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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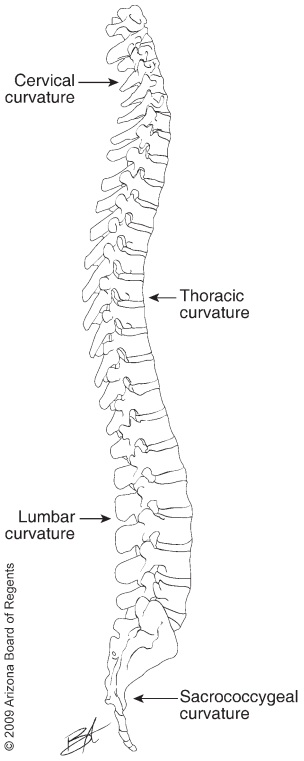

Fig. 1.1 Lateral view of the vertebral column, illustrating the spinal curvatures (Drawing by Brent Adrian)

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).

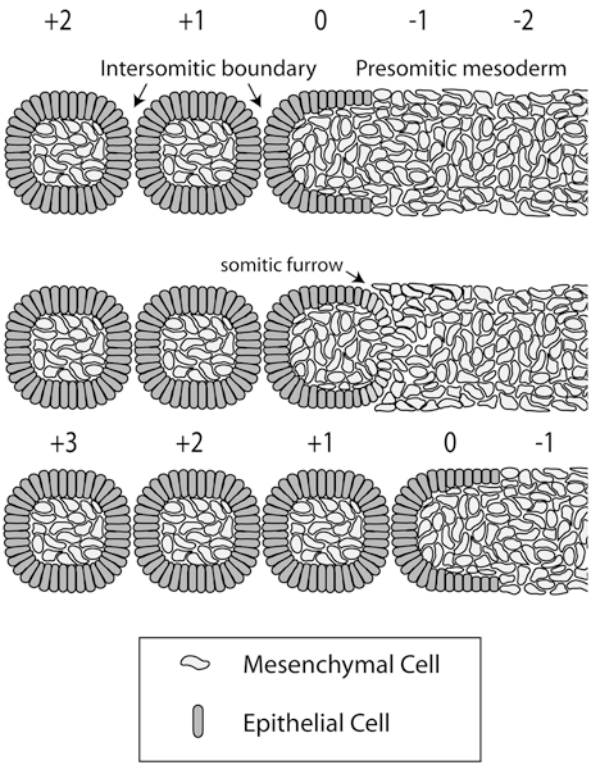

Fig. 1.2 Schematic of mouse somite formation. Lateral view of somites budding off the rostral end of the presomitic mesoderm demonstrates the stepwise transition of mesenchymal cells to epithelium. By convention, the forming somite is labeled "0" and the newest somite is "+1"

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.

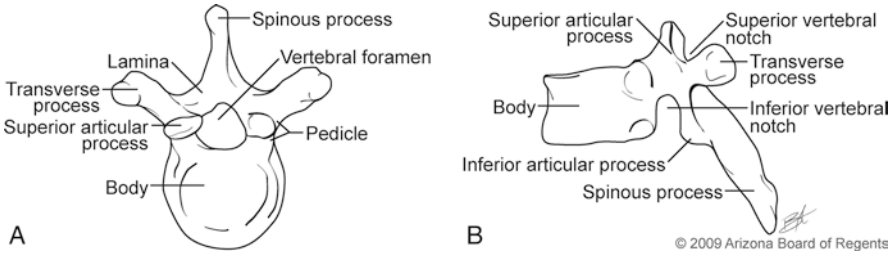

Fig. 1.3 Features of a typical human vertebra. (**A**) Superior and (**B**) lateral view (Drawing by Brent Adrian)

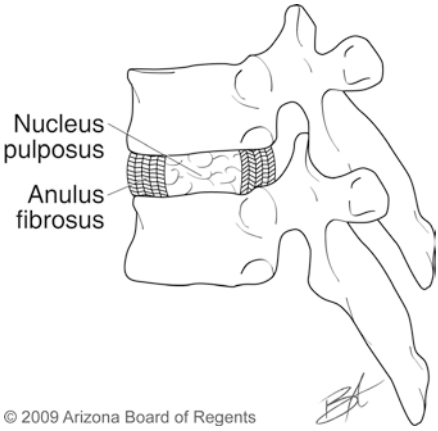

Fig. 1.4 Structure of the intervertebral disc (Drawing by Brent Adrian)

Functional Anatomy of the Vertebrae and IV Discs

The vertebral bodies consist of a shell of compact bone surrounding a core of trabecular bone and red marrow. In addition, hyaline cartilage forms vertebral end plates on the superior and inferior surfaces of each body. The vertebral bodies, in conjunction with the IV discs, bear and transmit weight; as a result, the bodies increase in size from the cervical to the lumbar region (Fig. 1.1). However, as weight is then transferred to the lower extremities via the sacrum, the bodies subsequently decrease in size.

The vertebral arch is located posterior to the vertebral body and consists of two pedicles and two laminae (Fig. 1.3A). The superior and inferior notches of adjacent pedicles form the intervertebral foramina, which transmit the spinal nerves (Figs. 1.1 and 1.3B). Disruption of these foramina (e.g., by a herniated disc) can compress the spinal nerves, leading to both sensory and motor deficits. In addition to protecting the spinal cord and spinal nerves, the vertebral arch also has a number of processes that provide sites for muscle and ligament attachment. The spinous process, located at the junction of the laminae, and the transverse processes, located at the pedicle-lamina junctions, provide attachment sites for ligaments as well as the erector spinae and transversospinalis muscles (Fig. 1.3A–B). In addition, in the thoracic region, the transverse processes articulate with the tubercles of the ribs to form the costovertebral joints. Finally, the superior and inferior articular processes of adjacent vertebrae interlock to form the zygapophysial (or facet) joints (Fig. 1.4). These synovial joints permit gliding movements and their orientation largely determines the ranges of motion that are possible between adjacent vertebrae.

The morphology and the functions of the vertebrae vary by region. The cervical spine is composed of seven vertebrae (Fig. 1.1). The bodies are small, reflecting their relatively minor weight-bearing role, while transverse foramina are present for the passage of the vertebral arteries and veins. In addition, the articular facets on the superior and inferior articular processes face superiorly and inferiorly, promoting

flexion, extension, lateral flexion, and rotation at the cervical facet joints. This region also includes two highly derived elements, the C1 and C2 vertebrae. The C1 vertebra, or atlas, lacks a body and spinous process. Instead, it features two lateral masses united by an anterior and posterior vertebral arch. The superior articular facets of the atlas articulate with the occipital condyles of the skull to form the atlanto-occipital joints. These synovial joints allow for flexion and extension of the head. The C2 vertebra, or axis, features a dens or odontoid process; this process represents the body of the atlas that fuses with the axis during development. The dens process articulates with the anterior arch of the atlas to form the median atlanto-axial joint while the facet joints between the C1 and C2 vertebrae form the lateral atlanto-axial joints. Together, these joints allow for rotation of the head.

The 12 thoracic vertebrae are distinct in featuring costal facets on their bodies and transverse processes (Fig. 1.3B). Typically, a thoracic vertebral body articulates with the heads of two ribs, while the transverse process articulates with the tubercle of one of these ribs; altogether, these articulations form the costovertebral joints. These synovial joints serve to elevate and depress the ribs, thus increasing the anterior-posterior and transverse diameters of the thoracic cavity during respiration. In the thoracic spine, the superior and inferior articular facets face anteriorly and posteriorly (Fig. 1.3B), permitting rotation and some lateral flexion. However, the orientation of these facets, as well as the inferiorly directed spinous processes and the costovertebral joints, severely restricts flexion and extension of the thoracic spine. In contrast, the medially and laterally facing articular facets of the five lumbar vertebrae allow for a great deal of flexion and extension, but restrict rotation. The lumbar vertebrae also exhibit robust vertebral bodies and well-developed spinous, transverse, and articular processes that provide attachment sites for ligaments as well as the erector spinae and transversospinalis muscles (Fig. 1.1).

The sacrum is typically formed by the fusion of five sacral vertebrae (Fig. 1.1). The sacral canal transmits the spinal roots of the caudal equina and ends at the sacral hiatus, an important landmark for administering a caudal epidural. In addition, pairs of sacral foramina transmit the ventral and dorsal rami of the sacral spinal nerves. The sacrum plays an important role in transmitting the weight of the body from the spine to the lower extremities; as a result, the sacroiliac joints are protected by extremely robust ligaments. The coccyx is typically formed by the fusion of four coccygeal vertebrae (Fig. 1.1). Although rudimentary in humans, the coccyx serves as a focal point for the attachment of the muscles of the pelvic floor as well as the sacrotuberous and sacrospinous ligaments.

Most of the vertebral bodies articulate superiorly and inferiorly with IV discs, forming secondary cartilaginous joints or symphyses (Fig. 1.4). However, an IV disc is not present between the atlas and axis, and the sacral and coccygeal IV discs ossify progressively into adulthood. Representing up to 25% of the total length of the spine, the IV discs act as shock absorbers and enhance spinal flexibility, particularly in the cervical and lumbar regions [100]. The IV discs are responsible for resisting compressive loads due to weight bearing as well as tensile and shearing stresses that arise with movements of the vertebral column, such as rotation and lateral flexion. The thoracic IV discs are relatively thin and uniform in shape, while

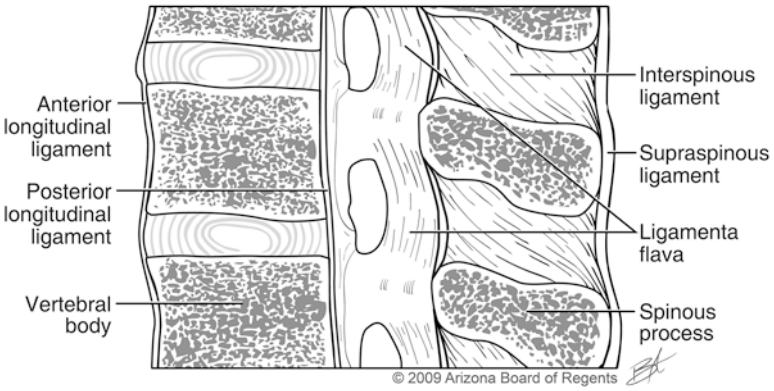

Fig. 1.5 Major ligaments of the spine. Lateral view illustrating the ligamentum flava, supraspinous, interspinous, and anterior and posterior longitudinal ligaments (Drawing by Brent Adrian)

the cervical and lumbar IV discs are wedge-shaped, contributing to the curvatures of the vertebral column (Fig. 1.1). Each IV disc is composed of an outer fibrocartilaginous ring, the annulus fibrosus, and a central gelatinous core, the nucleus pulposus (Fig. 1.4). Composed primarily of collagen fibers, the annulus fibrosus is characterized by a series of concentric layers, or lamellae (Fig. 1.4). The lamellae serve to resist the expansion of the nucleus pulposus during compression [25]. The nucleus pulposus is composed of water, proteoglycans, and scattered collagen fibers.

The vertebrae and IV discs are stabilized by robust spinal ligaments that function to restrict movements and to minimize the need for continual muscular contraction. The major spinal ligaments are illustrated in Fig. 1.5. The broad anterior longitudinal ligament is situated on the anterior surface of the vertebral bodies and IV discs and extends from the sacrum to the occipital bone (Fig. 1.5). This ligament, which prevents hyperextension of the spine and anterior herniation of the nucleus pulposus, is especially prone to injury in the cervical region due to whiplash (hyperextension) injuries. The posterior longitudinal ligament is slender compared to its counterpart. It lies within the vertebral canal, on the posterior surface of the vertebral bodies and IV discs (Fig. 1.5). This ligament prevents hyperflexion of the vertebral column and posterior herniation of the nucleus pulposus. In fact, due to the presence of the posterior longitudinal ligament, the nucleus pulposus tends to herniate in a posterolateral direction.

While the anterior and posterior longitudinal ligaments traverse the length of the spine, the ligamenta flava connect the laminae of adjacent vertebrae (Fig. 1.5). These ligaments contribute to the posterior wall of the vertebral canal, thus helping to protect the spinal cord. The ligamenta flava are highly elastic, supporting the normal curvatures of the spine, resisting separation of the laminae during flexion, and assisting in extending the spine from a flexed position. The vertebrae are also held together by the intertransverse and interspinous ligaments, which connect adjacent transverse and spinous processes, respectively (Fig. 1.5). More superficially,

the robust supraspinous ligament binds the spinous processes together. In the neck, the supraspinous ligament merges with the ligamentum nuchae, a fibroelastic structure that extends from the cervical spinous processes to the occiput, forming a midline raphe for muscle attachment [94]. The intertransverse, interspinous, and supraspinous ligaments help prevent hyperflexion and extreme lateral flexion of the vertebral column.

Development of the Vertebrae

The axial skeleton is derived from the sclerotome compartment of the somites, which first appear during the fourth week of development in humans as the epithelial cells in the ventral/medial quadrant of the somite undergo an epithelial-to-mesenchymal transition (EMT). These cells, along with the mesenchymal cells of the somitocoele, are initially specified to the chondrogenic lineage and form the cartilage models of the vertebrae (Fig. 1.6) (reviewed in [44]). Through endochondral ossification, the cartilage is replaced by bone. The molecular events that regulate this process are similar to those that regulate the appendicular skeleton and part of the cranium. These pathways are reviewed elsewhere [81]. In this chapter, we will focus on the signaling events that influence patterning of the newly formed vertebrae.

The transition from sclerotome to vertebrae can be divided into distinct processes for the ventral structures (vertebral body and intervertebral discs) and dorsal neural arch structures (pedicles, laminae, spinous and transverse processes) based on both cell origin and genetic regulation. Patterning along the dorsal/ventral axis is controlled by opposing gradients derived from the notochord and surface ectoderm overlying the neural tube. Sonic hedgehog (*Shh*) and the BMP inhibitor, noggin, have been identified as factors expressed in the notochord that are sufficient to promote the expression of the transcription factors *Pax1*, *Pax9*, and *Mfh1* in the sclerotome [49, 53, 91, 110]. *Pax1* and *Pax9* are essential for the maintenance of sclerotomal cells [53]. Compound mutations of these two genes in the mouse lead to loss of the vertebral body and proximal ribs [111]. In addition to signals from the notochord, the polycomb genes *Pbx1* and *Pbx2* and bHLH genes paraxis and *Mesp2* are also required for *Pax1* and *Pax9* transcription [28, 149]. The homeodomain-containing genes, *Meox1* and *Meox2*, that are essential for vertebrate development [84, 135] combine with *Pax1* and *Pax9* to activate the expression of *Nkx3.2*, a transcriptional repressor that triggers chondrogenesis [121, 122]. Chondrocyte differentiation is associated with the downregulation of *Pax1* in the cells of the sclerotome. Though *Pax1* is required for sclerotome specification, it is an inhibitor of chondrogenesis through the inhibition of *Sox9*, *Nkx3.2*, Indian hedgehog, and aggrecan [150]. This dual role for *Pax1* likely allows for further subdivision of the sclerotome as the cells that contribute the intervertebral disc and fail to undergo chondrogenesis maintain its expression.

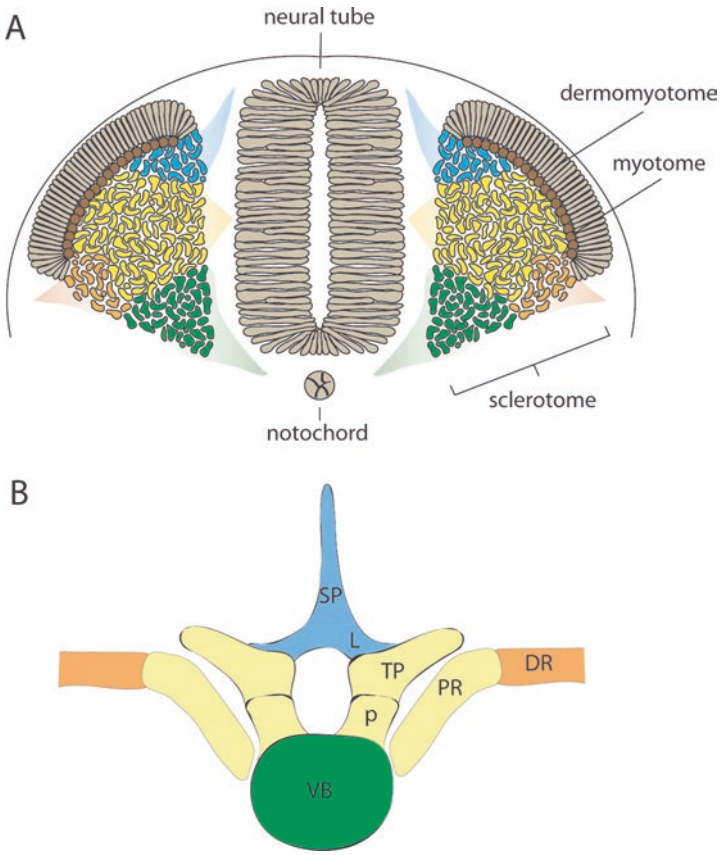

Fig. 1.6 Sclerotome origins of the vertebrae. (**A**) A schematic of the differentiating somites demarcating the domains of the sclerotome that migrate to form the individual elements of the vertebrae. (**B**) A diagram of a thoracic vertebra. Vertebral body (VB; green), pedicle (P; yellow), transverse process (TP; yellow), lamina (L; blue) and spinous processes (SP; blue), proximal rib (PR; yellow), and distal rib (DR; orange)

The formation of the vertebral body is dependent on the highly coordinated migration of sclerotomal cells both toward the midline and along the rostral/caudal axis (Fig. 1.6) (reviewed in [15]). Soon after EMT, cells from the ventral/medial sclerotome migrate toward the notochord. This is directed in part through an interaction with an extracellular matrix network (e.g., laminin, fibronectin, collagen I, aggrecan, and perlecan) radiating from the notochord [62]. Production of the matrix genes requires the expression of *Sox5* and *Sox6* [137]. Initially, the *Pax1 + ve* sclerotomal cells form an unsegmented sheath around the notochord that will give rise to both the future vertebral bodies and intervertebral discs. Segmentation appears as cells of the future intervertebral discs condense, and the intervening loose mesenchyme will give rise to the vertebral bodies [36]. The metameric pattern is also reflected in *Pax1* expression, which is maintained in the future intervertebral disc

and lost from the vertebral body anlagen. This is believed to promote differential chondrocyte maturation in the vertebral bodies, while maintaining the intervertebral cell in a mesenchymal state [150].

The formation of the neural arches is more complicated as the pedicles and transverse processes are derived from the central sclerotome while the lamina and spinous processes originate from the dorsal/medial sclerotome (Fig. 1.6). They are further distinguished by their contribution from the rostral and caudal halves of the sclerotome, which are morphologically distinguishable at this time (Fig. 1.7A). The pedicles and transverse processes originate almost solely from the caudal domain and the spinous process from the rostral domain. While the pedicles and transverse processes are dependent on *Pax1* for specification to the chondrogenic lineage, the lamina and spinous processes are dependent on *Msx1* and *Msx2* transcription. Thus, these structures still develop in *Pax1*/*Pax9* double knockouts where the vertebrae are absent [111]. *Msx1* and *Msx2* transcription is induced by BMP2 and BMP4 expressed in the surface ectoderm and roof plate of the neural tube [98, 99, 160]. SHH and the BMP's are mutually antagonistic in their actions [113]. Ectopic expression of BMP2 or BMP4 on the dorsal neural tube will increase dorsal chondrogenesis while ectopic expression lateral to the neural tube inhibits chondrogenesis [154, 160]. The corollary is also true with SHH-expressing cells grafted dorsally, inhibiting *Msx1* transcription and preventing chondrogenesis [160].

Resegmentation of the sclerotome is intimately linked to the specification of the rostral and caudal domains early in somitogenesis. As described previously, the interaction between the Notch signaling pathway and *Mesp2* leads to the specification of the rostral and caudal fate of the somite prior to overt segmentation. As such, the caudalization of the somite by inactivation of *Mesp2* leads to fusion of the vertebral bodies and neural arches along the length of the vertebral column [124]. In contrast, disruption of the somites' caudal identity through inactivation of the Notch pathway leads to fused vertebral bodies and an absence of neural arches. Mutations in genes regulating this process have been identified as the cause of spondylocostal dysostoses, a heterogeneous group of disorders with severe axial skeletal malformation characterized radiographically by multiple vertebral segmentation defects (reviewed in [139]). Disruption of rostral/caudal polarity after somite formation has also been shown to impact resegmentation, though to a lesser extent. In *paraxis*-deficient embryos, ventral cartilage fails to segment into vertebral bodies and IV discs, while the lateral neural arches are unaffected [65].

Rostral/Caudal Patterning

An additional layer of regulation is required to confer the distinctive regional characteristics of the cervical, thoracic, lumbar, sacral, and caudal vertebrae. Members of the Hox transcription factor family have been strongly implicated in establishing positional identity of vertebrae along the rostral/caudal axis (reviewed in [161]). From classic studies in *Drosophila*, the Hox genes have long been known to

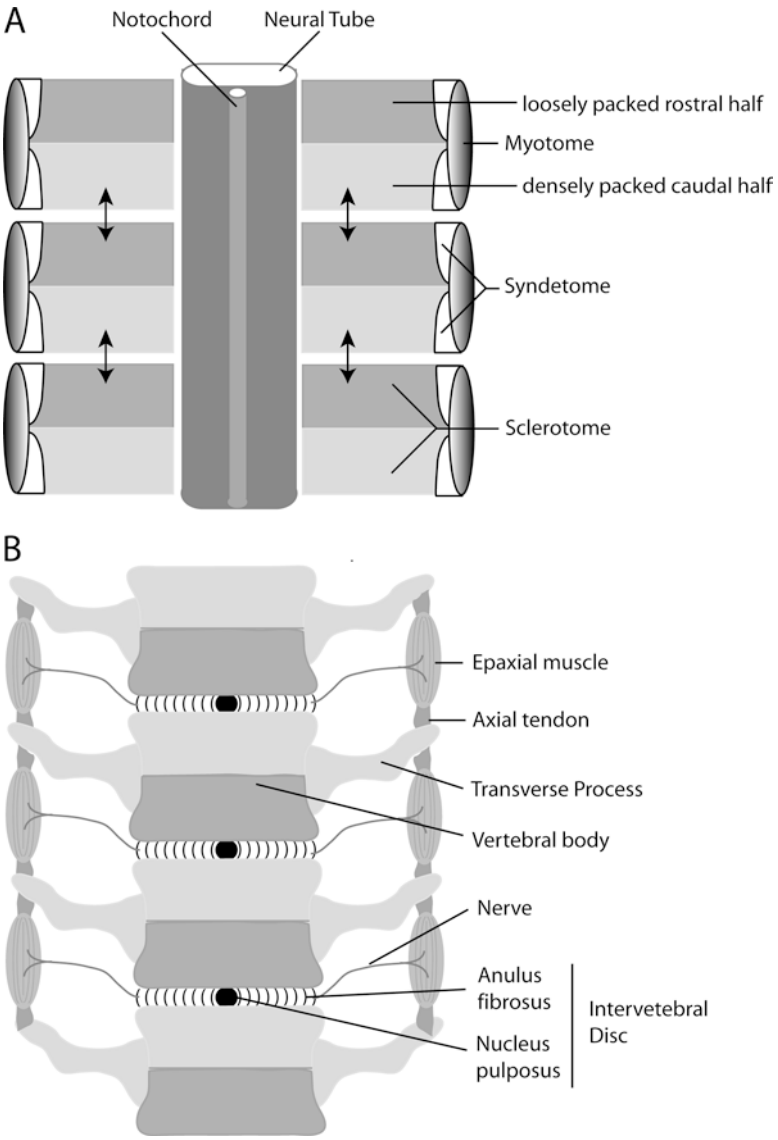

Fig. 1.7 Schematic of vertebral generation through sclerotome resegmentation. (**A**) Ventral view of the sclerotome, syndetome, and myotome compartments. The caudal half of the sclerotome grows into the rostral half of the adjacent somite. (**B**) Ventral view of the vertebral column with associated epaxial muscles and axial tendons. Shading represents the contribution of the rostral and caudal sclerotome to the vertebral bodies and transverse processes. The intervertebral disc forms at the site sclerotome separation. Note the relationship of the muscle and bone after resegmentation

regulate segmental identity in the insect body plan [77]. Compound mutations that inactivate more than one gene of a paralogous Hox group in mice lead to rostral homeotic transformation of the vertebrae. This was first observed with *Hoxa3*/*Hoxd3* double mutant embryos, where the prevertebral elements that normally contribute to the atlas form a bone contiguous with the occipital bone [37]. Since this observation, similar homeotic transformations have been reported for paralogous mutations in the Hox5, Hox6, Hox7, Hox8, Hox9, Hox10, and Hox11 group genes [31, 88, 156, 162]. Consistent with the colinear expression of these genes, the rostral homeotic transformations effect successively more caudal vertebrae with the Hox11 paralogous mutants displaying a transformation of sacral and early caudal vertebrae into a lumbar-like fate [162].

The positional identity conferred by the Hox genes during vertebral patterning is modified by members of the polycomb family and TALE class of homeodomain-containing transcription factors. The polycomb genes, *Bmi* and *Eed*, function as transcriptional repressors that limit the rostral transcription boundary of individual Hox genes. Inactivation of these genes leads to a rostral shift in gene expression and transformation of the vertebrae [72]. The TALE gene families, *Pbx* and *Meis* genes, are able to form dimer partners with the Hox genes, leading to modified transcription of target genes by altering DNA-specific binding specificity (reviewed in [96]). The TALE genes play a larger role in patterning, regulating the transcription of the 5 prime Hox genes by both a Hox-dependent and Hox-independent manner [11, 27, 82, 112].

Formation of the IV Discs

An IV disc is comprised of a proteoglycan-rich nucleus pulposus, the annulus fibrosus, and cartilage end plates that adhere to the adjacent vertebrae that collectively redistribute the compressive force generated by the vertebral column. Though originally thought to be derived solely from the sclerotome of the somite [60, 95], the nucleus pulposus has been shown to be derived from the notochord [34]. As a result, we must now invoke a more complicated model for the development of the IV discs that requires the coordination of multiple independent signaling pathways.

The notochord is a rodlike structure running the length of the embryonic ventral midline, where it serves as a signaling center for the patterning of the central nervous system, gut, and vertebral column. The notochord is comprised of highly vacuolated cells encapsulated in a sheath composed of collagen, aggrecan, fibronectin, laminin, cytokeratin, and sulfate glycosaminoglycans (GAGs). Components of the sheath including aggrecan and more than 100 sulfated GAGs are also found in the nucleus pulposus, where they maintain the osmolality essential for giving the tissue its gel-like characteristics [107, 134]. The signaling pathways that are required for nucleus pulposus formation remain poorly understood. Some insight has come from the study of *Shh*, which is required for the integrity of the notochordal sheath and cell proliferation [33]. In complete and conditional mutations, notochordal cells fail

to properly migrate to the nucleus pulposus [35]. Sheath stability and ultimately maintenance of the notochord are dependent on *Sox5/Sox6* and *Foxa1/Foxa2* expression [83, 137]. Single mutations in either of the *Sox* or *Foxa* genes did not have notochord defects, suggesting functional redundancy of sister genes. In the case of *Foxa*, the proteins have been shown to bind to the *Shh* promoter predicting a role in regulating the Shh pathway [63].

The annulus fibrosus of an IV disc forms from condensed mesenchyme derived from the somitocoele at the border of the rostral and caudal domains during resegmentation [60, 95]. Somitocoele cells cannot be replaced by sclerotomal cells derived from EMT in forming the IV disc predicting specification of a distinct lineage, now called the arthrotome [95]. Development of the annulus fibrosus and its maintenance in adults is dependent on members of the TGF-beta superfamily. Inactivation of *TGF-beta type II receptor* (*Tgfbr2*) in type II collagen expressing cells results in an expansion of *Pax1/Pax9* expression and the loss of IV discs [8]. GDF-5 and BMP-2 promote cell aggregation and expression of the chondrogenic genes instead of osteogenic genes in the IV discs [78, 167].

The Anatomy and Development of Spinal Muscles

A number of muscle groups act upon the spine. Those located anterior to the vertebral bodies act as flexors, including longus capitis and colli, sternocleidomastoid, psoas major, and rectus abdominis. In contrast, the extensors of the spine are located posterior to the vertebral bodies and include the splenius, erector spinae, and transversospinalis muscles (Fig. 1.8). Lateral (side to side) flexion is achieved by the scalenes, sternocleidomastoid, splenius capitis and cervicis, and the erector spinae in the cervical region and quadratus lumborum, transversus abdominis, the abdominal obliques, and the erector spinae in the lumbar region. While flexors of the spine are innervated by the ventral rami of spinal nerves or the spinal accessory nerve (CN XI), the extensors are innervated by the dorsal rami of spinal nerves. Since the lateral flexors include members from both of these groups, their innervation varies. The term "spinal muscles" typically refers to the dorsal rami innervated splenius, erector spinae, and transversospinalis muscles. In this section, the functional anatomy of the spinal muscles and the genetic basis for their development in the embryo will be discussed.

Functional Anatomy of the Spinal Muscles

Splenius capitis and cervicis occupy the posterior aspect of the cervical region, deep to the trapezius and the rhomboids (Fig. 1.8A). They take origin from the ligamentum nuchae and cervical and thoracic spinous processes and insert onto the mastoid process and occipital bone (capitis) or the cervical transverse processes (cervicis)

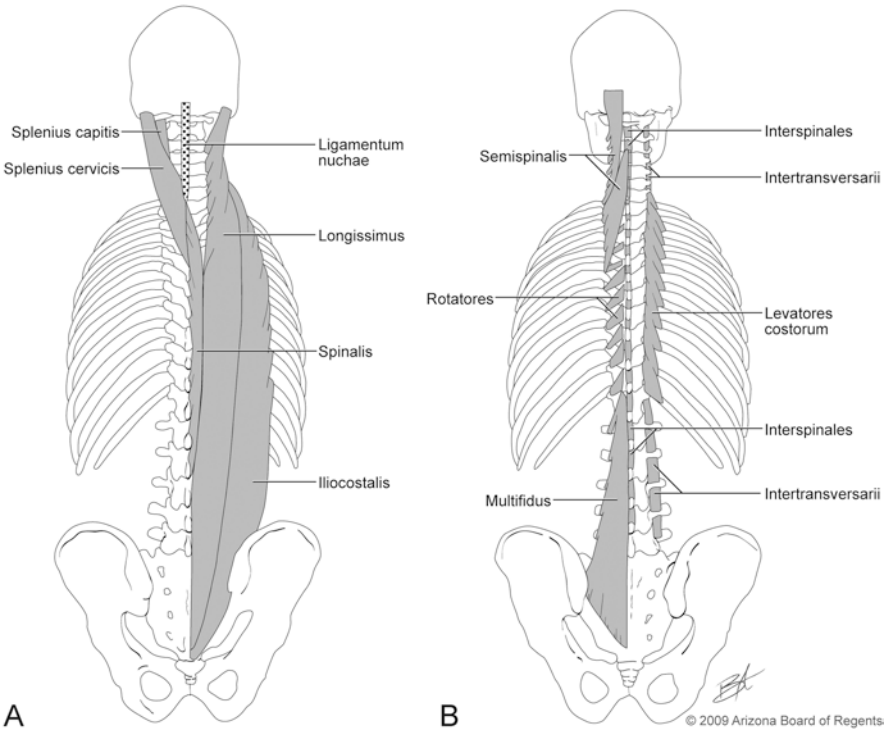

Fig. 1.8 Muscles of the back. (**A**) On the left, the superficial splenius muscles; on the right, the erector spinae muscles, including iliocostalis, longissimus, and spinalis. (**B**) On the left, the transversospinalis muscles, including semispinalis, multifidus, and rotatores; on the right, the levatores costarum, intertransversarii, and interspinales muscles (Drawing by Brent Adrian)

(Fig. 1.8A). Bilateral contraction of splenius capitis and cervicis extends the head and cervical spine while unilateral contraction laterally flexes and rotates the neck to the ipsilateral side.

Lying deep to the splenius layer, the erector spinae consist of three longitudinal columns of muscle (Fig. 1.8A). These muscles arise via a common tendon from the iliac crest, sacrum, and lumbar spinous processes. From lateral to medial, the columns include (1) iliocostalis, which attaches to the ribs and cervical transverse processes; (2) longissimus, which attaches to the ribs, thoracic and cervical transverse processes, and mastoid process; and (3) spinalis, which spans adjacent spinous processes and terminates on the occipital bone. Unilateral contraction of the erector spinae muscles laterally flexes and rotates the spine to the ipsilateral side while bilateral contraction extends the spine.

The transversospinalis muscles lie deep to the erector spinae. These muscles occupy the region between the transverse and spinous processes and include the semispinalis, multifidus, and rotatores muscles (Fig. 1.8B). The semispinalis muscles are located in the thoracic and cervical regions, while the rotatores are promi-

nent in the thoracic region. In contrast, the multifidus extends along the length of the spine but is most developed in the lumbar region. Unilateral contraction of the transversospinalis muscles rotates the spine to the contralateral side, while bilateral contraction extends the spine. These muscles also stabilize adjacent vertebrae and may have a proprioceptive function [23, 100].

Deep to the erector spinae are the levatores costarum, intertransversarii, interspinales, and the muscles of the suboccipital triangle (Fig. 1.8B). The levatores costarum are located between the transverse processes and the ribs and act as accessory muscles of respiration. The intertransversarii and the interspinales span the transverse and spinous processes, respectively, and help stabilize the spine. Finally, among the muscles of the suboccipital triangle, the rectus capitis posterior major and minor and the superior oblique extend the atlanto-occipital joints, while the inferior oblique rotates the atlanto-axial joints.

The extensor muscles of the spine may contribute to either the initiation or the progression of scoliotic curves [30, 50, 85, 92, 157]. Asymmetry of the spinal extensors, especially multifidus, has been reported in individuals with idiopathic scoliosis, including different degrees of hypertrophy, atrophy, fiber type distribution, centralization of nuclei, electromyographic activity, and disruption of sarcotubular and myofibrillar elements [1, 7, 22, 24, 30, 32, 50, 52, 71, 85, 92, 118, 120, 125, 140, 165, 168, 169]. Whether these conditions are responsible for the development of idiopathic scoliosis, its progression, or both, is unclear.

Development of Spinal Muscles

The spinal muscles that function to stabilize and extend the vertebral column are derived from the dorsal half of the myotome, from the occipital, thoracic, lumbar, and sacral somites. The origins of spinal muscles lie within a highly mitogenic myogenic progenitor cell (MPC) population located in the dorsomedial margin of the dermomyotome. These cells migrate subjacently to a space between the dermomyotome and the sclerotome where they exit the cell cycle and differentiate into mononucleated myocytes (Fig. 1.6, [43, 105]). The myotome expands along both the medial/lateral and dorsal/ventral axes by successive waves of MPC migration from the dermomyotome [42, 43, 66, 106]. This is followed by fusion of the myocytes into the multinucleated myotubes and morphogenic remodeling into the pattern of the adult spinal muscles [158].

The genetic basis of skeletal muscle development has been an area of intense study. The myogenic bHLH transcription factor family, including MyoD (*Myod1*), myf-5 (*Myf5*), myogenin (*Myog*), and MRF4 (*Myf6*), has been shown to be essential to initiate and maintain the myogenic program in cells fated to the myogenic lineage. The phenotypes of individual and compound null mutants reveal that these factors can be split into a specification subclass (myf-5 and MyoD) and a differentiation subclass (myogenin and MRF4). Interaction between the myogenic bHLH factors and members of the myocyte enhancer factor-2 (MEF2) family of MADS-

box transcription factors enhance muscle differentiation by increasing affinity of DNA binding and expanding the number of target genes that can be activated (reviewed in [4, 97]). The activity of Mef-2 and the myogenic factors are controlled in part by their association with chromatin remodeling proteins histone acetyltransferases (HATs) and histone deacetylases (HDAC) that promote and repress muscle-specific transcription, respectively. Calcium/calmodulin-dependent protein kinase (CaMK)-dependent phosphorylation of HDAC5 leads to its dissociation with MEF2 and transport out of the nucleus [89, 90]. Acetylation of MyoD and myf-5 through p300 or PCAF increases affinity of the transcription factors for its DNA target and promotes transcription of myogenin and MRF4 as well as induces cell cycle arrest [109, 115, 126].

Specification of MPCs within the somite fated to become the epaxial muscles is dependent on paracrine factors secreted by adjacent tissues. These signals direct the competence of the cells to initiate the myogenic program and promote the amplification of these committed progenitor cells in the dorsal/medial lip of the dermomyotome. Because of its role in specification, initiating *Myf5* transcription has been used as a readout of specification. A combination of sonic hedgehog (*Shh*) secreted from the notochord and Wnts from the dorsal neural tube and surface ectoderm are implicated in this process [14, 39, 117]. Based on explant experiments, *Wnt1* is able to induce the transcription of *Myf5* [145]. The activity is transduced by frizzled receptors 1 and 6 through the canonical β-catenin pathway [12]. The role of Shh in specification was first predicted by the absence of *Myf5* expression in the region of the epaxial myotome in *Shh* null embryos [13]. Further, mutations in Gli transcription factors, which transduce Shh signaling, also display a deficit in *Myf5* expression [87]. Consistent with these observations, the *Myf5* epaxial enhancer is dependent on a consensus binding sequence for Gli transcription factors and consensus binding sequence for Tcf/Lef, the β-catenin cofactor [12, 142, 153].

Though the cellular events associated with establishing the early muscle masses are now well described, as well as the genetic basis for muscle differentiation, less is known about subsequent events associated with establishing individual muscle groups from these masses. Embryonic muscles experience rapid growth, while the early muscle masses in the dorsal body wall, limb, hypoglossal chord, and head undergo several morphological processes (splitting, fusion, directional growth, and movement) in order to establish the appropriate shape, position, and fiber orientation of neonatal muscle. Further, they must coordinate with the growth and differentiation of tendons, ligaments, connective tissue, and skeletal elements to establish the appropriate origin and insertion sites on the bones. Patterning of muscle is dependent on innervation [164] and extrinsic signals from the surrounding tissue [61, 68]. This is mediated at least in part through mesodermal cells expressing *Tcf4* [68] and both intrinsic and extrinsic cues from members of the Hox gene family [2, 6]. In addition, the occurrence of defects in the multifidus muscles of mice with *Lfng* and *Dll3* mutations suggests a previously unappreciated role for Notch signaling in the patterning of the spinal muscles [51]. However, a clear understanding of the combination of local and global signals that directs individual and functional groups of muscles remains poorly understood.

Tendon Development

Tendons consist of fibroblast-like cells, called tenocytes, encased in a complex of collagen fibrils comprised of type I, III, IV, V, and VI collagen, Tenomodulin, and sulfated proteoglycans, including decorin, biglycan, fibromodulin, lumican, and aggrecan [133]. The embryonic formation of tendons occurs through the alignment of tenocytes along a linear track, followed by the deposition of the collagen fibrils. Tenocytes in mature tendon are thought to be in a non-proliferative quiescent state, with additional growth associated with an increase in collagen production [67]. Repair of tendons appears to be dependent on a localized stem cell population predicting an approach to injury repair similar to skeletal muscle [141].

The coordinated development of tendons along with muscle and skeletal elements is essential to the proper functioning of the musculoskeletal system [18]. However, the cellular origins of tendons and the regulator pathways that control their specification and differentiation are poorly understood. The identification of the bHLH transcription factor, scleraxis, as a tendon-specific marker accelerated research in this area [155]. Consistent with its intimate relationship to the epaxial muscles and vertebrae, the axial tendon is derived from a subdomain of the somite referred to as the syndetome, which is located between the myotome and sclerotome (Fig. 1.7) [17, 132]. The syndetomal cells are derived from an interaction between the sclerotome and myotome. Expression of *Fgf4* and *Fgf8* in the myotome is both necessary and sufficient for scleraxis expression in sclerotomal cells in the future syndetome region [17, 18]. Within the sclerotomal cells, the FGF induces an ERK MAP kinase-mediated cascade that requires activation of the ETS transcription factor, *Etv4/Pea3* [16, 136]. It appears that there are also inhibitory signals generated from the sclerotome that limit the size of the syndetome. Overexpression of *Pax1* reduces the scleraxis expression domain in the sclerotome, a compound mouse mutation in *Sox5/Sox6* lead to an expansion of the scleraxis-expressing domain [18].

Several regulators have been identified that are essential for tenocyte differentiation as well as tendon maturation and maintenance leading to a simple model for tendogenesis. TGFβ and FGF signaling specify tenocytes from mesenchymal progenitors in part by the induction of the bHLH transcription factor, Scleraxis (*Scx*) [48, 114]. This is followed by the expression of Mohawk (*Mkx*) and early growth response factors 1 and 2 (*Egr1* and *Egr2*) in tenocytes. These genes are maintained in the tendons after birth, while *Scx* transcription levels diminish [133]. This predicts distinct functions for the three transcription factors that ultimately promote the expression of elements of the collagen fibrils associated with tendon development and adult maintenance and repair [5].

Targeted null mutations in mice have been leveraged extensively to determine the function of these genes in tendogenesis. Inactivation of Scx (*Scx−/−*) resulted in a significant loss of tendons in the limbs, trunk, and tail [102]. However, this did not eliminate all tendons, suggesting the presence of additional factors that are differentially used in tenocyte differentiation. In contrast, *Mkx−/−* mice displayed a

reduction in tendon mass through hypoplastic tendon fibers but no reduction in tenocyte numbers [102]. This was recapitulated in rats, where *Mkx* was inactivated using the CRISPR-Cas9 system, suggesting an essential role for the gene in tendon maturation [143]. *Egr1* and *Egr2* mutations lead to a reduction of collagen fibrils and the expression of *Scx* and *Mkx* in embryonic tendons consistent with providing positive feedback in the tendon signaling cassette. It is important to note that none of these mutations lead to a complete ablation of tendon development. This predicts functional redundancy or the existence of additional regulators that have not yet been identified.

In adults, tendons participate in homeostatic sensing that matches force-transmission capacity to mechanical load through a mechanical sensing system. This leads to the differentiation of mesenchymal stem cells associated with the tendon to tenocytes [69]. This appears to recapitulate the embryonic signaling pathway, as it requires *Scx*, *Mkx*, and *Egr1* [54, 69, 93]. The general transcription factor II-I repeat domain-containing protein 1 (*Gtf2ird1*) has been found to be important in mechanical sensing. In response to stretching, *Gtf2ird1* translocates to the nucleus from the cytoplasm where it induces *Mkx* expression [69]. Interestingly, extreme stretching leads to tendon damage and a reduction of tendon-specific gene expression and an increase in osteogenic and chondrogenic gene markers [93]. This can be recapitulated under conditions of mild stress, including the expression of *Sox6*, *Sox9*, and aggrecan, by inactivation of *Mkx* [143]. This predicts that *Mkx* plays the dual role of promoting tendon differentiation and preventing chondrogenesis.

Summary

The vertebral column, spinal musculature, and associated tendons arise from paraxial mesoderm which undergoes segmentation in a rostral to caudal direction to form pairs of somites. Each somite differentiates into four cell lineage-specific compartments that contribute to the spine, including the sclerotome (vertebrae and ribs), myotome (skeletal muscle), dermomyotome (dermis and skeletal muscle progenitor cells), and syndetome (tendons). 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. In this chapter, we focused on three essential aspects of somite formation and patterning, including the establishment of the intersomitic boundary, somite epithelialization, and rostral/caudal polarity, and the subsequent development of the vertebrae, IV discs, and associated spinal muscles and tendons.

The vertebrae are derived from the sclerotome compartment of the somites. The transition from sclerotome to vertebrae can be divided into distinct processes for the ventral structures (vertebral body and intervertebral discs) and dorsal neural arch structures (pedicles, laminae, spinous and transverse processes) based on both cell origin and genetic regulation. An additional layer of regulation, via members of the Hox transcription factor family, is required to confer the distinctive regional

characteristics of the cervical, thoracic, lumbar, sacral, and caudal vertebrae. The IV discs, comprised of a nucleus pulposus, annulus fibrosus, and cartilage end plates, were originally thought to be derived solely from the sclerotome, but the nucleus pulposus has been shown to be derived from the notochord. As a result, we must now invoke a more complicated model for the development of the IV discs that requires the coordination of multiple independent signaling pathways such as the *Shh* and TGF-beta superfamily.

The spinal muscles that function to stabilize and extend the vertebral column are derived from a highly mitogenic myogenic progenitor cell population located in the dorsomedial margin of the dermomyotome. These cells migrate to a space between the dermomyotome and the sclerotome where they exit the cell cycle and differentiate into mononucleated myocytes, forming the myotome. Though the cellular events associated with establishing the early muscle masses are now well described, as well as the genetic basis for muscle differentiation, less is known about the events leading to the development of individual and functional groups of spinal muscles. The tendons associated with these muscles are derived from a subdomain of the somite referred to as the syndetome, which is located between the myotome and sclerotome. The cellular origins of tendons and the regulator pathways that control their specification and differentiation are also poorly understood, although recent work in this area has identified several regulators, such as TGFβ and FGF signaling, as essential for tenocyte differentiation as well as tendon maturation and maintenance.

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