Chapter 2
Development 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 the torso. The vertebral column is capable of extension, flexion, lateral flexion (side to side), and rotation. However, the degree to which the spine is capable of these movements varies by region. These regions, including the cervical, the thoracic, the lumbar, and the sacrococcygeal spine, form four curvatures (Fig. 2.1). The thoracic and the sacrococcygeal curvatures are established in fetal development, while the cervical and the thoracic curvatures develop during infancy. The cervical curvature arises in response to holding the head upright, while the lumbar curvature develops as an infant begins to sit upright and walk. Congenital defects and degenerative diseases can result in exaggerated, abnormal curvatures. The most common of these include a thoracic kyphosis (or hunchback deformity), a lumbar lordosis (or 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 vertebral column and skeletal muscle development from somites. In this chapter, we will review the embryonic development of the spine and associated muscles and link them to the functional anatomy of these structures in the adult.

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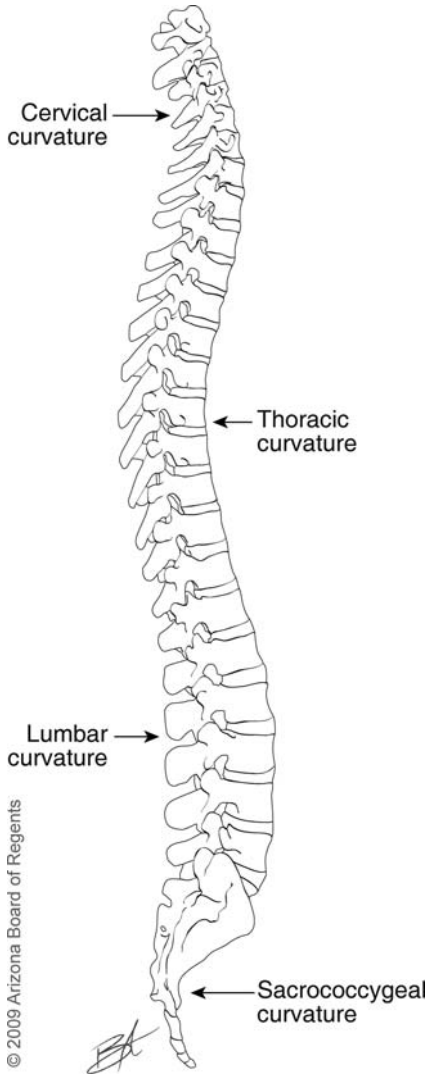

Fig. 2.1 Lateral view of the vertebral column, illustrating the spinal curvatures. Drawing by Brent Adrian

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: 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 the 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. This process is reviewed in Chapter 1. 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. Depending on the animal, this varies from the simple cleavage of the PSM by fissures initiated along either the medial or the lateral surfaces as seen in *Xenopus* and zebrafish to a more dynamic ball-and-socket shape with a reshuffling of cells across the presumptive somite–PSM boundary in chicks (Wood and Thorogood 1994, Henry et al. 2000, Jiang et al. 2000, Kulesa and Fraser 2002, Afonin et al. 2006, Kulesa et al. 2007). The activity is an intrinsic property of the PSM, as it will occur in explants in the absence of the adjacent ectoderm and endoderm (Palmeirim et al. 1998). 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 (Sato et al. 2002). Transcription factors *Mesp2* (and its chicken homologue *cMeso1*) and *Tbx18* have also been shown to play a role in forming boundaries (Saga et al. 1997, Buchberger et al. 1998, Tanaka and Tickle 2004, Takahashi and Sato 2008). 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 (Sato and Takahashi 2005). 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 Tam and Trainor 1994,

Keynes and Stern 1988), resulting in the condensation of mesenchyme into an epithelial ball, surrounding a mesenchymal core, called the somitocoel. This occurs in a gradual process with the cells along the rostral edge of somite 0 becoming epithelia at the time of boundary formation (Dubrulle and Pourquié 2004). Epithelialization is complete with the formation of the next boundary (Fig. 2.2). The transcription factors paraxis and *Pax3* are required to direct MET in cells of somite +1 (Burgess et al. 1995, 1996, Schubert et al. 2001). Inactivation of paraxis results in somites formed of loose clusters of mesenchyme separated by distinct intersomitic boundary formation (Fig. 2.2). 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 2 (*Snai1* and *Snai2*), which are expressed in oscillating patterns in the PSM (Dale et al. 2006). Snail genes are transcriptional repressors that are able to block the transcription of paraxis and cell adhesion molecules associated with epithelialization (Batlle et al. 2000, Cano et al. 2000, Barrallo-Gimeno and Nieto 2005, Dale et al. 2006). Overexpression of snail2 will prevent cells from contributing to epithelium in somite +1. Thus switching off snail gene expression may be essential for the timing of MET.

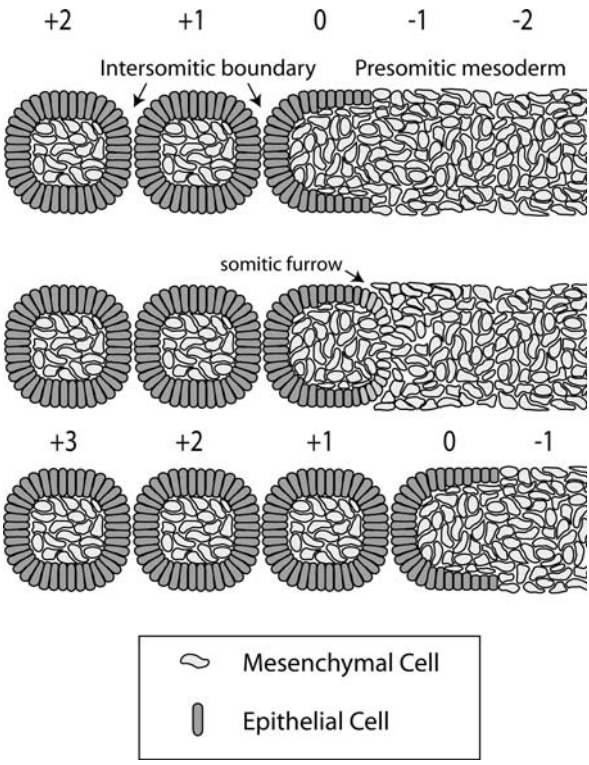

Fig. 2.2 Schematic of mouse somite formation. Lateral view of somites budding off of the rostral end of the presomitic mesoderm demonstrates the stepwise transition of mesenchymal cells to epithelium. By convention, the forming somite is labeled "0" and the newest somite is "+1"

In contrast to boundary formation, signals from the surface ectoderm are required to induce MET and the expression of paraxis (Duband et al. 1987, Sosic et al. 1997, Correia and Conlon 2000, Sato et al. 2002, Sato and Takahashi 2005, Linker et al. 2005). Wnt signaling has been implicated in regulating this process with Wnt6 and Wnt11 as the most likely candidates (Wagner et al. 2000, Linker et al. 2005, Geetha-Loganathan et al. 2006, Schmidt et al. 2004). Ectopic expression of *Wnt6* is able to rescue somite epithelialization where the ectoderm has been removed. Further, *Wnt6* is able to induce paraxis transcription in a beta-catenin-dependent manner, predicting a mechanism of action (Linker et al. 2005).

Somite epithelialization is associated with an increase in the expression of members of the cadherin superfamily and cell adhesion molecules (Duband et al. 1987, Tam and Trainor 1994). 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 (Radice et al. 1997, Horikawa et al. 1999). The phenotype of the cadherin mutations is 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 (Nakaya et al. 2004). Both the inhibition and the 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 (Nakaya et al. 2004).

Rostral/Caudal Polarity of Somites

Spatial identity along the rostral/caudal axis is established in each somite at the time of its formation (Aoyama and Asamoto1988). 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 leads to the loss of rostral- and caudal-specific gene expression, the fusion of the vertebrae, and the segmental pattern of the peripheral nerve pattern (Swiatek et al. 1994, Conlon et al. 1995, Oka et al. 1995, de la Pompa et al. 1997, Hrabe de Angelis et al. 1997, Kusumi et al. 1998, Barrantes et al. 1999, Koizumi et al. 2001, Dunwoodie et al. 2002, Schuster-Gossler et al. 2009). 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) (Saga et al. 1997, Takahashi et al. 2000). 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 (Yasuhiko et al. 2006), which in turn represses transcription of the *Dll1* ligand in the rostral domain through the transcriptional repressor, ripply2 (Morimoto et al. 2007). In the caudal half of somite 0, *Mesp2* transcription is repressed in a presenilin-1-dependent manner (Koizumi et al. 2001, Takahashi et al. 2003, Yasuhiko et al. 2006).

Maintenance of rostral/caudal polarity after somite formation requires paraxis, which is associated with the regulation of somite epithelialization (Johnson et al. 2001). 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 (*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 the caudal halves of the somite after their specification in the PSM (Johnson et al. 2001).

The Anatomy and Development of the Vertebrae and IV Discs

A typical vertebra consists of two parts: the body and the vertebral (or neural) arch (Fig. 2.3a). The vertebral body is located anteriorly and articulates with the adjacent IV discs (Figs. 2.1, 2.3, and 2.4). Together, the vertebral body and the arch form a central, vertebral foramen, and, collectively, the foramina create a vertebral canal, protecting 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.

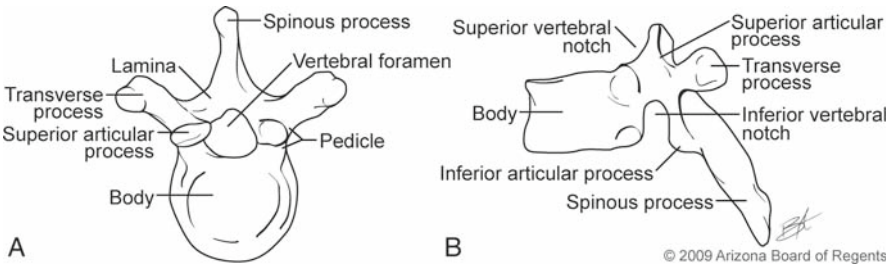

Fig. 2.3 Features of a typical human vertebra. **a**. Superior and **b**. lateral view. Drawing by Brent Adrian

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. 2.1). 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. 2.3a). The superior and inferior notches of adjacent pedicles form the intervertebral foramina, which transmit the spinal nerves (Figs. 2.1 and 2.3b). 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 processes, 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 muscle groups (Fig. 2.3a, b). In addition, the transverse processes articulate with the costal tubercles to form the costovertebral joints. Finally, the superior and the inferior articular processes of adjacent vertebrae interlock to form the zygapophysial (or facet) joints (Fig. 2.4). 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. 2.1). 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 the inferior articular processes face superiorly and inferiorly,

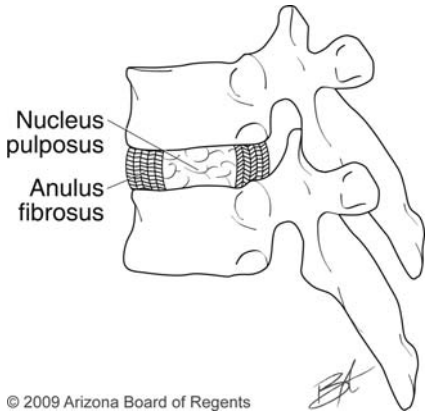

Fig. 2.4 Structure of the intervertebral disc. Drawing by Brent Adrian

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 a 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 an 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. 2.3b). Typically, a thoracic vertebral body articulates with two costal heads, 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. 2.3b), 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 superior articular processes that provide attachment sites for ligaments as well as the erector spinae and transversospinalis muscle groups (Fig. 2.1).

The sacrum is typically formed by the fusion of five sacral vertebrae (Fig. 2.1). The sacral canal transmits the spinal roots of the cauda equina and ends at the sacral hiatus, an important landmark for administering a caudal epidural. In addition, four 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. Similar to the sacrum, the coccyx is typically formed by the fusion of four coccygeal vertebrae (Fig. 2.1). Although the coccyx is rudimentary in humans, it 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. 2.4). However, an IV disc is not present between the atlas and the 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 (Moore and Dalley 2006). 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 the cervical and lumbar IV discs are wedge-shaped, contributing to the curvatures of the vertebral column (Fig. 2.1). Each IV disc is composed of an outer fibrocartilaginous ring, the anulus fibrosus, and a central gelatinous core, the nucleus pulposus (Fig. 2.4). Composed primarily of collagen fibers, the anulus fibrosus is characterized by a series of concentric layers, or lamellae (Fig. 2.4). The lamellae serve to resist the expansion of the nucleus pulposus during compression (Cailliet 1988). The nucleus pulposus is composed of water, proteoglycans, and scattered collagen fibers.

The vertebrae and IV discs are stabilized by robust spinal ligaments which function to restrict movements and to minimize the need for continual muscular contraction. The major spinal ligaments are illustrated in Fig. 2.5. 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. 2.5). This ligament prevents hyperextension of the spine and anterior herniation of the nucleus pulposus. This ligament 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. 2.5). 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. 2.5). These ligaments contribute to the posterior wall of the vertebral canal, thus helping to protect the spinal cord. The ligamenta flava are highly elastic; they support

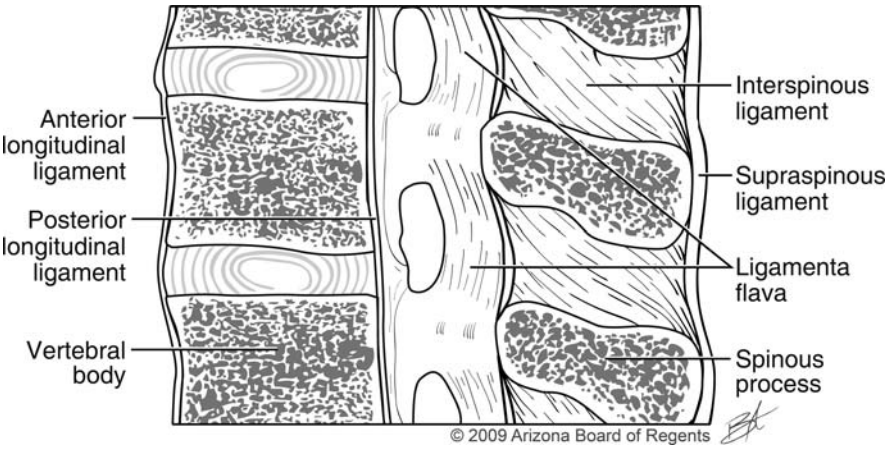

Fig. 2.5 Major ligaments of the spine. Lateral view illustrating the ligamentum flava, supraspinous, interspinous, and anterior and posterior longitudinal ligaments. Drawing by Brent Adrian

the normal curvatures of the spine, resist separation of the laminae during flexion, and assist 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. 2.5). 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. 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, in combination with the mesenchymal cells of the somitocoele, form the sclerotome (Fig. 2.6) (reviewed in Dockter 2000). Vertebrae are formed through the process of endochondral ossification. As such, a chondrogenic model of the vertebrae and ribs is developed from sclerotomal cells. Replacement of the cartilage with bone then follows. The molecular events that regulate this process are common with the appendicular and part of the cranial skeleton. These pathways are reviewed elsewhere (Mackie et al. 2008). 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 (1) the vertebral body and the intervertebral disc at the ventral midline, (2) lateral neural arches that will give rise to the pedicles and transverse process, and (3) the dorsal spinous process. Patterning of the vertebrae along the dorsal/ventral axis is controlled by opposing gradients derived from the notochord and the 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 (Fan and Tessier-Lavigne 1994, Peters et al. 1995, McMahon et al. 1998, Furumoto et al. 1999). *Pax1* and *Pax9* are essential for the maintenance of sclerotomal cells (Furumoto et al. 1999). Compound mutations of these two genes in the mouse lead to loss of the vertebral body and proximal ribs (Peters et al. 1999). 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 (Takahashi et al. 2007, Capellini et al. 2008). The homeodomain-containing genes *Meox1* and *Meox2* have also been implicated in vertebrae development. *Meox1*-deficient mice display segmental fusions in the occipital/cervical region and the *Meox1/Meox2* compound null mutations lead to a profound loss of vertebral structures (Mankoo et al. 2003, Skuntz et al. 2009).

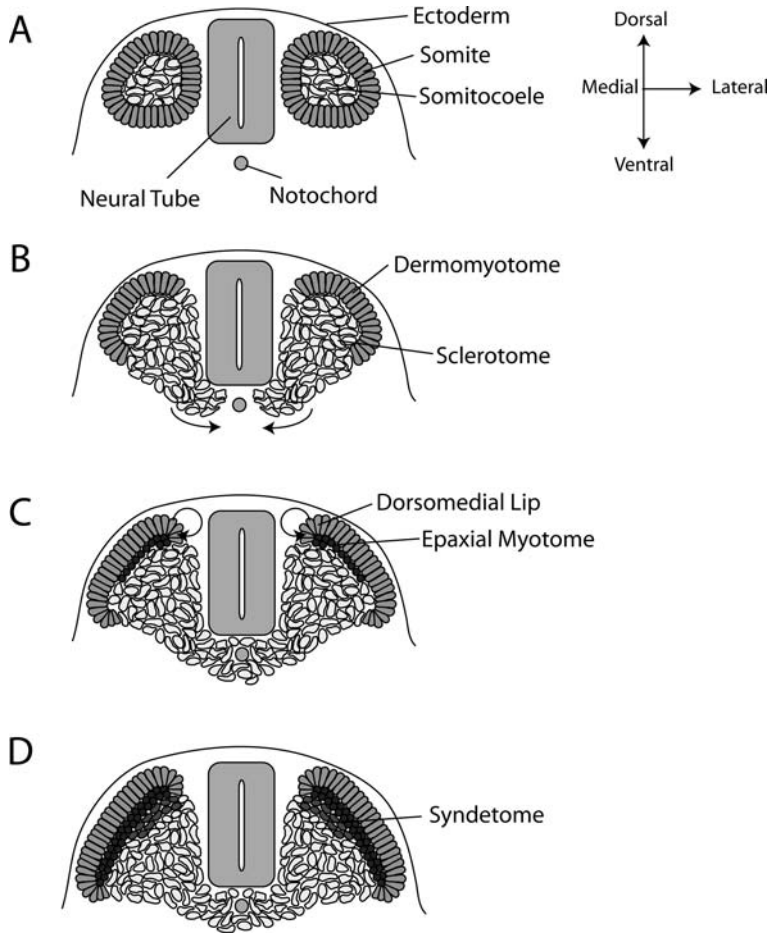

Fig. 2.6 Schematic of somite differentiation. **a**. Newly formed somites consist of an epithelium and a mesenchymal somitocoele. **b**. The sclerotome compartment forms as the medial/ventral region of the somite undergoes MET. **c**. The epaxial myotome forms as epithelial cells of the dorsomedial lip of the dermomyotome undergo MET and migrate subjacently. **d**. The syndetome appears as cells from the myotome interact with dorsal sclerotomal cells

The dorsal elements of the vertebrae, including the spinous process and the dorsal part of the neural arches, require *Msx1* and *Msx2* transcription induced by BMP2 and BMP4 expressed in the surface ectoderm and the roof plate of the neural tube (Monsoro-Burq et al. 1994, 1996, Watanabe et al. 1998). SHH and the BMPs are mutually antagonistic in their actions (Pourquié et al. 1993). 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 (Tonegawa et al. 1997, Watanabe et al. 1998). The corollary is also true with SHH-expressing cells grafted dorsally, inhibiting *Msx1* transcription and preventing chondrogenesis (Watanabe et al. 1998).

Remodeling the Sclerotome into Vertebrae

The formation of the vertebrae is dependent on the highly coordinated migration of sclerotomal cells both toward the midline and along the rostral/caudal axis (Fig. 2.6) (reviewed in Brand-Saberi and Christ 2000). Soon after EMT, cells from the ventral/medial sclerotome migrate toward the notochord, where they will contribute to the vertebral body and intervertebral discs. This is followed by the migration of the lateral sclerotomal cells dorsally to form the vertebral pedicles and the laminae of the neural arches. At this point, cells of the rostral and caudal halves of the sclerotome can be distinguished visually based on their density. The caudal half of the sclerotome will proliferate and migrate toward the rostral domain of the adjacent somite (Fig. 2.7a). Fate mapping in chick embryos revealed that the caudal and rostral sclerotome halves of adjacent somites contribute equally to the vertebral body (Goldstein and Kalcheim 1992, Aoyama and Asamoto 2000). In contrast, the neural arches are derived almost solely from the caudal domain and the spinous process from the rostral domain.

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 (Saga et al. 1997). In contrast, disruption of the caudal identity of somites through inactivation of the Notch pathway leads to fused vertebral bodies and an absence of neural arches. As will be discussed in detail in later chapters in this book, 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 Sparrow et al. 2007). 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 (Johnson et al. 2001).

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 Wellik 2007). From classic studies in *Drosophila*, the *Hox* genes have long been known to regulate segmental identity in the insect body plan (Lewis 1978). 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

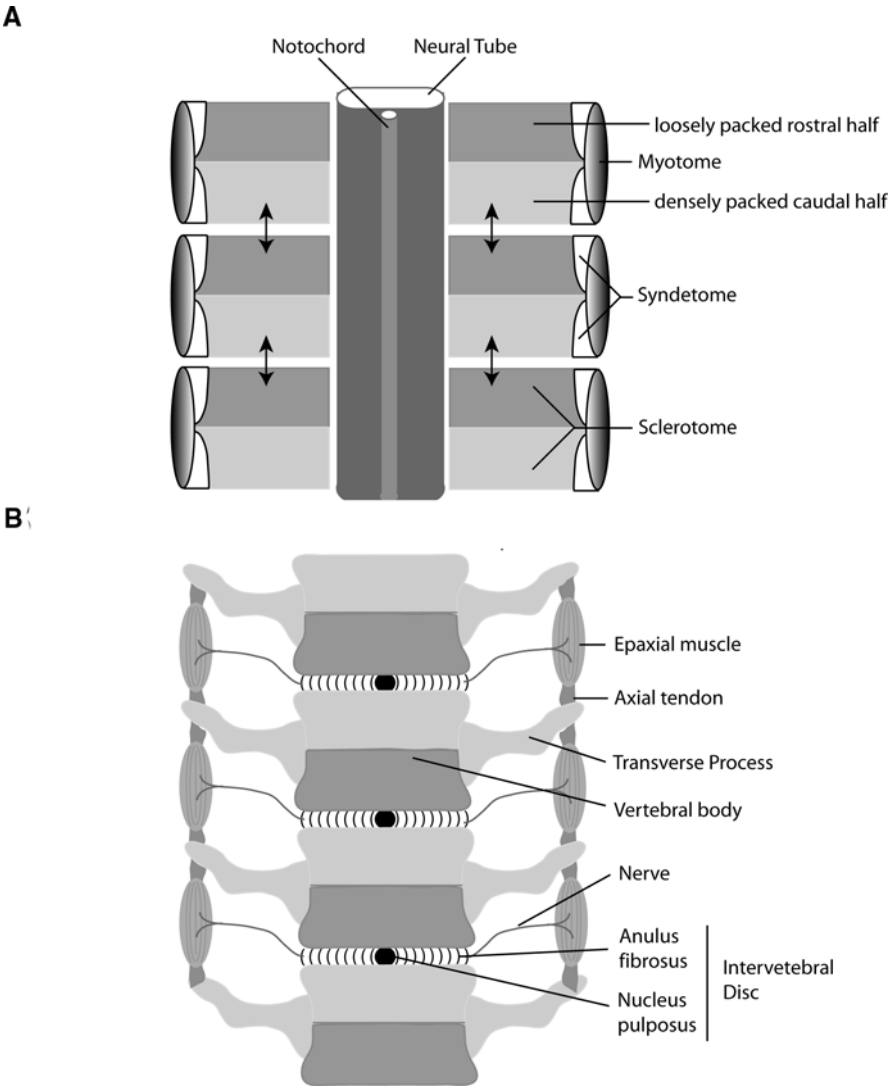

Fig. 2.7 Schematic of vertebral generation through sclerotome resegmentation. **a**. Ventral view of the sclerotome, syndetome, and myotome compartments. The caudal half of the sclerotome grows into the rostral half of the adjacent somite. **b**. Ventral view of the vertebral column with associated epaxial muscles and axial tendons. *Shading* represents the contribution of the rostral and the caudal sclerotomes to the vertebral bodies and transverse processes. The intervertebral disc forms at the site of sclerotome separation. Note the relationship of the muscle and bone after resegmentation

with *Hoxa3*/*Hoxd3* double mutant embryos, where the prevertebral elements that normally contribute to the atlas form a bone contiguous with the occipital bone (Condie and Capecchie 1994). Since this observation, similar homeotic transformations have been reported for paralogous mutations in the *Hox5*, *Hox6*, *Hox7*,

Hox8, *Hox9*, *Hox10*, and *Hox11* group genes (Chen et al. 1998, van den Akker et al. 2001, Wellik and Capecchi 2003, McIntyre et al. 2007). Consistent with the co-linear 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 (Wellik and Capecchi 2003).

The positional identity conferred by the *Hox* genes during vertebrae 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 (Kim et al. 2006). 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 Moens and Selleri 2006). The TALE genes play a larger role in patterning and regulating the transcription of the 5′ *Hox* genes in both a Hox-dependent and Hox-independent manner (Popperl et al. 1995, Maconochie et al. 1997, Berkes et al. 2004, Capellini et al. 2006).

Formation of the IV Discs

The genesis of the IV discs is intimately linked to somite polarity and sclerotome resegmentation and as such is dependent on the Notch/*Mesp2* signaling (Teppner et al. 2007). The annuli fibrosi of the IV discs forms from condensed mesenchyme derived from the somitocoele at the border of the rostral and caudal domains during resegmentation (Huang et al. 1996, Mittapalli et al. 2005). Somitocoele cells cannot be replaced by sclertomal cells derived from EMT in forming the IV disc predicting specification of a distinct lineage, now called the arthrotome (Mittapalli et al. 2005). Development of the annuli fibrosi 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 (Baffi et al. 2006). GDF-5 and BMP-2 promote cell aggregation and expression of the chondrogenic genes instead of osteogenic genes in the IV discs (Kim et al. 2003, Yoon et al. 2003, Li et al. 2004). The nucleus pulposus is derived from an expansion of notochord cells that move out of the cartilage of the adjacent vertebral body (Paavola et al. 1980). Survival of the notochord cells is dependent on the expression of *Sox5* and *Sox6* (Smits and Lefebvre 2003).

The Anatomy and Development of Spinal Muscles

The spinal muscles function to stabilize and achieve movements of the vertebral column. A number of muscle groups act on the spine. Those located anterior to the vertebral bodies act as flexors. These include longus capitis and colli, psoas

major, and rectus abdominis. Lateral flexion is achieved by the scalenes in the cervical region and quadratus lumborum, transversus abdominis, and the abdominal obliques in the lumbar region. The flexors and lateral flexors of the spine are innervated by the ventral rami of spinal nerves. In contrast, the extensors of the spine are located posterior to the vertebral bodies and are innervated by the dorsal rami of spinal nerves (Fig. 2.5). The term "spinal muscles" typically refers to the extensors of the spine. 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 trapezius and the rhomboids (Fig. 2.8a). They take origin from the ligamentum nuchae and cervical and thoracic spinous processes and insert onto the mastoid

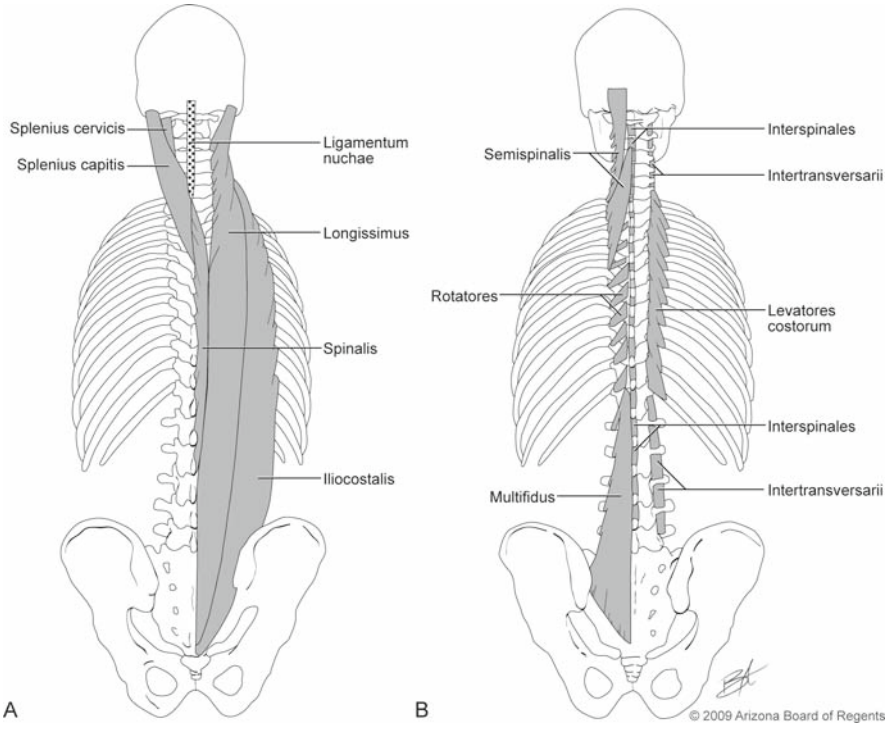

Fig. 2.8 Muscles of the back. **a**. On the *left*, the superficial splenius muscles; on the *right*, the erector spinae muscles, including iliocostalis, longissimus, and spinalis. **b**. On the *left*, the transversospinalis muscles, including semispinalis, multifidus, and rotatores; on the *right*, the levatores costarum, intertransversarii, and interspinales muscles. Drawing by Brent Adrian

process and occipital bone (capitis) or the cervical transverse processes (cervicis) (Fig. 2.8a). Bilateral contraction of splenius capitis and cervicis extends the head and the 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. 2.8a). 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. 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 the spinous processes, and include the semispinalis, multifidus, and rotatores muscles (Fig. 2.8b). The semispinalis muscles are located in the thoracic and cervical regions, while the rotatores are prominent 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 laterally flexes the spine and rotates it to the contralateral side, while bilateral contraction extends the spine. These muscles stabilize adjacent vertebrae and may have a proprioceptive function (Buxton and Peck 1989, Moore and Dalley 2006).

Deep to the erector spinae are the levatores costarum, intertransversarii, interspinales, and the muscles of the suboccipital triangle (Fig. 2.8b). 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 (Fidler and Jowett 1976, Meier et al. 1997, Mannion et al. 1998, Chan et al. 1999). Asymmetry of the spinal extensors, especially the multifidus muscle, has been reported in individuals with idiopathic scoliosis (Zuk 1962, Butterworth and James 1969, Fidler and Jowett 1976, Spencer and Zorab 1976, Alexander and Season 1978, Yarom and Robin 1979, Khosla et al. 1980, Reuber et al. 1983, Sahgal et al. 1983, Zetterberg et al. 1983, Ford et al. 1984, Bylund et al. 1987, Meier et al. 1997, Chan et al. 1999). Chan and colleagues (1999) analyzed MRI data for adolescents with idiopathic scoliosis and found multifidus abnormalities on the concave side of scoliotic curves. As multifidus achieves ipsilateral lateral flexion, this finding suggests that increased contractility or reduced fiber length may play a role in the development of a curvature. The documented increase in type II muscle fibers on the concave side of scoliotic curves, indicating a longer T2 relaxation time, could also contribute to the development of a lateral curve (Ford et al. 1984, Meier et al. 1997).

Development of Spinal Muscles

The spinal muscles that function to stabilize and achieve movements of 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. 2.6, Ordahl and Le Douarin 1992, Denetclaw et al. 1997). The myotome expands along both the medial/lateral and the dorsal/ventral axes by successive waves of MPC migration from the dermomyotome (Denetclaw et al. 1997, Kahane et al. 1998, Denetclaw and Ordahl 2000, Ordahl et al. 2001). This is followed by fusion of the myocytes into the multinucleated myotubes and morphogenic remodeling into the pattern of the adult spinal muscles (Venters et al. 1999).

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*) have 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 enhances muscle differentiation by increasing affinity of DNA binding and expanding the number of target genes that can be activated (reviewed in Molkentin and Olson 1996, Arnold and Braun 2000). 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 (HDACs) that promote and repress muscle-specific transcription, respectively. Calcium/calmodulin-dependent protein kinase (CaMK)-dependent phosphorylation of HDAC5 leads to its dissociation with MEF-2 and transport out of the nucleus (McKinsey et al. 2000, 2001). 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 (Puri et al. 1997, Sartorelli et al. 1999).

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 the surface ectoderm is implicated in this process (Reshef et al. 1998, Cossu and Borello 1999, Borycki et al. 2000). Based on explant experiments, Wnt1 is able to induce the transcription of *Myf5* (Tajbakhsh et al. 1998). The activity is transduced by Frizzled

receptors 1 and 6 through the canonical β-catenin pathway (Borello et al. 2006). 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 (Borycki et al. 1999). Further, mutations in Gli transcription factors, which transduce Shh signaling, also display a deficit in *Myf5* expression (McDermott et al. 2005). 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 (Summerbell et al. 2000, Teboul et al. 2003, Borello et al. 2006).

Though the cellular events associated with establishing the early muscle masses, as well as the genetic basis for muscle differentiation, are now well described, less is known about subsequent events associated with establishing individual muscle groups from these masses. Embryonic muscles experience rapid growth, while the early muscles 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 (Yang et al. 2001) and extrinsic signals from the surrounding tissue (Jacob and Christ 1980, Kardon et al. 2003). This is mediated at least in part through mesodermal cells expressing Tcf4 (Kardon et al. 2003) and both intrinsic and extrinsic cues from members of the *Hox* gene family (Ashby et al. 2002, Alvares et al. 2003). However, a clear understanding of the combination of local and global signals that direct individual and functional groups of muscles remains poorly understood.

Tendon Development

The coordinated development of tendons along with muscle and skeletal elements is essential to the proper functioning of the musculoskeletal system. However, the cellular origins of tendons and the regulator pathways that control their specification and differentiation are poorly understood. The recent identification of the bHLH transcription factor, scleraxis, as a tendon-specific marker has accelerated research in this area (Tozer and Duprez 2005). Consistent with its intimate relationship to the epaxial muscles and vertebrae, the axial tendon is derived from a subdomain of the domain of the somite referred to as the syndetome, which is located between the myotome and the sclerotome (Fig. 2.7) (Brent et al. 2003). The syndetomal cells are derived from an interaction between the sclerotome and the 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 (Brent et al. 2003, 2005). Within the sclerotomal cells, the FGF induces an ERK MAP kinase-mediated cascade that requires activation of the ETS transcription factor, *Etv4/Pea3* (Brent and

Tabin 2004, Smith et al. 2005). 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* leads to an expansion of the scleraxis-expressing domain (Brent et al. 2005).

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