Kenro Kusumi · Sally L. Dunwoodie
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The Genetics and Development of Scoliosis

Second Edition

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The editors thank the patients and their families who have participated in scoliosis genetic studies and the research collaborators who have made these efforts possible

Preface

Scoliosis is a lateral curvature of the spine that is frequently encountered by healthcare professionals. Scoliosis has historically been categorized into congenital, neuromuscular, and idiopathic forms, and related curves include kyphosis, kyphoscoliosis, and lordosis. Patients affected by scoliosis are concerned about prognosis, associated health conditions, and recurrence risks. Developmental genetic studies of the spine and next-generation sequencing-based genetic analysis have led to recent advances in understanding the genetic etiology of idiopathic and congenital scoliosis.

The inspiration for the First Edition was derived from the invited session, Straightening Out the Curves: Understanding the Genetics Basis of Idiopathic and Congenital Scoliosis organized at the 2008 American College of Medical Genetics, Annual Clinical Genetics Meeting in Phoenix, AZ, USA. The Second Edition presents significant progress in understanding the genetic etiology of adolescent idiopathic scoliosis, presented March 16–17, 2017, at the Genomic Approaches to Understanding and Treating Scoliosis Conference, in Dallas, TX, USA. This meeting was a joint session of the International Consortium for Vertebral Anomalies and Scoliosis and the International Consortium for Scoliosis Genetics. These groups have now combined forces and merged into the International Consortium for Spinal Genetics, Development, and Disease.

Our understanding of the genetic and developmental mechanisms underlying idiopathic and congenital scoliosis is rapidly evolving, and our goal in editing *The Genetics and Development of Scoliosis*, Second Edition, was to provide researchers, clinicians, and students with the emerging views in this field.

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Chapter 1
Developmental and Functional Anatomy of the Spine

Alan Rawls and Rebecca E. Fisher

Introduction

The vertebral column is composed of alternating vertebrae and intervertebral (IV) discs supported by robust spinal ligaments and muscles. All of these elements, bony, cartilaginous, ligamentous, and muscular, are essential to the structural integrity of the spine. The spine serves three vital functions: protecting the spinal cord and spinal nerves, transmitting the weight of the body, and providing a flexible axis for movements of the head and torso. The vertebral column is capable of extension, flexion, lateral (side to side) flexion, and rotation. However, the degree to which the spine is capable of these movements varies by region. These regions, including the cervical, thoracic, lumbar, and sacrococcygeal spine, form four curvatures (Fig. 1.1). The thoracic and sacrococcygeal curvatures are established during the fetal period while the cervical and thoracic curvatures develop during infancy. The cervical curvature is established in response to holding the head upright, while the lumbar curvature develops as an infant begins to sit upright and walk. However, congenital defects and degenerative diseases can result in exaggerated, abnormal curvatures. The most common of these include kyphosis (hunchback deformity), lordosis (swayback deformity), and scoliosis. Scoliosis involves a lateral curvature of greater than 10 °, often accompanied by a rotational defect. To appreciate the potential underlying causes of scoliosis, we need to understand the cellular and genetic basis of spinal development and patterning. In this chapter, we will review the embryonic

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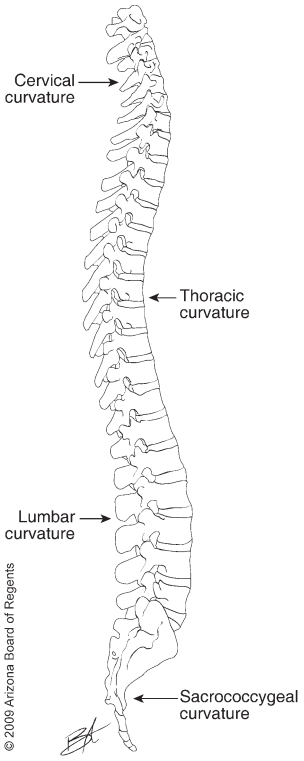

Fig. 1.1 Lateral view of the vertebral column, illustrating the spinal curvatures (Drawing by Brent Adrian)

development of the spine and associated muscles and the functional anatomy of these structures in the adult.

Embryonic Origins of the Spine

The origins of the vertebral column, spinal musculature, and associated tendons are two rods of paraxial mesoderm that fill in the space on either side of the neural tube at the time of gastrulation. Beginning at 20 days *post coitus*, paraxial mesoderm undergoes segmentation in a rostral to caudal direction to form 42–44 pairs of somites, which can be subdivided into 4 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 8–10 coccygeal somites. The first occipital and the last 5–7 coccygeal somites disappear during embryonic development. Each somite will differentiate into four cell lineage-specific compartments that contribute to the vertebral column and associated musculature, including the sclerotome (vertebrae and ribs), syndetome (tendons), myotome (skeletal muscle), and dermomyotome (dermis and skeletal muscle progenitor cells).

Somite formation can best be described as a continuous segmentation of mesenchymal cells from the rostral end of the paraxial mesoderm or presomitic mesoderm (PSM) that lays down the embryonic cells that will give rise to the axial skeleton. Intrinsic to this process is (1) an oscillating clock controlling the timing of somitogenesis, (2) the formation of intersomitic boundaries, (3) mesenchymal to epithelial transition (MET), and (4) positional identity (e.g., rostral/caudal and dorsal/ventral). Experimental disruption in any one of the processes in vertebrate model organisms (e.g., mouse and chick) can lead to an axial skeletal dysmorphogenesis that is phenotypically consistent with scoliosis. The timing of somite formation and the determination of the site of boundary formation are established by the interactions between the Notch, Wnt, and FGF signaling pathways. Here we will focus on the morphogenetic events associated with the physical separation of PSM during formation of the boundary, epithelialization, and positional identity.

Establishing the Intersomitic Boundary

Boundary formation occurs as somitic cells pull apart from the adjacent PSM. Dependent on the animal, this varies from the simple cleavage of the PSM by fissures initiated along either the medial or lateral surfaces as seen in *Xenopus* and zebra fish to a more dynamic ball-and-socket shape with a reshuffling of cells across the presumptive somite-PSM boundary in chicks [57, 64, 74, 75, 163]. The activity is an intrinsic property of the PSM, as it will occur in explants in the absence of the adjacent ectoderm and endoderm [108]. However, the underlying mechanism(s) remains poorly understood. In studies carried out in chick embryos, the fissure can be induced by activated Notch receptors and is stabilized by the presence of Lfng [128]. Transcription factors *Mesp2* (and its chicken homologue, *cMeso1*) and *Tbx18* have also been shown to play a role in forming boundaries [19, 124, 146, 152]. Ectopic expression of either *cMeso1* or *Tbx18* is sufficient to induce ectopic fissures in chick PSM. Additional signals derived from the ventral PSM coordinate fissure formation in the dorsal PSM, though the nature of the signal remains poorly understood [127]. It is likely that the physical separation of cells at the fissure is related to differential changes in cell adhesion.

Somite Epithelialization

Cells of the newly formed somites undergo an increase in cell number, density, and expression of extracellular matrix proteins (reviewed in [70, 151]), resulting in the condensation of mesenchyme into an epithelial ball, surrounding a mesenchymal core, called the somitocoele. This occurs in a gradual process with the cells along the rostral edge of somite 0 becoming epithelia at the time of boundary formation [46]. Epithelialization is complete with the formation of the next boundary (Fig. 1.2).

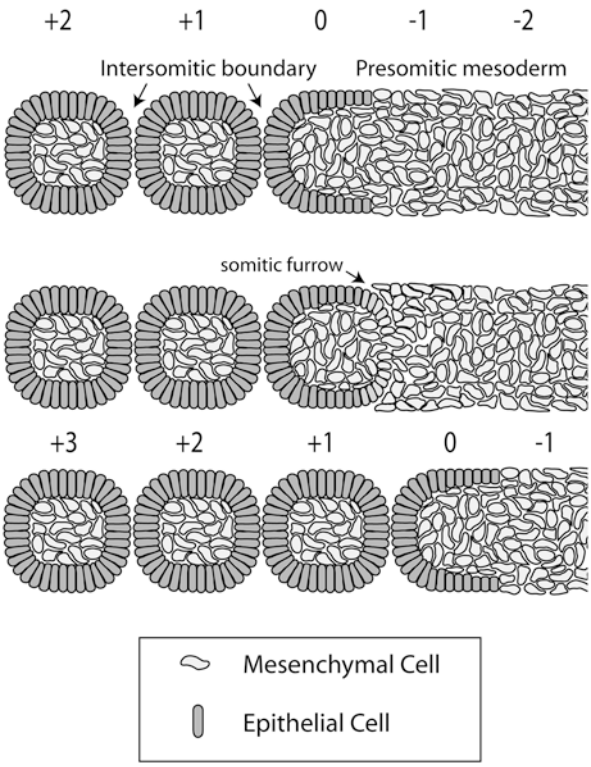

Fig. 1.2 Schematic of mouse somite formation. Lateral view of somites budding off the rostral end of the presomitic mesoderm demonstrates the stepwise transition of mesenchymal cells to epithelium. By convention, the forming somite is labeled "0" and the newest somite is "+1"

The transcription factors paraxis and *Pax3* are required to direct MET in cells of somite +1 [20, 21, 86, 130]. Inactivation of paraxis results in somites formed of loose clusters of mesenchyme separated by distinct intersomitic boundary formation (Fig. 1.2). This reveals that MET is not required for boundary formation. However, the two events are temporally linked, suggesting that they are both responsive to the oscillating segmental clock. Candidate genes for linking the two are *snail1* and *snail2* (*Snai1* and *Snai2*), which are expressed in oscillating patterns in the PSM [40]. Snail genes are transcriptional repressors that are able to block the transcription of paraxis and cell adhesion molecules associated with epithelialization [9, 10, 26, 40]. Overexpression of *Snai2* will prevent cells from contributing to epithelium in somite +1. Thus, switching off snail gene expression may be essential for the timing of MET.

In contrast to boundary formation, signals from the surface ectoderm are required to induce MET and the expression of paraxis [38, 45, 80, 127, 128, 138]. Wnt signaling has been implicated in regulating this process with *Wnt6* and *Wnt11* as the most likely candidates [55, 80, 129, 159]. Ectopic expression of *Wnt6* is able to rescue somite epithelialization where the ectoderm has been removed. Further, *Wnt6* is able to induce paraxis transcription through a beta-catenin-dependent manner, predicting a mechanism of action [80].

Somite epithelialization is associated with an increase in the expression of members of the cadherin superfamily and cell adhesion molecules [45, 151]. These cell surface molecules participate in the formation of focal adhesion and desmosomes at the apical junction of epithelium. Inactivation of N-cadherin (*Cdh2*), alone or in combination with cadherin 11 (*Cdh11*), leads to the disorganization of the somite epithelium into small clusters of cells [58, 79, 116]. Functional inactivation of *Cdh2* through increased endocytosis has been implicated in the formation of the new somitic boundary. The protocadherin, PAPC, which is dynamically expressed in the forming somites regulated by Notch/Mesp2 signaling, promotes clathrin-mediated endocytosis and the internalization of Cdh2 [29, 119]. This disrupts homotypic interaction of cadherins between adjacent cells leading to a fissure that will become the somitic boundary.

The phenotypes of the cadherin mutations are not as severe as either the paraxis or *Pax3*, predicting that additional factors associated with cell adhesion are required for epithelialization. The most likely candidates are the genes involved in cytoskeletal remodeling. Likely targets are members of the Rho family of GTPase. In the chick, overexpression of *Cdc42* promotes somitic cells to maintain their mesenchymal state [103]. Both the inhibition and over-activation of Rac1 disrupt somite epithelialization, demonstrating the sensitivity of the cells to disruption of this pathway. The activity of Rac1 cannot be rescued by paraxis predicting that Rac1 is acting downstream [103]. In the paraxis-null, localization of Rac1 is disrupted in the somites, and the regulation of the expression of Rac1 modifiers, including the guanine nucleotide exchange factor, Dock2, is disrupted reinforcing a role for paraxis downstream of Rac1 [123].

Differential gene expression studies with paraxis-null somites revealed a significant reduction in the expression of fibroblast activation protein alpha (*Fap*), encoding a dipeptidyl peptidase that regulates fibronectin and collagen fiber organization in extracellular matrix [123]. Further, downstream genes in the Wnt and Notch signaling pathways were downregulated in the absence of paraxis, predicting a positive feedback loops with both pathways.

Rostral/Caudal Polarity of Somites

Spatial identity along the rostral/caudal axis is established in each somite at the time of its formation [3, 56]. Rostral/caudal polarity is essential for imposing the segmental patterning of the peripheral nerves and the resegmentation of the sclerotome during vertebrae formation. This is regulated by an intricate feedback loop between cells in the rostral and caudal halves of the forming somite (somite 0). Consistent with the cyclical nature of somitogenesis, the feedback loop is also entrained with the oscillating segmental clock. Activation of the Notch pathway plays a central role in determining spatial identity. Disruption of *Notch1*, ligands *Dll1* and *Dll3*, or modifying gene peptide-O-fucosyltransferase 1 (*Pofut1*) and presenilin-1 lead to the

loss of rostral- and caudal-specific gene expression, fusion of the vertebrae, and disruption of the segmental pattern of the peripheral nerves [41, 47, 59, 73, 76, 104, 131, 144]. Spatial identity of the rostral half of the somite requires the expression of *Mesp2*, which is transcribed in a broad domain that encompasses presumptive somite −1 before becoming restricted to the rostral half of the presumptive somite (somite 0) [124, 147]. Mouse embryos deficient in *Mesp2* lead to expanded expression of caudal-specific genes and fused vertebrae. Transcription of *Mesp2* is upregulated by activated Notch in a *Tbx6*-dependent manner [166], which in turn represses transcription of the *Dll1* ligand in the rostral domain through the transcriptional repressor, Ripply2 [101]. In the caudal half of somite 0, *Mesp2* transcription is repressed by a presenilin-1-dependent manner [73, 148, 166].

Maintenance of rostral/caudal polarity after somite formation requires paraxis, which is associated with the regulation of somite epithelialization [65]. In paraxis-null embryos, the transcription pattern of *Mesp2* and components of the Notch signaling pathway are unaltered in somite 0 and − 1. However, the expression of caudal-specific genes, such as *Dll1* and *Uncx4.1*, is broadly transcribed in the newly formed somites. It has been proposed that paraxis participates in a cell adhesion-dependent mechanism of maintaining the intersomitic boundary between the rostral and caudal halves of the somite after their specification in the presomitic mesoderm [65].

The Anatomy and Development of the Vertebrae and Intervertebral Discs

A typical vertebra consists of two parts: the body and the vertebral (or neural) arch (Fig. 1.3A). The vertebral body is located anteriorly and articulates with the adjacent intervertebral (IV) discs (Figs. 1.1, 1.3, and 1.4). Together, the vertebral body and arch form a central, vertebral foramen, and, collectively, these foramina create a vertebral canal that protects the spinal cord. In this section, the functional anatomy of the vertebrae and IV discs in the adult and the genetic basis for their development in the embryo will be discussed.

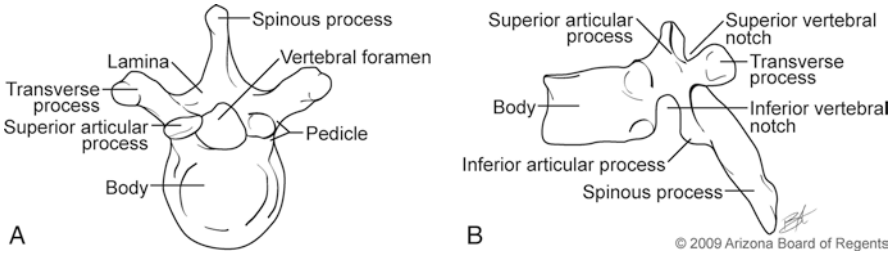

Fig. 1.3 Features of a typical human vertebra. (**A**) Superior and (**B**) lateral view (Drawing by Brent Adrian)