HHS Public Access

Author manuscript

Curr Opin Genet Dev. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Curr Opin Genet Dev. 2015 June ; 32: 106–111. doi:10.1016/j.gde.2015.02.005.

The tissue mechanics of vertebrate body elongation and segmentation

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Abstract

England's King Richard III, whose skeleton was recently discovered lying ignobly beneath a parking lot, suffered from a lateral curvature of his spinal column called scoliosis. We now know that his scoliosis was not caused by "imbalanced bodily humors", rather vertebral defects arise from defects in embryonic elongation and segmentation. This review highlights recent advances in our understanding of post-gastrulation biomechanics of the posteriorly advancing tailbud and somite morphogenesis. These processes are beginning to be deciphered from the level of gene networks to a cross-scale physical model incorporating cellular mechanics, the extracellular matrix, and tissue fluidity.

Introduction

The posterior leading edge of the growing vertebrate embryo, named the tailbud, consists of motile progenitors of the axial skeleton, musculature, blood, vasculature and spinal cord, as well as bipotential neural/mesodermal stem cells [1–3]. Musculoskeletal progenitors enter the paraxial mesoderm, which consists of two columns of cells that flank the notochord. Concomitant with axis elongation the paraxial mesoderm stiffens and is segmented into somites whose metameric organization patterns the vertebral column. The somites also give rise to the skeletal muscle of the trunk, tail and limbs, as well as tendons and the dermis. Body elongation and somite morphogenesis are powerful cross-scale models for studying how cellular processes including cell proliferation, cell migration, and cell adhesion organize the biomechanical landscape of a complex tissue.

Cell Proliferation

In zebrafish, the progenitors of the paraxial mesoderm undergo two rounds of cell division during gastrulation. After gastrulation and during body elongation the bipotential stem cells located in the dorsal posterior tailbud do not proliferate due to absence of expression of the

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cell cycle regulator *cdc25a* [4••]. Upon migration into the ventral posterior tailbud, the cells begin to express both *cdc25a* and mesoderm specific transcription factors such as *spadetail*/*tbx16*, *tbx6l* and *mesogenin* [4••, 5••, 6••, 7•]. These mesodermal progenitor cells undergo one round of cell division before both daughter cells differentiate [4••]. This modest level of proliferation in the tailbud is consistent with studies in zebrafish and chick that found that trunk and tail elongation is driven more by cell migration than cell proliferation [8–12].

Cell migration

Cell migration in the tailbud has been best described in zebrafish and chick with initial studies finding that cell motion is more disordered among the mesodermal progenitors in the posterior tailbud than in the presomitic mesoderm (PSM) [13,14]. More recent systematic studies have elaborated on these findings [12,15••,16••]. In both organisms, the instantaneous cell velocities are higher in the posterior tailbud, and there is extensive cell mixing. The PSM grows posteriorly as motile posterior progenitors decrease their cell motion and assimilate into the tissue. Thus, elongation of the paraxial mesoderm is not due to directed migration within the PSM. During trunk elongation in the chick, new cells are added to the posterior mesodermal progenitors from a more posterior pool of cells [12]. By contrast, during zebrafish trunk elongation, new cells enter the dorsal tailbud as a coherent posterior flow of cells dorsal to the notochord. As cells move from the dorsal to ventral posterior tailbud, this flow loses coherence resulting in an increase in cell mixing (Figure 1A) [15••,16••]. Computer simulations suggest that this cell mixing may help synchronize the segmentation clock that generates the segmental prepattern in the PSM [17•]. As mesodermal progenitors enter the posterior PSM, cell motion declines concomitantly with the assembly of an extracellular matrix (ECM) composed of Fibronectin and Laminin [15••, 18]. While cell-Fibronectin interactions are not required for this transition in cell migration, Cadherin 2 dependent cell-cell adhesion promotes coherent cell motion throughout the zebrafish tailbud [15••,16••].

Fgf and Wnt regulate transitions in tissue fluidity

Fgf and Wnt are expressed in gradients from the tip of the tailbud. Fgf signaling promotes the rapid movement and mixing of cells in the posterior chick tailbud and misregulation of Fgf slows body elongation [12]. Similarly, temporally controlled inhibition of Fgf signaling in zebrafish leads to a shorter body axis. These phenotypes contrast with inhibition of Wnt signaling which caused non-linear body elongation. Quantification of cell motion indicates that reduction of Wnt signaling leads to a premature loss of coherence in cell motion in the dorsal tailbud while Fgf inhibition causes both a loss of coherence and a reduction in flux of cells into the posterior tailbud. Computer modeling of cell motion suggests that loss of coherence combined with high flux, as after Wnt inhibition, leads to jamming within the flow of cells. When the jam resolves, bilaterally symmetric flow can be disrupted leading to a bend in the body axis. In contrast, the loss of flux following Fgf inhibition compensates for the loss of coherence to prevent jamming, and the trunk elongates linearly albeit more slowly than wild type [16••].

The notochord

The notochord, which is located between the two columns of the paraxial mesoderm, also contributes to body elongation. It is assembled from axial mesoderm precursors during trunk elongation as cells intercalate along the dorsal midline [19]. This elongation persists in the absence of normal paraxial mesoderm growth but causes the notochord to buckle as it presses against the posterior tailbud [15••]. As tail formation begins at the 16 somite stage, notochord vacuoles begin to enlarge via endosomal trafficking [20]. An ECM consisting of collagen, elastin and laminin forms around the notochord and prevents radial expansion [19]. Thus, vacuole maturation causes elongation of the notochord along the anterior-posterior axis in a process called directed dilation [19]. Failure of vacuole maturation or of peri-notochord ECM integrity can lead to a shorter body axis, scoliosis and fusion of vertebrae [20,21].

ECM and the Mechanics of trunk elongation

Fibronectin is a prominent ECM protein in early vertebrate embryos, and cell-Fibronectin interactions are required for embryonic axis elongation and segmentation [15••,18,22–33]. Live imaging of both the Fibronectin matrix and cell migration during avian embryogenesis found that the ECM undergoes complex movements that closely mirror the pattern of cell migration [12,34–36]. These latter studies suggest that rather than acting primarily as a substrate for cell migration, the ECM may have a greater structural/mechanical function in force transmission or force generation during morphogenesis [36]. Indeed, cell-Fibronectin interactions in the paraxial mesoderm are required for trunk elongation but not cell migration. The Fibronectin matrix coats the zebrafish paraxial mesoderm and mechanically couples the bilateral halves of the paraxial mesoderm to the notochord and periderm [15••]. In *Xenopus*, the boundary between the paraxial mesoderm and notochord is induced by Eph/Ephrin signaling which increases cytoskeletal contractility and prevents Cadherin clustering along the boundary between the two tissues [37]. Eph/Ephrin also induces membrane blebbing along the tissue boundary, and in zebrafish, reduction of Fibronectin matrix increases cellular blebbing along the tissue boundary of the paraxial mesoderm [15••,37]. Cellular blebbing is driven by intracellular hydrostatic pressure [38]. Thus the appearance of blebbing behavior on the surface of the paraxial mesoderm suggests linkages between Eph/Ephrin signaling and cell-Fibronectin interactions in generating local cellular mechanics along the surface of the tissue.

Fibronectin forms a dense matrix on the surface of the paraxial mesoderm and the ability to assemble this ECM is an intrinsic property of both the zebrafish and chick paraxial mesoderm [39,40]. In zebrafish, removal of the Fibronectin receptors Integrin α5 and αV strongly reduces the amount of Fibronectin matrix, and the fibers that do form exhibit an abnormal medial-lateral anisotropy [15••]. Fibronectin fibrillogenesis is driven by cytoskeletal contractile forces transmitted to Fibronectin via linkage to the cytoplasmic domain of Integrins [41]. In 2D cell culture, mechanical stress is applied to the ECM in the direction of cell motion prior to migration [42], and ECM fibers often align along the direction of cell motion in both cell culture and the *Xenopus* gastrula [43,44]. The anisotropy of Fibronectin fibers in embryos lacking Integrin α5 and αV suggests a medial-lateral

alignment of stresses on the surface of the paraxial mesoderm. This phenotype implies that a countervailing anterior-posterior stress produces a Fibronectin matrix with no bias in fiber alignment in wild-type embryos [15••].

Mechanical stiffening of the axis

Tissue stiffening within the *Xenopus* gastrula and neurula has been quantified using explant culture. During this developmental time period, the tissues stiffen by more than four fold with the paraxial mesoderm being twice as stiff as the notochord or neural plate and an order of magnitude stiffer than the endoderm [45,46]. Partial knockdown of Fibronectin does not affect the stiffening, but manipulation of the actin cytoskeleton does [46,47]. The morphology of the *Xenopus laevis* paraxial mesoderm is distinguished by medial-laterally elongated cells in the PSM whereas the other model systems have mesenchymal PSM [48]. Mechanical stiffening of the paraxial mesoderm has not been directly measured in other model species. Thus, it is unclear whether the apparent discrepancy between the role of Fibronectin in paraxial mesoderm mechanics in *Xenopus* and zebrafish is real and perhaps due to differences in tissue morphology.

The cells and ECM appear to exist as an integrated mechanical unit whose fluidity is modulated throughout tissue morphogenesis. Tissues and cell aggregates can be modeled as viscoelastic materials which have both flow and elastic characteristics. The response of viscoelastic materials to stress (force per unit area) changes with time. Under stress, viscoelastic fluids will eventually deform (flow) until stress equals zero while viscoelastic solids deform over time but retain some elastic form and ability to bear stress. Prior *in vitro* analyses of cell aggregates found that modulating levels of Fibronectin and Itgα5β1 leads to tissue phase transitions between a viscoelastic-fluid and a viscoelastic-solid. The reduction in matrix fibers may reduce apparent tissue viscosity by diminishing global interconnectivity within the cell aggregates [49]. The assembly of the paraxial mesoderm during trunk elongation can be thought of as a transition from a viscoelastic fluid to a viscoelastic solid (Figure 1B).

Somite morphogenesis

The segmentation clock creates metameric stripes of gene expression that initiate somite morphogenesis (Figure 2). In mouse, chick and zebrafish, somite morphogenesis entails a mesenchymal to epithelial transition, giving rise to an epithelial somite with a core of mesenchyme. The internal mesenchyme is not necessary for morphological segmentation as somites containing only anterior and posterior border cells can form in the absence of convergence-extension [50]. In *Xenopus laevis* and some other amphibians, somite morphogenesis involves a 90° rotation of elongated cells to give rise to muscle cells that span the anterior-posterior length of the somite [48]. There is a 10 fold variation in the number of cells per somite with zebrafish somites containing 100–150 cells while chick somites comprise ~1000 cells. The larger number of cells would take longer to sort during somite morphogenesis. in silico studies have modeled the mechanics of segmentation at the tissue, cellular and molecular scales [51•,52]. One of the studies suggests that the temporal delay after segmental patterning and before somite morphogenesis is functional. The delay

facilitates error correction as cells sort along the segment boundary according to changes in cell adhesion [51].

In response to patterning by the segmentation clock, the receptor tyrosine kinase *ephA4* is segmentally expressed along the posterior of the nascent somite border while its membrane bound ligand *ephrinB2* is transcribed along the anterior of the border in mice, chick and zebrafish [53–57]. This juxtaposed expression of the receptor and ligand activates EphA4 along nascent somite borders [39]. In turn, Eph/Ephrin signaling can induce the mesenchymal to epithelial transition, inside-out Itgα5 activation and FN matrix assembly [39,53,54,57,58]. Formation of the Fibronectin matrix is necessary for the completion of somite epithelialization and cell sorting [28,29,39,59].

A number of other proteins have been implicated in somite morphogenesis, though an integrated understanding of the system at the molecular level remains elusive. *rap1b* is a GTPase known to regulate Integrin activation and epithelial cell morphology, and it functions with *itgα5* to promote Fibronectin assembly at the zebrafish somite border [60]. In *Xenopus*, the cytoskeletal regulator Ena/Vasp is necessary for Fibronectin matrix assembly and cell rearrangement. In chick, the small GTPase Cdc42 restricts the mesenchymal to epithelial transition, while proper Rac1 levels are necessary for epithelialization [61]. Focal Adhesion Kinase (FAK), which mediates Integrin signaling is also required for boundary matrix formation [62].

Conclusion

The field of developmental biomechanics and biophysics has been reinvigorated by recent advances in instrumentation and conceptually by interdisciplinary research combining biology, physics and engineering. In fact, in September of 2014 the Lorentz Center at Leiden University in the Netherlands hosted a weeklong interdisciplinary workshop focusing specifically on the mechanobiology of somitogenesis. More broadly, integrating gene network function and biomechanics across multiple spatial and temporal scales promises to be a fertile field of inquiry that will contribute to our fundamental understanding of organogenesis, homeostasis and tissue engineering.

Acknowledgments

We thank Abeer Obaid for comments on the manuscript. SAH is supported by grants from the Eunice Kennedy Shriver National Institute of Child Health (1R21HD076173-01A1) and Human Development and the National Institute of General Medical Sciences (1R01GM107385-01A1 and 1R33GM114257-01).

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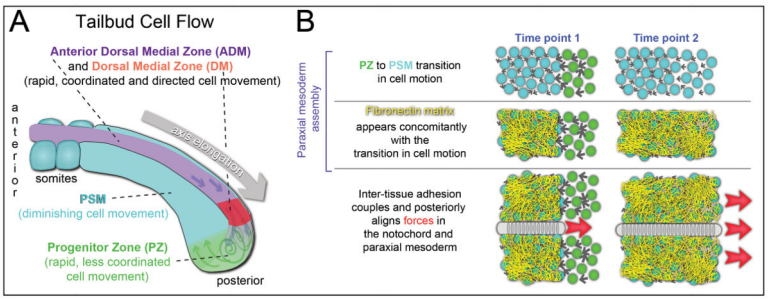

Figure 1. Tissue mechanics during zebrafish trunk elongation

(A) Cell motion in the tailbud can be quantified using metrics from fluid mechanics and thus described as cell flow. The first major transition in cell flow occurs as mesodermal progenitors migrate from the Dorsal Medial Zone into the Progenitor Zone (PZ) where they begin to express mesoderm specific transcription factors such as *tbx16*, *tbx6* (in the mouse and chick) */tbx6l* (in zebrafish) and *mesogenin*. The second transition in tissue fluidity occurs as Progenitor Zone cells assimilate into the Presomitic Mesoderm (PSM). **(B)** During assembly of the PSM, rapidly moving PZ cells (green; time point 1), reduce their instantaneous velocities, (time point 2). This transition coincides with the assembly of a Fibronectin matrix on the surface of the paraxial mesoderm, but the Fibronectin matrix is not necessary for the transition in cell motion. The Fibronectin matrix is required for normal elongation of the bilateral columns of paraxial mesoderm. In addition, the Fibronectin matrix mechanically couples the paraxial mesoderm and elongating notochord.

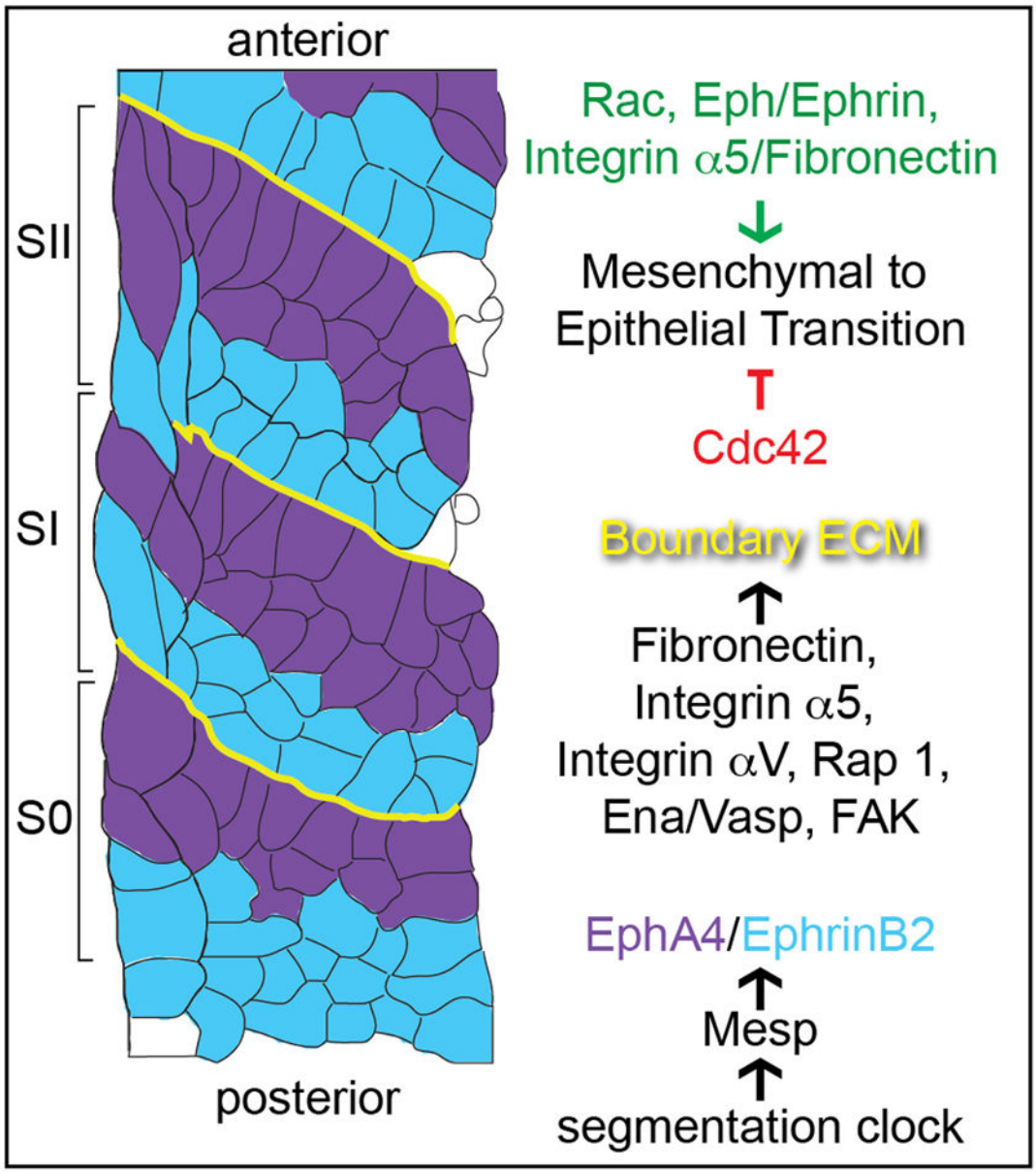

Figure 2. Somite morphogenesis

The S0 is the region in the anterior presomitic mesoderm that will form the next somites. The SI is the most recently formed somite and the SII is the preceding somite. In the presomitic mesoderm, the segmentation clock creates a segmental prepattern and stripes of expression of the transcription factor Mesp, which in turn sets up stripes of EphA4 and EphrinB2 expression flanking the somite boundary. Eph/Ephrin signaling activates Integrin α5 which then initiates assembly of Fibronectin matrix along the somite boundary. Integrin αV, Rap1, Ena/Vasp and FAK also promote Fibronectin matrix assembly. Upon initiation of boundary formation, the somite boundary cells undergo a mesenchymal to epithelial transition. This transition is inhibited by Cdc42 in the presomitic mesoderm and promoted

by Rac1 in the forming somite. Eph/Ephrin signaling and Integrin α5/Fibronectin also promote the mesenchymal to epithelial transition.