 2013. Accelerating the tempo of the segmentation clock by reducing the number of introns in the *Hes7* gene. *Cell Rep*. 3:1–7

Hatada Y, Stern CD. 1994. A fate map of the epiblast of the early chick embryo. *Development* 120:2879–89

Henry CA, Urban MK, Dill KK, Merlie JP, Page MF, et al. 2002. Two linked *hairy/Enhancer of split*-related zebrafish genes, *her1* and *her7*, function together to refine alternating somite boundaries. *Development* 129:3693–704

Herrmann BG, Labeit S, Poustka A, King TR, Lehrach H. 1990. Cloning of the *T* gene required in mesoderm formation in the mouse. *Nature* 343:617–22

Hinsch CW, Hamilton HL. 1956. The developmental fate of the first somite in the chick embryo. *Anat. Rec.* 125:225–46

Holmdahl DE. 1925. Experimentelle Untersuchungen über die lage der Grenze zwischen Primärer und sekundärer Körperentwicklung beim Huhn. *Anat. Anz.* 59:393–96

Hsieh J-C, Lee L, Zhang L, Wefer S, Brown K, et al. 2003. Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* 112:355–67

Iimura T, Denans N, Pourquié O. 2009. Establishment of Hox vertebral identities in the embryonic spine precursors. *Curr. Top. Dev. Biol.* 88:201–34

Iimura T, Pourquié O. 2006. Collinear activation of *Hoxb* genes during gastrulation is linked to mesoderm cell ingression. *Nature* 442:568–71

Iimura T, Yang X, Weijer CJ, Pourquié O. 2007. Dual mode of paraxial mesoderm formation during chick gastrulation. *Proc. Natl. Acad. Sci. USA* 104:2744–49

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Iulianella A, Beckett B, Petkovich M, Lohnes D. 1999. A molecular basis for retinoic acid-induced axial truncation. *Dev. Biol.* 205:33–48
Joubin K, Stern CD. 1999. Molecular interactions continuously define the organizer during the cell movements of gastrulation. *Cell* 98:559–71
Jouve C, Iimura T, Pourquié O. 2002. Onset of the segmentation clock in the chick embryo: evidence for oscillations in the somite precursors in the primitive streak. *Development* 129:1107–17
Kanki JP, Ho RK. 1997. The development of the posterior body in zebrafish. *Development* 124:881–93
Keller PJ, Schmidt AD, Wittbrodt J, Stelzer EH. 2008. Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. *Science* 322:1065–69
Keller R, Danilchik M. 1988. Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. *Development* 103:193–209
Keller R, Shih J, Sater A. 1992. The cellular basis of the convergence and extension of the *Xenopus* neural plate. *Dev. Dyn.* 193:199–217
Keller R, Shook D, Skoglund P. 2008. The forces that shape embryos: physical aspects of convergent extension by cell intercalation. *Phys. Biol.* 5:015007
Kelly OG, Pinson KI, Skarnes WC. 2004. The Wnt co-receptors Lrp5 and Lrp6 are essential for gastrulation in mice. *Development* 131:2803–15
Kessel M. 1992. Respecification of vertebral identities by retinoic acid. *Development* 115:487–501
Kessel M, Gruss P. 1991. Homeotic transformations of murine vertebrae and concomitant alteration of *Hox* codes induced by retinoic acid. *Cell* 67:89–104
Kirby ML, Lawson A, Stadt HA, Kumiski DH, Wallis KT, et al. 2003. Hensen's node gives rise to the ventral midline of the foregut: implications for organizing head and heart development. *Dev. Biol.* 253:175–88
Knezevic V, De Santo R, Mackem S. 1998. Continuing organizer function during chick tail development. *Development* 125:1791–801
Kondoh H, Takemoto T. 2012. Axial stem cells deriving both posterior neural and mesodermal tissues during gastrulation. *Curr. Opin. Genet. Dev.* 22:374–80
Krol AJ, Roellig D, Dequeant ML, Tassy O, Glynn E, et al. 2011. Evolutionary plasticity of segmentation clock networks. *Development* 138:2783–92
Kulesa PM, Fraser SE. 2002. Cell dynamics during somite boundary formation revealed by time-lapse analysis. *Science* 298:991–95
Lauschke VM, Tsiairis CD, François P, Aulhela A. 2013. Scaling of embryonic patterning based on phase-gradient encoding. *Nature* 493:101–5
Lawson A, Schoenwolf GC. 2001. Cell populations and morphogenetic movements underlying formation of the avian primitive streak and organizer. *Genesis* 29:188–95
Lawson KA, Pedersen RA. 1992. Clonal analysis of cell fate during gastrulation and early neurulation in the mouse. *Ciba Found. Symp.* 165:3–21; discussion pp. 21–26
Lawton AK, Nandi A, Stulberg MJ, Dray N, Sneddon MW, et al. 2013. Regulated tissue fluidity steers zebrafish body elongation. *Development* 140:573–82
Leslie NR, Yang X, Downes CP, Weijer CJ. 2007. PtdIns(3,4,5)P_3-dependent and -independent roles for PTEN in the control of cell migration. *Curr. Biol.* 17:115–25
Lewis J. 2003. Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. *Curr. Biol.* 13:1398–408
Liu C, Knezevic V, Mackem S. 2004. Ventral tail bud mesenchyme is a signaling center for tail paraxial mesoderm induction. *Dev. Dyn.* 229:600–6
Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. 1999. Requirement for *Wnt3* in vertebrate axis formation. *Nat. Genet.* 22:361–65
Mallo M, Wellik DM, Deschamps J. 2010. *Hox* genes and regional patterning of the vertebrate body plan. *Dev. Biol.* 344:7–15
Martin BL, Kimelman D. 2010. Brachyury establishes the embryonic mesodermal progenitor niche. *Genes Dev.* 24:2778–83
Martins GG, Rifes P, Amândio R, Rodrigues G, Palmeirim I, Thorsteinsdóttir S. 2009. Dynamic 3D cell rearrangements guided by a fibronectin matrix underlie somitogenesis. *PLoS ONE* 4:e7429

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Masamizu Y, Ohtsuka T, Takashima Y, Nagahara H, Takenaka Y, et al. 2006. Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells. *Proc. Natl. Acad. Sci. USA* 103:1313–18

Mathis L, Kulesa PM, Fraser SE. 2001. FGF receptor signalling is required to maintain neural progenitors during Hensen's node progression. *Nat. Cell Biol.* 3:559–66

McGrew MJ, Sherman A, Lillico SG, Ellard FM, Radcliffe PA, et al. 2008. Localised axial progenitor cell populations in the avian tail bud are not committed to a posterior Hox identity. *Development* 135:2289–99

Morelli LG, Ares S, Herrgen L, Schroter C, Julicher F, Oates AC. 2009. Delayed coupling theory of vertebrate segmentation. *HFSP J.* 3:55–66

Moreno TA, Kintner C. 2004. Regulation of segmental patterning by retinoic acid signaling during *Xenopus* somitogenesis. *Dev. Cell* 6:205–18

Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang J, Ahnert SE, Benfey PN. 2010. Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. *Science* 329:1306–11

Morimoto M, Sasaki N, Oginuma M, Kiso M, Igarashi K, et al. 2007. The negative regulation of Mesp2 by mouse Ripply2 is required to establish the rostro-caudal patterning within a somite. *Development* 134:1561–69

Naiche LA, Holder N, Lewandoski M. 2011. FGF4 and FGF8 comprise the wavefront activity that controls somitogenesis. *Proc. Natl. Acad. Sci. USA* 108:4018–23

Nakaya Y, Sheng G. 2008. Epithelial to mesenchymal transition during gastrulation: an embryological view. *Dev. Growth Differ.* 50:755–66

Nakaya Y, Sukowati EW, Wu Y, Sheng G. 2008. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nat. Cell Biol.* 10:765–75

Nicolet G. 1971. Avian gastrulation. *Adv. Morphog.* 9:231–62

Nieto MA. 2011. The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annu. Rev. Cell Dev. Biol.* 27:347–76

Nishimura T, Honda H, Takeichi M. 2012. Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell* 149:1084–97

Niwa Y, Masamizu Y, Liu T, Nakayama R, Deng C-X, Kageyama R. 2007. The initiation and propagation of Hes7 oscillation are cooperatively regulated by Fgf and Notch signaling in the somite segmentation clock. *Dev. Cell* 13:298–304

Niwa Y, Shimojo H, Isomura A, González A, Miyachi H, Kageyama R. 2011. Different types of oscillations in Notch and Fgf signaling regulate the spatiotemporal periodicity of somitogenesis. *Genes Dev.* 25:1115–20

Oates AC, Ho RK. 2002. *Hairy/E(spl)*-related (*Her*) genes are central components of the segmentation oscillator and display redundancy with the Delta/Notch signaling pathway in the formation of anterior segmental boundaries in the zebrafish. *Development* 129:2929–46

Oginuma M, Niwa Y, Chapman DL, Saga Y. 2008. Mesp2 and Tbx6 cooperatively create periodic patterns coupled with the clock machinery during mouse somitogenesis. *Development* 135:2555–62

Oginuma M, Takahashi Y, Kitajima S, Kiso M, Kanno J, et al. 2010. The oscillation of Notch activation, but not its boundary, is required for somite border formation and rostro-caudal patterning within a somite. *Development* 137:1515–22

Ohta S, Suzuki K, Tachibana K, Tanaka H, Yamada G. 2007. Cessation of gastrulation is mediated by suppression of epithelial-mesenchymal transition at the ventral ectodermal ridge. *Development* 134:4315–24

Olivera-Martinez I, Harada H, Halley PA, Storey KG. 2012. Loss of FGF-dependent mesoderm identity and rise of endogenous retinoid signalling determine cessation of body axis elongation. *PLoS Biol.* 10:e1001415

Olivier N, Luengo-Oroz MA, Duloquin L, Faure E, Savy T, et al. 2010. Cell lineage reconstruction of early zebrafish embryos using label-free nonlinear microscopy. *Science* 329:967–71

Ozbudak EM, Pourquié O. 2008. The vertebrate segmentation clock: the tip of the iceberg. *Curr. Opin. Genet. Dev.* 18:317–23

Palmeirim I, Henrique D, Ish-Horowicz D, Pourquié O. 1997. Avian *hairy* gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* 91:639–48

Pasteels J. 1937. Etudes sur la gastrulation des Vertébrés méroblastiques. III. Oiseaux. IV. Conclusions générales. *Arch. Biol.* 48:381–488

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Pourquié O. 2011. Vertebrate segmentation: from cyclic gene networks to scoliosis. *Cell* 145:650–63
Psychoyos D, Stern CD. 1996a. Fates and migratory routes of primitive streak cells in the chick embryo. *Development* 122:1523–34
Psychoyos D, Stern CD. 1996b. Restoration of the organizer after radical ablation of Hensen's node and the anterior primitive streak in the chick embryo. *Development* 122:3263–73
Rashbass P, Cooke LA, Herrmann BG, Beddington RS. 1991. A cell autonomous function of Brachyury in *T/T* embryonic stem cell chimaeras. *Nature* 353:348–51
Rifes P, Carvalho L, Lopes C, Andrade RP, Rodrigues G, et al. 2007. Redefining the role of ectoderm in somitogenesis: a player in the formation of the fibronectin matrix of presomitic mesoderm. *Development* 134:3155–65
Rifes P, Thorsteinsdóttir S. 2012. Extracellular matrix assembly and 3D organization during paraxial mesoderm development in the chick embryo. *Dev. Biol.* 368:370–81
Rong PM, Teillet MA, Ziller C, Le Douarin NM. 1992. The neural tube/notochord complex is necessary for vertebral but not limb and body wall striated muscle differentiation. *Development* 115:657–72
Rossant J, Zirngibl R, Cado D, Shago M, Giguère V. 1991. Expression of a retinoic acid response element-*hsplacZ* transgene defines specific domains of transcriptional activity during mouse embryogenesis. *Genes Dev.* 5:1333–44
Roszko I, Sawada A, Solnica-Krezel L. 2009. Regulation of convergence and extension movements during vertebrate gastrulation by the Wnt/PCP pathway. *Semin. Cell Dev. Biol.* 20:986–97
Saga Y. 2007. Segmental border is defined by the key transcription factor Mesp2, by means of the suppression of Notch activity. *Dev. Dyn.* 236:1450–55
Saga Y. 2012. The mechanism of somite formation in mice. *Curr. Opin. Genet. Dev.* 22:331–38
Saga Y, Hata N, Koseki H, Taketo MM. 1997. *Mesp2*: a novel mouse gene expressed in the presegmented mesoderm and essential for segmentation initiation. *Genes Dev.* 11:1827–39
Sakai Y, Meno C, Fujii H, Nishino J, Shiratori H, et al. 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.* 15:213–25
Sanders EJ, Khare MK, Ooi VC, Bellairs R. 1986. An experimental and morphological analysis of the tail bud mesenchyme of the chick embryo. *Anat. Embryol.* 174:179–85
Sandersius SA, Chuai M, Weijer CJ, Newman TJ. 2011. A 'chemotactic dipole' mechanism for large-scale vortex motion during primitive streak formation in the chick embryo. *Phys. Biol.* 8:045008
Sarrazin AF, Peel AD, Averof M. 2012. A segmentation clock with two-segment periodicity in insects. *Science* 336:338–41
Sausedo RA, Schoenwolf GC. 1993. Cell behaviors underlying notochord formation and extension in avian embryos: quantitative and immunocytochemical studies. *Anat. Rec.* 237:58–70
Sausedo RA, Schoenwolf GC. 1994. Quantitative analyses of cell behaviors underlying notochord formation and extension in mouse embryos. *Anat. Rec.* 239:103–12
Sawada A, Shinya M, Jiang Y-J, Kawakami A, Kuroiwa A, Takeda H. 2001. Fgf/MAPK signalling is a crucial positional cue in somite boundary formation. *Development* 128:4873–80
Schoenwolf GC. 1977. Tail (end) bud contributions to the posterior region of the chick embryo. *J. Exp. Zool.* 201:227–46
Schoenwolf GC. 1978. Effects of complete tail bud extirpation on early development of the posterior region of the chick embryo. *Anat. Rec.* 192:289–95
Schoenwolf GC. 1979a. Histological and ultrastructural observations of tail bud formation in the chick embryo. *Anat. Rec.* 193:131–47
Schoenwolf GC. 1979b. Observations on closure of the neuropores in the chick embryo. *Am. J. Anat.* 155:445–65
Schoenwolf GC, Alvarez IS. 1989. Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. *Development* 106:427–39
Schoenwolf GC, Alvarez IS. 1992. Role of cell rearrangement in axial morphogenesis. *Curr. Top. Dev. Biol.* 27:129–73
Schoenwolf GC, Chandler NB, Smith JL. 1985. Analysis of the origins and early fates of neural crest cells in caudal regions of avian embryos. *Dev. Biol.* 110:467–79

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Schoenwolf GC, Garcia-Martinez V, Dias MS. 1992. Mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dyn.* 193:235–48

Schroter C, Ares S, Morelli LG, Isakova A, Hens K, et al. 2012. Topology and dynamics of the zebrafish segmentation clock core circuit. *PLoS Biol.* 10:e1001364

Schroter C, Oates AC. 2010. Segment number and axial identity in a segmentation clock period mutant. *Curr. Biol.* 20:1254–58

Selleck MA, Stern CD. 1991. Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* 112:615–26

Selleck MA, Stern CD. 1992. Commitment of mesoderm cells in Hensen's node of the chick embryo to notochord and somite. *Development* 114:403–15

Serra-Picamal X, Conte V, Vincent R, Anon E, Tambe DT, et al. 2012. Mechanical waves during tissue expansion. *Nat. Phys.* 8:628–34

Shih J, Keller R. 1992a. Cell motility driving mediolateral intercalation in explants of *Xenopus laevis*. *Development* 116:901–14

Shih J, Keller R. 1992b. Patterns of cell motility in the organizer and dorsal mesoderm of *Xenopus laevis*. *Development* 116:915–30

Shum ASW, Poon LLM, Tang WWT, Koide T, Chan BWH, et al. 1999. Retinoic acid induces down-regulation of *Wnt-3a*, apoptosis and diversion of tail bud cells to a neural fate in the mouse embryo. *Mech. Dev.* 84:17–30

Skoglund P, Keller R. 2010. Integration of planar cell polarity and ECM signaling in elongation of the vertebrate body plan. *Curr. Opin. Cell Biol.* 22:589–96

Solnica-Krezel L, Sepich DS. 2012. Gastrulation: making and shaping germ layers. *Annu. Rev. Cell Dev. Biol.* 28:687–717

Sparrow DB, Chapman G, Smith AJ, Mattar MZ, Major JA, et al. 2012. A mechanism for gene-environment interaction in the etiology of congenital scoliosis. *Cell* 149:295–306

Spratt NT Jr. 1946. Formation of the primitive streak in the explanted chick blastoderm marked with carbon particles. *J. Exp. Zool.* 103:259–304

Spratt NT Jr. 1947. Regression and shortening of the primitive streak in the explanted chick blastoderm. *J. Exp. Zool.* 104:69–100

Sun X, Meyers EN, Lewandoski M, Martin GR. 1999. Targeted disruption of *Fgf8* causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev.* 13:1834–46

Sweetman D, Wagstaff L, Cooper O, Weijer C, Munsterberg A. 2008. The migration of paraxial and lateral plate mesoderm cells emerging from the late primitive streak is controlled by different Wnt signals. *BMC Dev. Biol.* 8:63

Szeto DP, Kimelman D. 2006. The regulation of mesodermal progenitor cell commitment to somitogenesis subdivides the zebrafish body musculature into distinct domains. *Genes Dev.* 20:1923–32

Tada M, Heisenberg CP. 2012. Convergent extension: using collective cell migration and cell intercalation to shape embryos. *Development* 139:3897–904

Takada S, Stark KL, Shea MJ, Vassileva G, McMahon JA, McMahon AP. 1994. *Wnt-3a* regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* 8:174–89

Tam PP. 1981. The control of somitogenesis in mouse embryos. *J. Embryol. Exp. Morphol.* 65(Suppl.): 103–28

Tam PP, Beddington RS. 1992. Establishment and organization of germ layers in the gastrulating mouse embryo. *Ciba Found. Symp.* 165:27–41

Tenin G, Wright D, Ferjentsik Z, Bone R, McGrew MJ, Maroto M. 2010. The chick somitogenesis oscillator is arrested before all paraxial mesoderm is segmented into somites. *BMC Dev. Biol.* 10:24

Trepat X, Fredberg JJ. 2011. Plithotaxis and emergent dynamics in collective cellular migration. *Trends Cell Biol.* 21:638–46

Trepat X, Wasserman MR, Angelini TE, Millet E, Weitz DA, et al. 2009. Physical forces during collective cell migration. *Nat. Phys.* 5:426–30

Trinkaus JP, Trinkaus M, Fink RD. 1992. On the convergent cell movements of gastrulation in *Fundulus*. *J. Exp. Zool.* 261:40–61

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Truong TV, Supatto W, Koos DS, Choi JM, Fraser SE. 2011. Deep and fast live imaging with two-photon scanned light-sheet microscopy. *Nat. Methods* 8:757–60

Tucker AS, Slack JM. 1995. Tail bud determination in the vertebrate embryo. *Curr. Biol.* 5:807–13

Tzouanacou E, Wegener A, Wymeersch FJ, Wilson V, Nicolas JF. 2009. Redefining the progression of lineage segregations during mammalian embryogenesis by clonal analysis. *Dev. Cell* 17:365–76

Uriu K, Morishita Y, Iwasa Y. 2010. Random cell movement promotes synchronization of the segmentation clock. *Proc. Natl. Acad. Sci. USA* 107:4979–84

Vasiev B, Balter A, Chaplain M, Glazier JA, Weijer CJ. 2010. Modeling gastrulation in the chick embryo: formation of the primitive streak. *PLoS ONE* 5:e10571

Voiculescu O, Bertocchini F, Wolpert L, Keller RE, Stern CD. 2007. The amniote primitive streak is defined by epithelial cell intercalation before gastrulation. *Nature* 449:1049–52

von Dassow M, Strother JA, Davidson LA. 2010. Surprisingly simple mechanical behavior of a complex embryonic tissue. *PLoS ONE* 5:1

Wei Y, Mikawa T. 2000. Formation of the avian primitive streak from spatially restricted blastoderm: evidence for polarized cell division in the elongating streak. *Development* 127:87–96

Wetzel R. 1929. Untersuchungen am Hühnchen. Die Entwicklung des Keims während der ersten beiden Bruttage. *Arch. EntwMech. Org.* 119:188–321

Williams M, Burdsal C, Periasamy A, Lewandoski M, Sutherland A. 2012. Mouse primitive streak forms in situ by initiation of epithelial to mesenchymal transition without migration of a cell population. *Dev. Dyn.* 241:270–83

Wilson V, Beddington R. 1997. Expression of T protein in the primitive streak is necessary and sufficient for posterior mesoderm movement and somite differentiation. *Dev. Biol.* 192:45–58

Wilson V, Manson L, Skarnes WC, Beddington RS. 1995. The *T* gene is necessary for normal mesodermal morphogenetic cell movements during gastrulation. *Development* 121:877–86

Wilson V, Olivera-Martinez I, Storey KG. 2009. Stem cells, signals and vertebrate body axis extension. *Development* 136:1591–604

Wilson V, Rashbass P, Beddington RS. 1993. Chimeric analysis of *T* (*Brachyury*) gene function. *Development* 117:1321–31

Yamaguchi TP, Takada S, Yoshikawa Y, Wu N, McMahon AP. 1999. *T* (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev.* 13:3185–90

Yamanaka Y, Tamplin OJ, Beckers A, Gossler A, Rossant J. 2007. Live imaging and genetic analysis of mouse notochord formation reveals regional morphogenetic mechanisms. *Dev. Cell* 13:884–96

Yanagawa N, Sakabe M, Sakata H, Yamagishi T, Nakajima Y. 2011. Nodal signal is required for morphogenetic movements of epiblast layer in the pre-streak chick blastoderm. *Dev. Growth Differ.* 53:366–77

Yang JT, Bader BL, Kreidberg JA, Ullman-Cullere M, Trevithick JE, Hynes RO. 1999. Overlapping and independent functions of fibronectin receptor integrins in early mesodermal development. *Dev. Biol.* 215:264–77

Yang JT, Rayburn H, Hynes RO. 1993. Embryonic mesodermal defects in α_5 integrin-deficient mice. *Development* 119:1093–105

Yang X, Chrisman H, Weijer CJ. 2008. PDGF signalling controls the migration of mesoderm cells during chick gastrulation by regulating N-cadherin expression. *Development* 135:3521–30

Yang X, Dormann D, Munsterberg AE, Weijer CJ. 2002. Cell movement patterns during gastrulation in the chick are controlled by positive and negative chemotaxis mediated by FGF4 and FGF8. *Dev. Cell* 3:425–37

Yen WW, Williams M, Periasamy A, Conaway M, Burdsal C, et al. 2009. PTK7 is essential for polarized cell motility and convergent extension during mouse gastrulation. *Development* 136:2039–48

Yin C, Kiskowski M, Pouille PA, Farge E, Solnica-Krezel L. 2008. Cooperation of polarized cell intercalations drives convergence and extension of presomitic mesoderm during zebrafish gastrulation. *J. Cell Biol.* 180:221–32

Young T, Rowland JE, van de Ven C, Bialecka M, Novoa A, et al. 2009. *Cdx* and *Hox* genes differentially regulate posterior axial growth in mammalian embryos. *Dev. Cell* 17:516–26

Yue Q, Wagstaff L, Yang X, Weijer C, Munsterberg A. 2008. Wnt3a-mediated chemorepulsion controls movement patterns of cardiac progenitors and requires RhoA function. *Development* 135:1029–37

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Zamir EA, Czirok A, Cui C, Little CD, Rongish BJ. 2006. Mesodermal cell displacements during avian gastrulation are due to both individual cell-autonomous and convective tissue movements. *Proc. Natl. Acad. Sci. USA* 103:19806–11

Zamir EA, Rongish BJ, Little CD. 2008. The ECM moves during primitive streak formation—computation of ECM versus cellular motion. *PLoS Biol.* 6:e247

Zhou J, Kim HY, Davidson LA. 2009. Actomyosin stiffens the vertebrate embryo during crucial stages of elongation and neural tube closure. *Development* 136:677–88

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Annual Review of Cell and Developmental Biology

Volume 29, 2013

Contents

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Annu. Rev. Cell Dev. Biol. 2013.29:1-26. Downloaded from www.annualreviews.org
by Brandeis University on 10/07/13. For personal use only.

Indexes

Errata

An online log of corrections to *Annual Review of Cell and Developmental Biology* articles may be found at http://cellbio.annualreviews.org/errata.shtml

Cellular and Molecular Life Sciences (2019) 76:89–98
https://doi.org/10.1007/s00018-018-2927-4

REVIEW

Dynamics and mechanisms of posterior axis elongation in the vertebrate embryo

Bertrand Bénazéraf[1]

Received: 3 July 2018 / Revised: 24 September 2018 / Accepted: 25 September 2018 / Published online: 3 October 2018
© Springer Nature Switzerland AG 2018

Abstract

During development, the vertebrate embryo undergoes significant morphological changes which lead to its future body form and functioning organs. One of these noticeable changes is the extension of the body shape along the antero-posterior (A–P) axis. This A–P extension, while taking place in multiple embryonic tissues of the vertebrate body, involves the same basic cellular behaviors: cell proliferation, cell migration (of new progenitors from a posterior stem zone), and cell rearrangements. However, the nature and the relative contribution of these different cellular behaviors to A–P extension appear to vary depending upon the tissue in which they take place and on the stage of embryonic development. By focusing on what is known in the neural and mesodermal tissues of the bird embryo, I review the influences of cellular behaviors in posterior tissue extension. In this context, I discuss how changes in distinct cell behaviors can be coordinated at the tissue level (and between tissues) to synergize, build, and elongate the posterior part of the embryonic body. This multi-tissue framework does not only concern axis elongation, as it could also be generalized to morphogenesis of any developing organs.

Keywords Live imaging · Bird embryo · Axis elongation · Proliferation · PSM · Multi-tissue · Tissue deformations · Morphogenesis

Introduction

Vertebrate embryos develop according to an anterior–posterior (A–P) sequence of events. The most anterior structures, i.e., the head region, are formed first, followed by the successive formation of more posterior structures: cervical, thoracic, trunk, and tail regions. This sequence of events takes place concomitantly with the A–P extension of the different tissues (i.e., the neural tube, the paraxial mesoderm, and the notochord) of the embryonic body. Distinct cellular behaviors such as cell proliferation, cell migration, and cell rearrangements are known to be involved in tissue elongation. Early in development, the vertebrate embryo transforms from a rounded shape (either spherical or discoidal) into an oblong shape. It is generally considered that this shape change is due to convergent extension of the different tissues of the embryo. During convergent extension, embryonic tissue converges (or narrows) along one axis and extends (or elongates) along a perpendicular axis by cellular movement. One of these rearrangements is called mediolateral intercalation, a process in which cells orient their main axis perpendicular to the A–P axis of the embryo and intercalate with one another. Convergent extension by cellular intercalation has been described in detail in frog embryos [1, 2] and is conserved in different embryonic territories among vertebrate embryos (for review [3]). The early phase of vertebrate embryo axial extension is thought to be mainly driven by convergence. The following phase, which gives rise to the posterior part of the body (i.e., posterior elongation), requires the addition of cells from a posterior growth zone. Therefore, the elongation of the posterior tissues of the vertebrate embryo depends mainly on growth and also on precursor's migration/rearrangements.

During posterior axis elongation, the spatial organization of embryonic tissues is stereotypical among vertebrate embryos and can be schematized as a general framework (Fig. 1). The neural tube and the notochord are located axially. The paraxial mesoderm is located on each side of the neural tube and the lateral mesoderm located even more laterally. These tissues are sandwiched in between two

✉ Bertrand Bénazéraf
bertrand.benazeraf@univ-tlse3.fr

[1] Centre de Biologie du Développement (CBD), Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, Toulouse, France

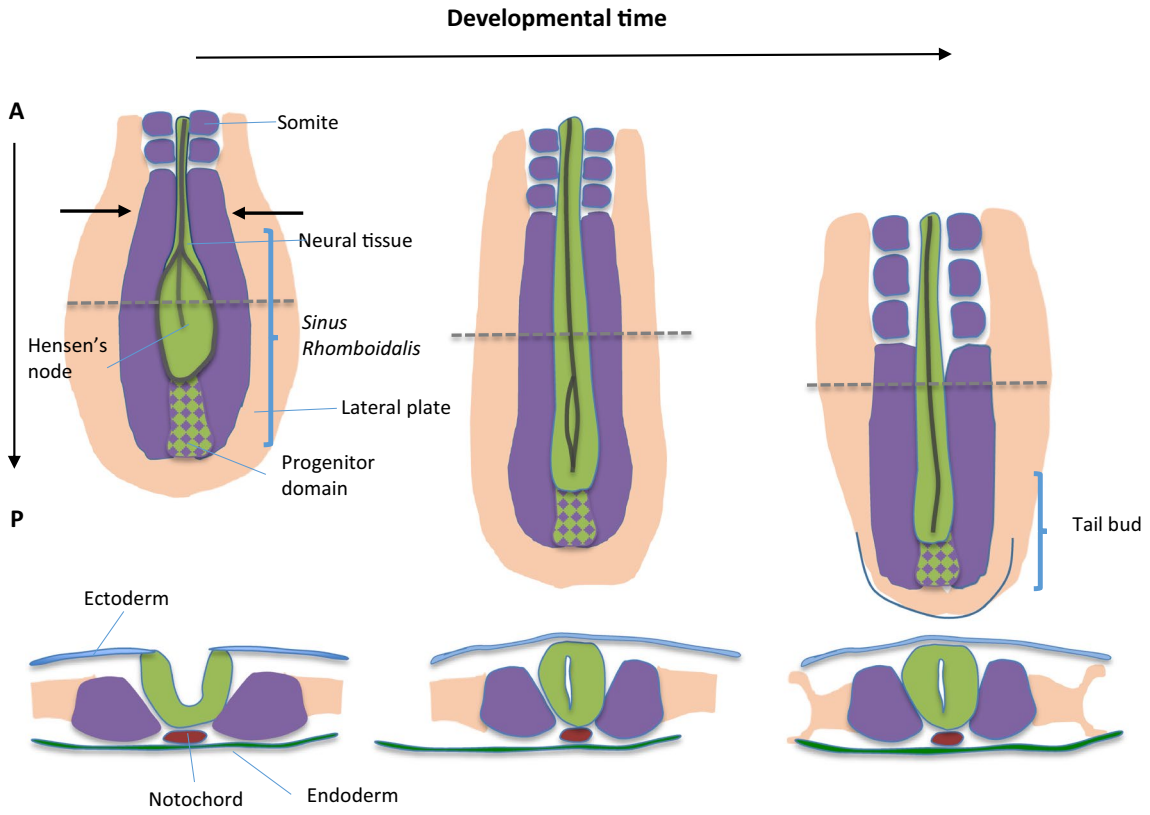


Fig. 1 Schema of posterior tissue morphologies and organization during posterior elongation. Dorsal view (top) and transverse sections (bottom) of the posterior part of higher vertebrate embryo during different times of axis elongation (from left to right: equivalent to stage 9HH, 11HH, and 15HH in chicken embryo). Paraxial mesoderm is in purple, neural tissue in green, notochord in red, lateral mesoderm in pink, ectoderm in blue, and endoderm in dark green, progenitor domain in purple and green. Ectoderm is not represented on the dorsal views. Note that tissues have a higher width posteriorly than anteriorly in the early phase (black arrows), whereas they are becoming more straight during later phases. General tissue organization remains conserved throughout the stages (transverse section); *A* anterior, *P* Posterior

epithelial tissues: the ectoderm dorsally and the endoderm ventrally. In its most posterior part, the embryonic body is terminated by a group of posterior progenitors, a stem zone that becomes the tail bud during the latest phases of axis extension [4, 5]. Signaling pathways active in the most posterior part of the embryo, such as Wnt and FGF, have been identified to maintain the posterior progenitors in an undifferentiated state [6–9]. While leaving the posterior region, neural and paraxial mesoderm cells that are progressively moving away from the influence of these signaling pathways acquire a positional identity and differentiate. Notably, neuronal diversity will be generated in the neural tube, and the paraxial mesoderm will become periodically segmented into somites (the metameric units that will pattern the spine) (for reviews [10, 11]). Therefore, posterior axial morphogenesis is highly coordinated with tissue patterning, specification, and differentiation to ensure the laying down of precursors that will form the future functioning organs.

What do we know about the cellular behavior allowing for the elongation of the different tissues composing the posterior part of the embryo? What are their dynamics? What is the nature of the mechanical forces implicated in tissue deformation and elongation? How are elongation and growth coordinated between different tissues? While lineage analysis has provided some clues in the past, the recent development of live imaging techniques, transgenic animals, and genetic experiments has allowed researchers to re-explore these questions in more depth. Here, I will review the current knowledge concerning the dynamics and the mechanisms of posterior tissue elongation. I will mainly focus on examples taken from bird embryo development. This embryonic model, due to its accessibility, flat shape, and progressive maturation along the A–P axis, has been a key system to study posterior axis extension. First, I will consider each posterior embryonic region (posterior progenitor domain, notochord, neural tube, and paraxial mesoderm) by describing their formation. For each region, I will review the main cellular behaviors contributing to posterior axial elongation that have been analyzed by live imaging. Then, I will elaborate on a blueprint of how these different

embryonic regions could interact to ensure the formation of the posterior part of the body axis.

Origins of axial and paraxial tissues: the progenitor region and tail bud

During the beginning of posterior axis elongation, the progenitor region giving rise to axial and paraxial tissues spans an area located around Hensen's node called the *sinus rhomboidalis* at stage HH 8–9 in chicken (Fig. 1) [12]. The fate of cells from this area (and its equivalent in younger stages) has been mapped extensively. Hensen's node is a group of cells located at the most anterior part of the primitive streak that move posteriorly during axis elongation. During its caudal movement, Hensen's node lays down precursors of axial tissues: the notochord, the dorsal endoderm, and, according to some lineage studies, the floor plate of the neural tube [13–15]. The dorsal cells located proximal to Hensen's node, posteriorly (neural/streak border) and laterally stay positioned dorsally during axis elongation and give rise to the neural tissue [16, 17]. Cells located posteriorly to Hensen's node in the primitive streak undergo gastrulation movements to form mesodermal tissues. The precursors located in the more anterior streak are migrating in the paraxial mesoderm, while the precursors located more posteriorly will migrate into the lateral mesoderm [15, 18, 19]. Their antero-posterior position in the streak will, therefore, be translated into medio-lateral position in the maturating tissues. During axis extension, some progenitors remain residents of the *sinus rhomboidalis,* as they will be integrated into a structure called the tail bud during later phases of axis elongation [20]. The tail bud forms a mass of apparently undifferentiated mesenchymal cells and is located at the posterior tip of the embryo. Based on their origin, location, and potential, different regions of the tail bud have been distinguished. The Chordo-Neural Hinge (CNH), which derives from the primitive streak and Hensen's node, is located in the internal region of the tail bud. This region gives rise to notochord, neural tube, and paraxial mesoderm cells [20–22]. Cells from more dorsal regions of the tail bud give rise to neural tube, whereas cells from the lateral wall of the tail bud participate in the production of the paraxial mesoderm [22]. It has been shown that gastrulating-like movements continue in the tail bud until stage HH13 with cells migrating from the posterior axial region to form the paraxial mesoderm [23].

Because of its location at the caudal end of the embryonic body, the progenitor region has been at the center of attention in the field of posterior axis extension. Live imaging combined with ex ovo culture and electroporation techniques enable direct observation of cell movements within the posterior structures of the bird embryo [24–26]. These techniques allow the monitoring of both the caudal-ward movements of resident axial progenitor cells and the exit of progenitors to the paraxial mesoderm or the neural tube. Furthermore, they have allowed for deciphering different molecular mechanisms regulating the migration of cells out of the progenitor region. For instance, FGF8 signaling, active in the most posterior region of the embryo [7, 9], is implicated in the migration of mesoderm progenitor cells away from the streak [27]. FGF signaling has an opposite effect on neural precursors, where its activity is needed to maintain neural precursors in the regressing tail bud [28]. Wnt3a and Wnt5a are also involved in the migration and segregation of mesoderm progenitors into paraxial and lateral plate mesoderm tissues of the chicken embryo [29]. Genetic experiments in mice confirm the implication of FGF and WNT signaling pathways in the ingression and migration of mesodermal precursors [6, 30]. Finally, the decision for a precursor cell to leave the primitive streak for the paraxial mesoderm in the chicken embryo has been shown to depend on the activity of *Hox* genes, whose expression is progressively activated in the posterior region of the embryo in a collinear manner [31, 32]. Indeed, premature expression of "posterior" Hox genes delays gastrulation movements and shifts mesodermal cell final location posteriorly. Interestingly, a similar mechanism-linking Hox gene ant cell location along the A/P axis has been proposed to be at work during Xenopus axis elongation, suggesting a conservation of this process among vertebrates [33]. These experiments provide a general mechanism to explain how the timing of *Hox* genes expression could be translated into cell positional information along the AP axis. Interestingly, it also opens up the possibility that Hox functions on morphogenesis could be accompanying their more deeply explored functions on axial patterning (for review on this field [34, 35]).

Recently, many efforts have been made to better understand the mechanisms that maintain a progenitor pool in the tail bud to produce tissue for the entire length of the body axis. The territory surrounding the anterior part of the primitive streak and, later on, the CNH (or equivalent regions in other vertebrate) has been shown to contain cells capable of giving rise to both the paraxial mesoderm and the neuro-ectoderm cells [36–38]. These cells, because they can self-renew and give progeny, seem particularly relevant to the process of tissue production during posterior elongation [22, 38]. Retrospective lineage tracing in mouse embryo has shown that a single progenitor in the tail bud can give rise to both neural and mesoderm cells demonstrating the existence of a neuro-mesodermal progenitor (NMP) lineage [39]. These data suggest that the regulation of the NMP specification can influence axial extension via differential production of paraxial mesoderm and neural tissues. NMPs co-express the transcription factors Sox2 (pan-neural factor) and T/brachyury (an essential gene in mesodermal development), revealing a molecular signature suggestive of a

mixed identity [40–43]. Gene regulatory networks involving Sox2 and T/brachyury, Cdx, OCT4, as well as signaling and metabolic pathways have been proposed to regulate the balance of self-renewal and specification of NMPs [44–49]. The relative contribution of NMPs vs. other neural only and mesoderm only progenitors in the building of the axis remains, however, to be precisely quantified. NMPs seem to integrate signaling pathways which influence both their specification and migration. For instance, the FGF pathway promotes specification of NMPS toward mesoderm while maintaining neural precursors in the stem zone [28, 42, 50, 51]. Understanding how these progenitors respond differentially to signaling pathways might be particularly meaningful in understanding how specification and morphogenesis can be regulated and coordinated within the tail bud to produce the different tissues of the body.

Axial progenitors located within the most posterior region of the embryo are at the "forefront" of posterior axis elongation by their potential to produce progeny in different tissues. However, the mechanical causes of their caudal movements are still poorly understood. These progenitors, which are epithelial or densely packed mesenchymal cells, do not show active signs of cell migration themselves (such as oriented lamellipodia); instead, their movements seem intimately linked with the production and the movements of cells belonging to neighboring elongating tissues.

Axial tissues and axial elongation

The notochord is a rod-like structure composed of axial mesodermal cells that extend during antero-posterior body axis elongation. In fish and frogs, the notochord is known to undergo convergence extension at an early phase of development and to later vacuolize, building up the pressure inside the tissue and elongating the structure [52–55]. In contrast, the process of notochord elongation in bird and mouse is proposed to be the result of several cell behaviors: cell rearrangement (i.e., intercalation), cell division (proliferation), and addition of new cells at the posterior end of the notochord by the Hensen's node and later by the CNH [56–58]. The relative importance of each of these cell behaviors in the elongation of the A–P axis remains to be precisely determined.

Located dorsal to the notochord lies another axial structure from ectodermal origin: the neural tissue. The neural tube is a pseudo-stratified epithelium composed of proliferating neural precursors. The anterior part of the central nervous system is formed by bending of the neural plate into the neural tube. This phenomenon, called primary neurulation, is occurring during the early phase of posterior axis elongation (for review [59]). Subsequent neurulation that will form the most posterior part of the central nervous system is called secondary neurulation. In this process, a group of mesenchymal cells located internally in the tail bud progressively undergoes epithelialization and organizes into a neuro-epithelium by cavitation [60, 61]. Interestingly, there is no sudden switch between primary and secondary neurulations. During a transition period called junctional neurulation and corresponding to the formation of the thoracic level in chicken or human embryo, neural tissue is formed by a mixed process of elevation and folding of the neural folds with local ingression and accretion [62].

During primary neurulation, the elongation of the neural plate occurs in parallel to its bending and is, therefore, accompanied by a drastic change in tissue shape. These shape changes involve convergent extension of the tissue and cell intercalation [63]. Convergent extension has been documented by time-lapse imaging in the chick embryo. As demonstrated in the mouse embryo, it involves the Wnt/PCP pathway [64, 65]. Time-lapse imaging of avian neural tube closure during the transition phase of elongation has shown the persistence of convergence extension movements at the tissue level but with only few cell intercalations following cell division [66]. Another mechanism that is involved in the lengthening of the tissue is the orientation of cell division. When they divide, neural cells tend to orient their axis parallel to the A–P axis. Therefore, divisions directly contribute to the lengthening of the tissue [67, 68]. Finally, during the late phase of axis extension, convergent movements of the neural tissue are becoming less important, whereas lineage analysis and imaging of neural progenitors have shown that cells from the posterior neural stem zone (later the tail bud) are leaving the stem zone to be integrated into the neural tube [28, 66, 69, 70]. Altogether, these data suggest that elongation of the neural tube relies preferentially on convergence during early development and on growth and accretion of neural precursors from the neural stem zone during late stages of axial extension.

Paraxial tissue elongation

The paraxial mesoderm consists of two columns of tissue located on both sides of the neural tube. In its caudal part, the paraxial mesoderm is unsegmented and called the presomitic mesoderm (PSM). During axis elongation, the PSM elongates posteriorly, while its anterior region sub-divides periodically into somites (the metameric units that will pattern the spine) (for review [11, 71]).

PSM cell rearrangements are known to trigger tissue elongation. During the beginning of posterior axial elongation, cells migrate from the progenitor region into the posterior part of the PSM with a medio-lateral movement. Simultaneously, cells located more anteriorly in the PSM tissue converge toward the midline. These two different movements

correlate with the global shape of the PSM at these stages, large posteriorly and thin anteriorly (Fig. 1). Two types of FGF signaling drive these movements. FGF8, secreted at the level of the primitive streak, acts as a chemo-repulsive signal, whereas FGF4, secreted by the notochord, attracts PSM cells toward the midline anteriorly [27]. Convergence in the anterior PSM is a conserved mechanism among vertebrates that have been observed in zebrafish and mouse embryos [72, 73]. After stage HH10–11, while convergence is progressively diminishing, other types of cellular movements have been observed in the caudal PSM of the chicken embryo. In particular, within the expanding caudal PSM tissue, cells migrate extensively with no apparent directionality, exchanging neighbors frequently [74–76] and, therefore, explaining the spreading of PSM cells previously observed by single-cell lineage analysis (Fig. 2) [77]. Tracking of these movements and comparing them to the deformation of the tissue highlighted the existence of a gradient of non-directional motility within the PSM tissue [74]. Cells in the caudal PSM spread more than cells located anteriorly. This posterior graded "diffusion" is under the influence of FGF8 signaling coming from the tail bud. By itself, this gradient of motion can explain how PSM cells re-arrange to elongate the PSM tissue without extreme variations in tissue width. An equivalent gradient of non-directional movements has been observed in zebrafish embryos, although the zone of high non-directional cellular motility seems to be located more axially, where it could also play a role in segregating cells on both sides of the embryo [78, 79]. However, cell movements cannot alone explain PSM tissue elongation, as the tissue volume is expanding with constant cell density and few convergence movements. Over short time scales, cell rearrangements appear to induce tissue elongation more than the addition of new cells [74]. However, over longer time scales (several hours), increased numbers of paraxial mesoderm cells are becoming a critical part of tissue elongation [80]. This increase in cell number is the result of two different mechanisms: the PSM is actively proliferating, and new cells from the progenitor zone are added in the posterior end of the PSM. As seen previously, the gradient of non-directional migration induces a posterior bias in the direction of cell spreading within the PSM tissue. Because this mode of elongation comprises a significant increase in the number of total cells, it does not involve a drastic diminution

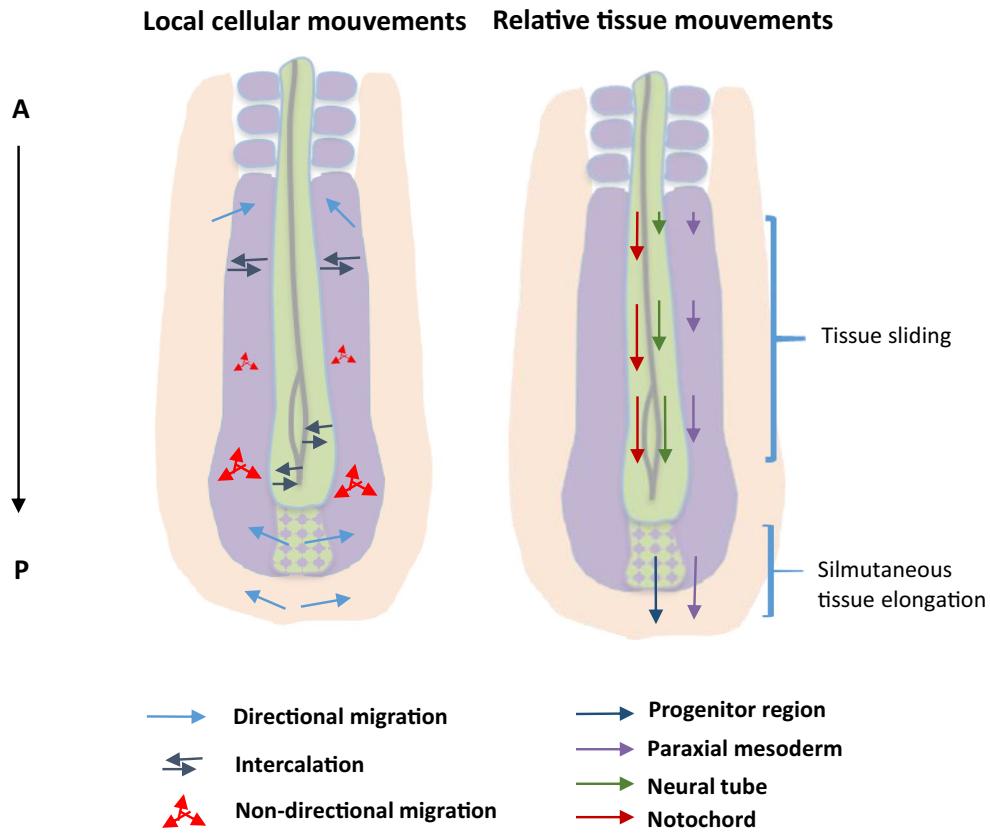


Fig. 2 Schema of the main cell and tissue movements during posterior elongation. Dorsal view of a schematized vertebrate embryo during posterior axial elongation. Local cellular movements on the left schema, relative tissue movements on the right schema. Note that tissue movements are shown relative to the last formed somite. Note that for a matter of simplicity, the elongation phase represented is a transition phase in which early and late phase movements are both present (equivalent of stage 10–11HH in chicken embryo)

of the width of the tissue to achieve elongation (as observed in convergence extension movements). Therefore, graded non-directional migration and an increase in cell number might prevent convergence while creating the cellular rearrangements and physical forces which are both necessary to elongate the tissue. These data suggest an increasing role of growth and non-directional cellular motion vs. convergence movements during PSM tissue elongation over the course of development.

Embryo axis elongation as a multi-tissue system

Most tissue morphogenesis analyses focus only on cellular behaviors taking place within one tissue, paying little attention to interactions with neighboring tissues. Axial elongation in vertebrates is an excellent paradigm to assess how different tissues can interact to promote a global morphogenetic behavior. Indeed, during this process tissues that share some common lineage in the tail bud, deform simultaneously while being in close physical proximity. Therefore, even though all posterior tissues display cellular behaviors that could by themselves account for tissue elongation, it is likely that some tissues influence or regulate behaviors and deformations of their neighboring tissues. When considering axial elongation as a multi-tissue problem, it is interesting to note that posterior tissues are elongating at different rates and sliding past each other, a characteristic that is visible in different species [52, 81, 82]. Time-lapse imaging of transgenic quail embryos, in which all nuclei are fluorescent [83], permits these differential tissue movements along the A–P axis to be visualized and quantitated. At the level of the last formed somite, the neural tube and the notochord move posteriorly faster than the paraxial mesoderm. In the most posterior region of the embryo, tissue sliding diminishes until all tissues move together (Fig. 2) [80]. The mechanisms leading to the complexity of these tissue dynamics are not well understood. In zebrafish, it has been proposed that regulation of cell adhesion and coherence in cellular movements is driving the choreography of corresponding tissue flows [78]. Another non-exclusive possibility is that some tissues might be more active than others in controlling multi-tissue kinetics and axis extension.

Deletion experiments, although being very invasive and susceptible to misinterpretations due to regulative properties of the embryo, point out differences in the importance of certain embryonic regions in elongation. For instance, experiments of deletion of the anterior part of the node in chick embryos lead to elongated embryos without a notochord, suggesting a non-essential role of these structures in axis extension [84–87]. On the contrary, experiments of deletion of the region at the interface between the posterior node and anterior primitive streak (that contain PSM progenitors) have been shown to result in axis truncation [87]. Furthermore, analyses of time-lapse movies have shown that deletion of caudal paraxial mesoderm more drastically affects axis elongation than deletion of adjacent tissues [74, 88]. Data coming from mouse genetics also highlight a crucial role of the paraxial mesoderm in posterior body axial elongation as numerous mouse mutants for mesoderm specification and migration factors display posterior axial truncation [47, 89–91]. The importance of the paraxial mesoderm in embryonic axis extension correlates with the fact that this tissue expands and proliferates faster than other posterior tissues in bird embryos [80]. Paraxial mesoderm growth/posterior expansion is, therefore, a good candidate for having a global influence on the movement and deformations of surrounding tissues including the regression of the tail bud itself. From this point of view, the tail bud can be seen as a stem zone that is displaced caudally by the forces produced from its daughter's cells (PSM), which are actively amplified by proliferation and rearranging.

How many forces applied by neighboring tissues on one another also affect axis elongation? Posterior tissues are separated by extracellular matrix (ECM) including fibronectin and fibrillin [92]. ECM is organized in a highly dynamic network embedding tissues and undergoing the same global deformations [74, 93–95]. Interestingly, cellular movements are locally decoupled from the ECM movements in some embryonic regions suggesting that cell migration can drive tissue deformations and ECM kinetics [74, 93]. Data obtained in several vertebrate species suggest that the ECM network is an integral part in the coordination of both the kinetics between tissues and the whole axial elongation process. Mouse mutants for integrin and fibronectin display axis truncation among other posterior phenotypes [96–98]. A study in zebrafish shows that disruption between cells and fibronectin at the notochord paraxial mesoderm interface leads to an extension defect and undulation of the notochord tissues. These results suggest that ECM is responsible for an inter-tissue mechanical link during zebrafish axis elongation [99]. Collectively, these data suggest that inter-tissue forces could be an active player during axial elongation. The ECM that separates tissues could, in that context, play a crucial role in the regulation of these forces.

Concluding remarks and open questions

Axial elongation is an essential process in vertebrate embryonic development. This morphogenetic process can be deconstructed in space and time, as it takes place in different tissues and at distinct developmental stages. The elongation of the different tissues of the vertebrate posterior embryonic body relies on the same basic cellular mechanisms: cell

rearrangements, cell proliferation, and influx of cells from neighboring tissues (i.e., tail bud). However, the relative weight of these variables in generating tissue elongation is not always known and, when known, appears to differ from tissue to tissue. In the future, quantification of the effect of these variables on the elongation of the different tissues will be crucial for a deeper understanding of axis elongation. Neuro-mesodermal and other types of precursors located in the posterior stem zone are essential in fueling the growth of tissues that compose the body axis. Testing the relationships between their fate decisions and cellular behavior (migration and proliferation) will allow for a better understanding of their role in tissue elongation. It will require the combination of functional experiments disrupting their specification and behavior and time-lapse experiments to analyze consequences in these cells and their progeny.

Some tissues, such as the caudal PSM, seem to play a prominent role over other tissues in vertebrate posterior axis elongation. Measuring forces and biophysical properties of this tissue compared to other tissues are necessary next steps to understanding how tissues can trigger their own elongation and influence the elongation of others. Recent development of techniques allowing measuring forces and biophysical tissue properties during axis elongation are, therefore, particularly relevant to understand how intra- and inter-tissue mechanics elicit axial elongation in vertebrates [100–102].

Several mechanisms that contribute to axial elongation seem conserved between different phyla of vertebrate embryos. For instance, convergence extension in the early phase of elongation or the existence of neuro-mesodermal precursors participating in the formation of the posterior structures of the embryo might be conserved between fish, birds, and mammals. Interestingly, the dynamics of these processes can differ between vertebrate embryos. For instance, while growth is playing a central role in the formation of the posterior body in mouse or fishes having a long body type (i.e., dogfish), it is not the case for zebrafish embryos (which have a shorter body) [82]. These data highlight the fact that the regulation of cellular behavior dynamics can be a key mechanism in generating body-type diversity among vertebrate embryos. In the future, imaging of different species and inter-species comparison will be crucial in understanding how regulation of axis elongation mechanism can lead to body shape diversity across evolution.

During the revision process of this review a research article has been published that is very relevant to understand the physical forces driving axis elongation [103]. In this article the authors measure mechanical properties of the zebrafish paraxial mesoderm and identify a fluid-to-solid jamming transition in this tissue. They propose that rigidification of the anterior presomitic mesoderm physically supports posterior tissue remodeling to drive body axis elongation posteriorly.

Acknowledgements The author thanks Rusty Lansford, David Huss, Cathy Soula, Eric Theveneau, Ben Steventon, Daniela Roellig and Octavian Voiculescu for reading and giving critical comments on the manuscript.

References

1. Keller RE, Danilchik M, Gimlich R, Shih J (1985) The function and mechanism of convergent extension during gastrulation of Xenopus laevis. J Embryol Exp Morphol 89(Suppl):185–209
2. Keller R, Davidson L, Edlund A et al (2000) Mechanisms of convergence and extension by cell intercalation. Philos Trans R Soc Lond B Biol Sci 355:897–922. https://doi.org/10.1098/rstb.2000.0626
3. Shindo A (2018) Models of convergent extension during morphogenesis. Wiley Interdiscip Rev Dev Biol. https://doi.org/10.1002/wdev.293
4. Beck CW (2015) Development of the vertebrate tailbud. Wiley Interdiscip Rev Dev Biol 4:33–44. https://doi.org/10.1002/wdev.163
5. Griffith CM, Wiley MJ, Sanders EJ (1992) The vertebrate tail bud: three germ layers from one tissue. Anat Embryol (Berl) 185:101–113
6. Takada S, Stark KL, Shea MJ et al (1994) Wnt-3a regulates somite and tailbud formation in the mouse embryo. Genes Dev 8:174–189. https://doi.org/10.1101/gad.8.2.174
7. Bertrand N, Médevielle F, Pituello F (2000) FGF signalling controls the timing of Pax6 activation in the neural tube. Development 127:4837–4843
8. Diez del Corral R, Olivera-Martinez I, Goriely A et al (2003) Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. Neuron 40:65–79
9. Dubrulle J, McGrew MJ, Pourquié O (2001) FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. Cell 106:219–232
10. Wilson L, Maden M (2005) The mechanisms of dorsoventral patterning in the vertebrate neural tube. Dev Biol 282:1–13. https://doi.org/10.1016/j.ydbio.2005.02.027
11. Hubaud A, Pourquié O (2014) Signalling dynamics in vertebrate segmentation. Nat Rev Mol Cell Biol 15:709–721. https://doi.org/10.1038/nrm3891
12. Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morphol 88:49–92. https://doi.org/10.1002/jmor.1050880104
13. Patten I, Kulesa P, Shen MM et al (2003) Distinct modes of floor plate induction in the chick embryo. Development 130:4809–4821. https://doi.org/10.1242/dev.00694
14. Catala M, Teillet MA, De Robertis EM, Le Douarin ML (1996) A spinal cord fate map in the avian embryo: while regressing, Hensen's node lays down the notochord and floor plate thus joining the spinal cord lateral walls. Development 122:2599–2610
15. Selleck MA, Stern CD (1991) Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. Development 112:615–626
16. Hatada Y, Stern CD (1994) A fate map of the epiblast of the early chick embryo. Development 120:2879–2889
17. Brown JM, Storey KG (2000) A region of the vertebrate neural plate in which neighbouring cells can adopt neural or epidermal fates. Curr Biol 10:869–872

18. Iimura T, Yang X, Weijer CJ, Pourquié O (2007) Dual mode of paraxial mesoderm formation during chick gastrulation. Proc Natl Acad Sci USA 104:2744–2749. https://doi.org/10.1073/pnas.0610997104
19. Psychoyos D, Stern CD (1996) Fates and migratory routes of primitive streak cells in the chick embryo. Development 122:1523–1534
20. Catala M, Teillet MA, Le Douarin NM (1995) Organization and development of the tail bud analyzed with the quail-chick chimaera system. Mech Dev 51:51–65
21. Cambray N, Wilson V (2007) Two distinct sources for a population of maturing axial progenitors. Development 134:2829–2840. https://doi.org/10.1242/dev.02877
22. McGrew MJ, Sherman A, Lillico SG et al (2008) Localised axial progenitor cell populations in the avian tail bud are not committed to a posterior Hox identity. Development 135:2289–2299. https://doi.org/10.1242/dev.022020
23. Knezevic V, De Santo R, Mackem S (1998) Continuing organizer function during chick tail development. Development 125:1791–1801
24. Iimura T, Pourquié O (2008) Manipulation and electroporation of the avian segmental plate and somites in vitro. Methods Cell Biol 87:257–270. https://doi.org/10.1016/S0091-679X(08)00213-6
25. Rupp PA, Rongish BJ, Czirok A, Little CD (2003) Culturing of avian embryos for time-lapse imaging. Biotechniques 34:274–278
26. Chapman SC, Collignon J, Schoenwolf GC, Lumsden A (2001) Improved method for chick whole-embryo culture using a filter paper carrier. Dev Dyn 220:284–289. https://doi.org/10.1002/1097-0177(20010301)220:3%3c284:AID-DVDY1102%3e3.0.CO;2-5
27. Yang X, Dormann D, Münsterberg AE, Weijer CJ (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
28. Mathis L, Kulesa PM, Fraser SE (2001) FGF receptor signalling is required to maintain neural progenitors during Hensen's node progression. Nat Cell Biol 3:559–566. https://doi.org/10.1038/35078535
29. Sweetman D, Wagstaff L, Cooper O et al (2008) The migration of paraxial and lateral plate mesoderm cells emerging from the late primitive streak is controlled by different Wnt signals. BMC Dev Biol 8:63. https://doi.org/10.1186/1471-213X-8-63
30. Ciruna B, Rossant J (2001) FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. Dev Cell 1:37–49. https://doi.org/10.1016/S1534-5807(01)00017-X
31. Iimura T, Pourquié O (2006) Collinear activation of Hoxb genes during gastrulation is linked to mesoderm cell ingression. Nature 442:568–571. https://doi.org/10.1038/nature04838
32. Denans N, Iimura T, Pourquié O (2015) Hox genes control vertebrate body elongation by collinear Wnt repression. Elife. https://doi.org/10.7554/eLife.04379
33. Wacker SA, McNulty CL, Durston AJ (2004) The initiation of Hox gene expression in Xenopus laevis is controlled by Brachyury and BMP-4. Dev Biol 266:123–137. https://doi.org/10.1016/j.ydbio.2003.10.011
34. Mallo M, Wellik DM, Deschamps J (2010) Hox genes and regional patterning of the vertebrate body plan. Dev Biol 344:7–15. https://doi.org/10.1016/j.ydbio.2010.04.024
35. Deschamps J, Duboule D (2017) Embryonic timing, axial stem cells, chromatin dynamics, and the Hox clock. Genes Dev 31:1406–1416. https://doi.org/10.1101/gad.303123.117
36. Brown JM, Storey KG (2000) A region of the vertebrate neural plate in which neighbouring cells can adopt neural or epidermal fates. Curr Biol 10:869–872
37. Martin BL, Kimelman D (2012) Canonical Wnt signaling dynamically controls multiple stem cell fate decisions during vertebrate body formation. Dev Cell 22:223–232. https://doi.org/10.1016/j.devcel.2011.11.001
38. Cambray N, Wilson V (2002) Axial progenitors with extensive potency are localised to the mouse chordoneural hinge. Development 129:4855–4866
39. Tzouanacou E, Wegener A, Wymeersch FJ et al (2009) Redefining the progression of lineage segregations during mammalian embryogenesis by clonal analysis. Dev Cell 17:365–376. https://doi.org/10.1016/j.devcel.2009.08.002
40. Beddington RS, Rashbass P, Wilson V (1992) Brachyury–a gene affecting mouse gastrulation and early organogenesis. Development 116:157–165
41. Graham V, Khudyakov J, Ellis P, Pevny L (2003) SOX2 functions to maintain neural progenitor identity. Neuron 39:749–765. https://doi.org/10.1016/S0896-6273(03)00497-5
42. Olivera-Martinez I, Harada H, Halley PA, Storey KG (2012) Loss of FGF-dependent mesoderm identity and rise of endogenous retinoid signalling determine cessation of body axis elongation. PLoS Biol 10:e1001415. https://doi.org/10.1371/journal.pbio.1001415
43. Wymeersch FJ, Huang Y, Blin G et al (2016) Position-dependent plasticity of distinct progenitor types in the primitive streak. Elife 5:e10042. https://doi.org/10.7554/eLife.10042
44. Aires R, Jurberg AD, Leal F et al (2016) Oct4 is a key regulator of vertebrate trunk length diversity. Dev Cell 38:262–274. https://doi.org/10.1016/j.devcel.2016.06.021
45. Gouti M, Delile J, Stamataki D et al (2017) A gene regulatory network balances neural and mesoderm specification during vertebrate trunk development. Dev Cell 41(243–261):e7. https://doi.org/10.1016/j.devcel.2017.04.002
46. Koch F, Scholze M, Wittler L et al (2017) Antagonistic activities of Sox2 and brachyury control the fate choice of neuro-mesodermal progenitors. Dev Cell 42(514–526):e7. https://doi.org/10.1016/j.devcel.2017.07.021
47. Amin S, Neijts R, Simmini S et al (2016) Cdx and T brachyury co-activate growth signaling in the embryonic axial progenitor niche. Cell Rep 17:3165–3177. https://doi.org/10.1016/j.celrep.2016.11.069
48. Oginuma M, Moncuquet P, Xiong F et al (2017) A gradient of glycolytic activity coordinates FGF and Wnt signaling during elongation of the body axis in amniote embryos. Dev Cell 40(342–353):e10. https://doi.org/10.1016/j.devcel.2017.02.001
49. Takemoto T, Uchikawa M, Yoshida M et al (2011) Tbx6-dependent Sox2 regulation determines neural or mesodermal fate in axial stem cells. Nature 470:394–398. https://doi.org/10.1038/nature09729
50. Goto H, Kimmey SC, Row RH et al (2017) FGF and canonical Wnt signaling cooperate to induce paraxial mesoderm from tailbud neuromesodermal progenitors through regulation of a two-step epithelial to mesenchymal transition. Development 144:1412–1424. https://doi.org/10.1242/dev.143578
51. Akai J, Halley PA, Storey KG (2005) FGF-dependent Notch signaling maintains the spinal cord stem zone. Genes Dev 19:2877–2887. https://doi.org/10.1101/gad.357705
52. Glickman NS, Kimmel CB, Jones MA, Adams RJ (2003) Shaping the zebrafish notochord. Development 130:873–887
53. Keller R, Cooper MS, Danilchik M et al (1989) Cell intercalation during notochord development in Xenopus laevis. J Exp Zool 251:134–154. https://doi.org/10.1002/jez.1402510204
54. Ellis K, Bagwell J, Bagnat M (2013) Notochord vacuoles are lysosome-related organelles that function in axis and

spine morphogenesis. J Cell Biol 200:667–679. https://doi.org/10.1083/jcb.201212095
55. Adams DS, Keller R, Koehl MA (1990) The mechanics of notochord elongation, straightening and stiffening in the embryo of Xenopus laevis. Development 110:115–130
56. Catala M, Teillet MA, Le Douarin NM (1995) Organization and development of the tail bud analyzed with the quail-chick chimaera system. Mech Dev 51:51–65
57. Sausedo RA, Schoenwolf GC (1994) Quantitative analyses of cell behaviors underlying notochord formation and extension in mouse embryos. Anat Rec 239:103–112. https://doi.org/10.1002/ar.1092390112
58. Sausedo RA, Schoenwolf GC (1993) Cell behaviors underlying notochord formation and extension in avian embryos: quantitative and immunocytochemical studies. Anat Rec 237:58–70. https://doi.org/10.1002/ar.1092370107
59. Schoenwolf GC (2018) Contributions of the chick embryo and experimental embryology to understanding the cellular mechanisms of neurulation. Int J Dev Biol 62:49–55. https://doi.org/10.1387/ijdb.170288gs
60. Schoenwolf GC (1984) Histological and ultrastructural studies of secondary neurulation in mouse embryos. Am J Anat 169:361–376. https://doi.org/10.1002/aja.1001690402
61. Schoenwolf GC, Delongo J (1980) Ultrastructure of secondary neurulation in the chick embryo. Am J Anat 158:43–63. https://doi.org/10.1002/aja.1001580106
62. Dady A, Havis E, Escriou V et al (2014) Junctional neurulation: a unique developmental program shaping a discrete region of the spinal cord highly susceptible to neural tube defects. J Neurosci 34:13208–13221. https://doi.org/10.1523/JNEUROSCI.1850-14.2014
63. Schoenwolf GC (1985) Shaping and bending of the avian neuroepithelium: morphometric analyses. Dev Biol 109:127–139
64. Nishimura T, Honda H, Takeichi M (2012) Planar cell polarity links axes of spatial dynamics in neural-tube closure. Cell 149:1084–1097. https://doi.org/10.1016/j.cell.2012.04.021
65. López-Escobar B, Caro-Vega JM, Vijayraghavan DS et al (2018) The non-canonical Wnt-PCP pathway shapes the mouse caudal neural plate. Development. https://doi.org/10.1242/dev.157487
66. Roszko I, Faure P, Mathis L (2007) Stem cell growth becomes predominant while neural plate progenitor pool decreases during spinal cord elongation. Dev Biol 304:232–245. https://doi.org/10.1016/j.ydbio.2006.12.050
67. Sausedo RA, Smith JL, Schoenwolf GC (1997) Role of nonrandomly oriented cell division in shaping and bending of the neural plate. J Comp Neurol 381:473–488. https://doi.org/10.1002/(SICI)1096-9861(19970519)381:4%3c473:AID-CNE7%3e3.0.CO;2-%23
68. Ciruna B, Jenny A, Lee D et al (2006) Planar cell polarity signalling couples cell division and morphogenesis during neurulation. Nature 439:220–224. https://doi.org/10.1038/nature04375
69. Shimokita E, Takahashi Y (2011) Secondary neurulation: fate-mapping and gene manipulation of the neural tube in tail bud. Dev Growth Differ 53:401–410. https://doi.org/10.1111/j.1440-169X.2011.01260.x
70. Le Douarin NM, Teillet MA, Catala M (1998) Neurulation in amniote vertebrates: a novel view deduced from the use of quail-chick chimeras. Int J Dev Biol 42:909–916
71. Chal J, Pourquié O (2009) Patterning and differentiation of the vertebrate spine. Cold Spring Harbor Laboratory, New York
72. Yin C, Kiskowski M, Pouille P-A et al (2008) Cooperation of polarized cell intercalations drives convergence and extension of presomitic mesoderm during zebrafish gastrulation. J Cell Biol 180:221–232. https://doi.org/10.1083/jcb.200704150
73. Yen WW, Williams M, Periasamy A et al (2009) PTK7 is essential for polarized cell motility and convergent extension during mouse gastrulation. Development 136:2039–2048. https://doi.org/10.1242/dev.030601
74. Bénazéraf B, Francois P, Baker RE et al (2010) A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. Nature 466:248–252. https://doi.org/10.1038/nature09151
75. Delfini M-C, Dubrulle J, Malapert P et al (2005) Control of the segmentation process by graded MAPK/ERK activation in the chick embryo. Proc Natl Acad Sci USA 102:11343–11348. https://doi.org/10.1073/pnas.0502933102
76. Kulesa PM, Fraser SE (2002) Cell dynamics during somite boundary formation revealed by time-lapse analysis. Science 298:991–995. https://doi.org/10.1126/science.1075544
77. Stern CD, Fraser SE, Keynes RJ, Primmett DR (1988) A cell lineage analysis of segmentation in the chick embryo. Development 104(Suppl):231–244
78. Lawton AK, Nandi A, Stulberg MJ et al (2013) Regulated tissue fluidity steers zebrafish body elongation. Development 140:573–582. https://doi.org/10.1242/dev.090381
79. Das D, Chatti V, Emonet T, Holley SA (2017) Patterned disordered cell motion ensures vertebral column symmetry. Dev Cell 42(170–180):e5. https://doi.org/10.1016/j.devcel.2017.06.020
80. Bénazéraf B, Beaupeux M, Tchernookov M et al (2017) Multiscale quantification of tissue behavior during amniote embryo axis elongation. Development 144:4462–4472. https://doi.org/10.1242/dev.150557
81. Wilson PA, Oster G, Keller R (1989) Cell rearrangement and segmentation in Xenopus: direct observation of cultured explants. Development 105:155–166
82. Steventon B, Duarte F, Lagadec R et al (2016) Species-specific contribution of volumetric growth and tissue convergence to posterior body elongation in vertebrates. Development 143:1732–1741. https://doi.org/10.1242/dev.126375
83. Huss D, Benazeraf B, Wallingford A et al (2015) A transgenic quail model that enables dynamic imaging of amniote embryogenesis. Development 142:2850–2859. https://doi.org/10.1242/dev.121392
84. Schoenwolf GC, Yuan S (1995) Experimental analyses of the rearrangement of ectodermal cells during gastrulation and neurulation in avian embryos. Cell Tissue Res 280:243–251
85. Smith JL, Schoenwolf GC (1989) Notochordal induction of cell wedging in the chick neural plate and its role in neural tube formation. J Exp Zool 250:49–62. https://doi.org/10.1002/jez.1402500107
86. Psychoyos D, Stern CD (1996) Restoration of the organizer after radical ablation of Hensen's node and the anterior primitive streak in the chick embryo. Development 122:3263–3273
87. Charrier J-B, Catala M, Lapointe F et al (2005) Cellular dynamics and molecular control of the development of organizer-derived cells in quail-chick chimeras. Int J Dev Biol 49:181–191. https://doi.org/10.1387/ijdb.041962jc
88. Charrier JB, Teillet MA, Lapointe F, Le Douarin NM (1999) Defining subregions of Hensen's node essential for caudalward movement, midline development and cell survival. Development 126:4771–4783
89. van Nes J, de Graaff W, Lebrin F et al (2006) The Cdx4 mutation affects axial development and reveals an essential role of Cdx genes in the ontogenesis of the placental labyrinth in mice. Development 133:419–428. https://doi.org/10.1242/dev.02216
90. Takada S, Stark KL, Shea MJ et al (1994) Wnt-3a regulates somite and tailbud formation in the mouse embryo. Genes Dev 8:174–189. https://doi.org/10.1101/gad.8.2.174
91. Herrmann BG, Labeit S, Poustka A et al (1990) Cloning of the T gene required in mesoderm formation in the mouse. Nature 343:617–622. https://doi.org/10.1038/343617a0

92. Duband JL, Dufour S, Hatta K et al (1987) Adhesion molecules during somitogenesis in the avian embryo. J Cell Biol 104:1361–1374
93. Zamir EA, Czirók A, Cui C et al (2006) Mesodermal cell displacements during avian gastrulation are due to both individual cell-autonomous and convective tissue movements. Proc Natl Acad Sci USA 103:19806–19811. https://doi.org/10.1073/pnas.0606100103
94. Filla MB, Czirók A, Zamir EA et al (2004) Dynamic imaging of cell, extracellular matrix, and tissue movements during avian vertebral axis patterning. Birth Defects Res C Embryo Today 72:267–276. https://doi.org/10.1002/bdrc.20020
95. Czirók A, Rongish BJ, Little CD (2004) Extracellular matrix dynamics during vertebrate axis formation. Dev Biol 268:111–122. https://doi.org/10.1016/j.ydbio.2003.09.040
96. George EL, Georges-Labouesse EN, Patel-King RS et al (1993) Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. Development 119:1079–1091
97. Yang JT, Rayburn H, Hynes RO (1993) Embryonic mesodermal defects in alpha 5 integrin-deficient mice. Development 119:1093–1105
98. Girós A, Grgur K, Gossler A, Costell M (2011) α5β1 integrin-mediated adhesion to fibronectin is required for axis elongation and somitogenesis in mice. PLoS One 6:e22002. https://doi.org/10.1371/journal.pone.0022002
99. Dray N, Lawton A, Nandi A et al (2013) Cell-fibronectin interactions propel vertebrate trunk elongation via tissue mechanics. Curr Biol 23:1335–1341. https://doi.org/10.1016/j.cub.2013.05.052
100. Serwane F, Mongera A, Rowghanian P et al (2017) In vivo quantification of spatially varying mechanical properties in developing tissues. Nat Methods 14:181–186. https://doi.org/10.1038/nmeth.4101
101. Agero U, Glazier JA, Hosek M (2010) Bulk elastic properties of chicken embryos during somitogenesis. Biomed Eng Online 9:19. https://doi.org/10.1186/1475-925X-9-19
102. Zhou J, Kim HY, Davidson LA (2009) Actomyosin stiffens the vertebrate embryo during crucial stages of elongation and neural tube closure. Development 136:677–688. https://doi.org/10.1242/dev.026211
103. Mongera A, Rowghanian P, Gustafson HJ, et al. (2018) A fluid-to-solid jamming transition underlies vertebrate body axis elongation. Nature. https://doi.org/10.1038/s41586-018-0479-2