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The Lateral Somitic Frontier in Ontogeny and Phylogeny*

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Abstract

The vertebrate musculoskeletal system comprises the axial and appendicular systems. The postcranial axial system consists of the vertebrae, ribs and associated muscles, and the appendicular system comprises the muscles and skeleton of the paired appendages and their respective girdles. The morphology, proportions, and arrangements of these parts have undergone tremendous variation during vertebrate history. Despite this vertebrate diversity, the cells that form all of the key parts of the musculoskeletal system during development arise from two populations of embryonic mesoderm, the somites and somatic lateral plate. Nowicki et al. (2003. *Mech Dev* 120:227–240) identified two dynamic domains in the developing chick embryo. The primaxial domain is populated exclusively by cells from the somites. The abaxial domain includes muscle and bone that develop within lateral plate-derived connective tissue. The boundary between the two domains is the lateral somitic frontier. We hypothesize that the primaxial and abaxial domains are patterned independently and that morphological evolution of the musculoskeletal system is facilitated by partially independent developmental changes in the abaxial and primaxial domain. Here we present our hypothesis in detail and review recent experimental and comparative studies that use the concept of the lateral somitic frontier in the analysis of the evolution of the highly derived chelonian and limbless squamate body plans.

There are more than 50,000 extant vertebrate species, representing over 500 million years of evolution. During that time, the vertebrate musculoskeletal systems have adapted to aquatic, terrestrial, fossorial, and arboreal lifestyles, while simultaneously retaining functionally integrated axial and appendicular skeletal systems. All limbed vertebrates have a postcranial axial system consisting of vertebrae, ribs and associated muscles, and an appendicular system comprising muscles and bones of the paired appendages and their respective girdles. Primitively, vertebrates lacked paired appendages and their skeletons were comprised only of a cranium and vertebral column. As vertebrates evolved novel modes of locomotion (e.g. flight, burrowing, jumping) this primitive body plan was elaborated upon. Paired appendages appeared first in jawless vertebrates (Janvier, '96). With the origin of tetrapods, the axial system evolved a more extensive rib cage and a distinct neck (Daeschler et al., 2006). Elements of the appendicular system became adapted for weight bearing, and the articulation between the skull and pectoral girdle, characteristic of fish, was lost (Jarvik, '80; Janvier, '96). Additional modifications of the vertebrate body include the evolution of wings for active flight in reptiles (pterosaurs and birds) and mammals, and the loss of limbs in various lineages of fish, amphibians, squamates, and mammals (Fig. 1). By necessity, these evolutionary innovations occurred while the functional integrity of the musculoskeletal system was maintained.

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E. A. Buchholz and C. C. Stepien (2009) argue that the unusual number of 'cervical' vertebrae in sloths result from a shift of abaxial structures relative to primaxial structures.

Despite the diversity in adult form, the development of the vertebrate body plan remains quite conserved. The cells that give rise to the post-cranial musculoskeletal system originate from two populations of embryonic mesoderm. The axial skeletal elements arise from the paraxial somites, whereas the appendicular skeleton and sternum arise from the somatic lateral plate mesoderm. However, all the postcranial, striated, muscle of both systems arises from the somites, with somitic cells infiltrating the lateral plate to form limb and body wall muscles. This demands extensive integration between the two mesodermal populations to ensure that somitic cells are patterned appropriately for their region of the body, e.g. neck vs. limb vs. thorax.

Muscles of the vertebrate body are classically described as epaxial or hypaxial according to the innervation from either dorsal or ventral rami of the spinal nerves, respectively. This classification is based on adult positional and functional criteria. Nowicki et al. (2003) defined new terminology using embryonic criteria to distinguish domains relative to embryonic patterning. They (Nowicki et al., 2003) identified two mesodermal domains in the developing musculoskeletal system based on the mixing of somitic and lateral plate cells during morphogenesis. The primaxial domain comprises somitic cells that develop within somite-derived connective tissue, and the abaxial domain includes muscle and bone that develop within lateral plate-derived connective tissue. The boundary between the two domains is the lateral somitic frontier (Fig. 2).

In this review we present our hypothesis on the developmental and evolutionary significance of the frontier and the perspective it provides on how the vertebrate body plan may evolve while retaining the essential integration between the axial and appendicular systems. We also review recent studies that use the conceptual framework of the lateral somitic frontier in the analysis of the evolution of the chelonian and limbless squamate body plans.

Development and the evolution of morphology

Embryonic pattern formation occurs as cells respond to an environment generated by their own transcriptional history and signals provided by their surroundings. We are now aware of many of the molecular players involved in local signaling environments, including transcription factors, matrix proteins, and secreted signaling factors (Carroll et al., 2005). The fact that these players are used over and over again in different tissues and organs reflects the evolution of increasingly complex regulatory machinery that enables the same toolkit genes to be utilized in multiple contexts (Carroll et al., 2005; Davidson and Erwin, 2006). An excellent example of this phenomenon is the expression of the Abdominal B-related Hox genes along the caudal body axis and in the limb fields. This has been particularly well documented for the HoxD cluster (Kmita et al., 2002; Spitz et al., 2003; Deschamps, 2007). The genomic regulatory regions responsible for the primitive axial expression are independent from novel elements that drive expression of the same Hox genes in the appendicular limb fields (reviewed in Duboule, 2007; Deschamps, 2007). The orchestrated regulation of the Hox cluster genes during development suggests that they represent a system that organizes local signals into global pattern of the fundamental body plan (Duboule and Dollé, '89; Krumlauf, '94; Burke, 2000).

Whereas our knowledge of the targets of Hox genes in vertebrates remains unsatisfying (cf. Svingen and Tonissen, 2006), there can be no doubt that they provide a critical component of the embryonic environment in which tissue patterning occurs. We are proposing that the Hox code and its downstream read-out are different in the abaxial and primaxial domains. Thus, as somitic cells cross the frontier, they come under the influence of a differently regulated network of genes that direct cell behavior resulting in morphological pattern. The distinct regulatory elements that drive differential expression of the HoxD genes mentioned above is an example of this phenomenon, and the results of both genetic and tissue level

embryonic perturbations (discussed below) also support this hypothesis. Any degree of independence between the abaxial and primaxial environments could facilitate the appearance of morphological novelties and subsequent adaptations during vertebrate evolution.

Patterning information and dynamic interactions at the frontier

The key distinction between the abaxial and primaxial domains is the embryonic origin of the investing connective tissue. Somitic cells that form the vertebral column and axial muscles develop within a somitic environment that includes the surrounding connective tissue. Cells that delaminate from the dermomyotome and migrate into the limb or ventral body wall leave their parental somitic environment and enter mesenchyme of the lateral plate. This new environment, the lateral plate mesoderm, includes the putative connective tissue lineage of the abaxial domain. Therefore, all of musculoskeletal elements that develop within the abaxial domain do so within lateral plate-derived connective tissue (Fig. 2).

Experimental data from model systems show that connective tissue is specifically involved in musculoskeletal patterning. Early studies on the limb field reveal that somites from any axial level, when transplanted to limb levels, can populate the limb bud with a normal complement of muscles (Chevallier et al., '77; Gumpel-Pinot, '84; Hayashi and Ozawa, '95; Winslow et al., 2007). The myoblasts are not committed to form any specific muscle. Instead, the lateral plate-derived connective tissue, which forms limb bones and the fascia and tendons of individual muscles, dictates the patterning. Furthermore, muscle connective tissue forms normally in the experimental absence of muscle and can pattern nonmuscle cells to form muscle-like patterns in the limb (Jacob and Christ, '80; Lancer and Fallon, '87; Grim and Wachtler, '91). The molecular players involved in this patterning remain largely unknown, however, Kardon et al. (2003) suggest that the transcription factor Tcf4, likely plays a role. Tcf4 is expressed in a muscle-like pattern in the lateral plate-derived limb connective tissue even when muscle is absent. There is also strong precedent for connective tissue controlling the patterning of both muscle and bone in other systems. For example, neural crest-derived connective tissue dictates the pattern of mesoderm-derived muscle and bone in the developing head (Noden, '86; Borue and Noden, 2004).

Experimental data have also consistently demonstrated that there is a degree of independence in the patterning of abaxial and primaxial domains. Kieny et al. ('72) and more recently Nowicki and Burke (2000) show that, in chicks, primaxial thoracic vertebrae and ribs form from thoracic presomitic mesoderm when transplanted to the cervical region. In the inverse experiment, cervical somites do not form ribs when transplanted to the thorax. Nowicki and Burke (2000) also show that the Hox expression of transplanted somites remains autonomous to the site of origin, consistent with the morphological outcome. In the abaxial domain, the patterning autonomy observed in primaxial structures was not observed. As stated above, vertebral ribs develop from donor thoracic somites when transplanted to the cervical region of a host. However, the sternal component of the rib does not form in the cervical lateral plate of the host. Sternal ribs normally develop within thoracic lateral plate connective tissue (Christ et al., '83; Sudo et al., 2001; Nowicki et al., 2003). Their failure to form indicates that the cervical lateral plate is not competent to pattern the development of these abaxial elements. Cervical to thoracic level transplants produce graft-specific cervical muscles in the primaxial domain whereas thoracic muscles from the graft are formed in the abaxial domain of the host (Murakami and Nakamura, '91; Nowicki and Burke, 2000). We interpret these data to mean that once donor cells migrate across the frontier, they conform to the patterning information inherent in the host lateral plate mesenchyme and form abaxial structures appropriate to the level of the transplant.

Independent patterning of primaxial and abaxial domains can also explain phenotypes produced by several different Hox gene mutations. For example, full paralogue knockouts of Hox6 and Hox9 show various homeotic transformations of primaxial structures, accompanied by dramatic and contrasting patterning disruption in the abaxial sternum and the distal ribs, which form under the influence of abaxial intercostal muscles (McIntyre et al., 2007). These data indicate that homeosis is confined to the primaxial domain, and that the loss of Hox expression in somite cells that cross the frontier has a very different outcome when differentiating in the patterning environment of the lateral plate.

THE LATERAL SOMITIC FRONTIER IN MODEL SYSTEMS

As described above, several developmental studies offer evidence that connective tissue plays a role in musculoskeletal patterning and indicate partial independence of primaxial and abaxial domains. To understand how these developmental phenomena may influence the evolution of the vertebrate body plan, we take a comparative approach to studying the frontier and identify differences in the primaxial and abaxial domains across distantly related taxa. To this end we have been mapping the lateral somitic frontier in the chick and mouse.

During somitogenesis the frontier is coincident with the boundary between the somitic and lateral plate mesoderm. This “incipient” frontier begins as a smooth surface separating these two populations of mesoderm (Fig. 2). Deviations from this smooth cross-sectional profile of the frontier result from the expansion of the primaxial domain. As the embryo grows, the primaxial domain displaces the frontier away from the axis. The elements that establish an early connection with the lateral plate mesenchyme preserve the original point of contact, which becomes visible as the investing, lateral plate-derived connective tissue later in development. Given a lineage marker to identify either somitic or lateral plate cells, we can visualize the position of the frontier in older embryos well after the developmental stages and patterning events crucial for axial and appendicular formation have occurred. The late stage position of the frontier provides a watermark of the incipient frontier and reflects the morphological outcome of the information exchange that occurred at the incipient frontier.

Nowicki et al. (2003) first used chick–quail chimeras to identify the frontier in birds and Durland et al. (2008) more recently mapped the frontier using Prx1Cre transgenic mice. In both mice and chicks, deep axial muscles, vertebrae, and ribs are primaxial, whereas muscles and bone of the appendages are abaxial. The position of the frontier in the dorsal–ventral plane changes along the anterior–posterior axis. As the embryo grows, the primaxial domain expands and the abaxial domain becomes dramatically enlarged at the levels of the fore and hind limbs relative to other anterior–posterior positions along the axis (Fig. 3).

Use of the Prx1 Cre Z/AP mice allowed Durland et al. (2008) to describe the frontier in detail in a developmental series of embryos. In these mice, reporter expression activated in the postcranial somatic lateral plate mesoderm was used to visualize the abaxial domain, providing a proxy for the frontier. Durland et al. (2008) identify several muscles and bones that have both primaxial and abaxial components, that is, portions of these musculoskeletal elements are invested in somite-derived connective tissue and other portions are invested in lateral plate-derived connective tissue. We describe these structures as “incorporating” the frontier. In the thorax, the scapula, intercostal muscles, latissimus dorsi and spinotrapezius incorporate the frontier. In the pelvic region of the mouse embryo, the sacral ribs and gluteus maximus also possess both primaxial and abaxial components. The boundaries between primaxial and abaxial domains in several of these structures represent discontinuities in the profile of the frontier. These discontinuities illustrate the extensive expansion of the primaxial domain during the development of the embryo (Fig. 3).

The position of the lateral somitic frontier differs in mice and chicks, notably in the post-cranial skeleton. The avian rib cage is formed from ossified, jointed ribs. Each rib comprises a dorsal vertebral rib and a ventral, sternal rib. Based on quail–chick chimeras, the vertebral ribs are primaxial and the sternal ribs are abaxial (Nowicki et al., 2003). The mouse rib cage has a different morphology and ontogeny. Each rib has two regions, the dorsal ossified region that articulates with the vertebrae, and the ventral chondrogenic region that fuses with the sternum. Most of the ribs are entirely primaxial, however, the first rib has an abaxial portion. Within this abaxial region of rib one, chondrocytes are derived from somites yet the investing connective tissue is of lateral plate origin (Fig. 4). This relationship between somitic chondrocytes and lateral plate connective tissue is similar to that of an avian sternal rib (Nowicki et al., 2003).

Like the rib cage, the position of the frontier in the shoulder girdle appears to differ in mice and chicks. As Durland et al. (2008) describe, the vertebral margin of the scapula is somitic and the remainder of the scapula derives from lateral plate mesoderm. Furthermore, the frontier in the mouse shoulder is coincident with the contact between these two tissues at early developmental stages (Fig. 4). Although the position of the frontier in the avian scapula has not been mapped, the majority of chick scapular blade is derived from somites (Chevallier, '77, '79; Huang et al., 2000), and only the head and neck are derived from lateral plate mesoderm (Huang et al., 2000). If, as in mice, the frontier in the chick scapula is coincident with the boundary between the lateral plate and somitic portions of the blade, the position of the frontier in the avian scapula will be quite different from that in mice. These differences in the position of the frontier would reflect the different evolutionary histories of the avian and mammalian body. Specifically, the differences may reflect the morphological adaptations that occurred to the avian limb, pectoral girdle, and thorax as birds evolved flight (Burke, '91).

THE LATERAL SOMITIC FRONTIER IN NONMODEL SYSTEMS

The lateral somitic frontier has only been preliminarily mapped in chicks and mice. However, two recent studies have used the concept of the lateral somitic frontier to explain the origin of two highly derived body plans, the chelonian carapace and the elongate, limbless form of many squamates.

Turtles are often used as an example of evolutionary novelty because their ribs contribute to the carapace and are superficial to the pectoral girdle (Fig. 5A). This derived morphology appears without any intermediates in the fossil record. The earliest known turtle, *Proganochelys*, is from the late Triassic, and looks very similar to a modern turtle (Gaffney, '90). Ontogenetic studies have been a productive, alternative source of information on the evolution of the turtle body plan (Ruckes, '29; Burke, '89, '91; Gilbert et al., 2001; Nagashima et al., 2007). In most vertebrates, the pectoral girdle does not articulate with the vertebrae, and it sits in a muscular sling lateral to the ribs (Romer and Parsons, '77). The inverted relationship of the ribs and pectoral girdle in turtles results from the lateral, rather than ventral, trajectory of the growing ribs. However, development of the chelonian shoulder and limb muscles remains similar to that of other amniotes (Ruckes, '29; Walker, '47).

The carapacial ridge (CR), an embryonic ectodermal thickening that forms the outer margin of the carapace, is unique to turtles (Burke, '89, '91). The CR is essential to the development of the turtle bodyplan, however, the causal role of the ridge in the lateral growth of the ribs remains a matter of debate (Burke '91; Cebra-Thomas et al., 2005; Nagashima et al., 2007). Nagashima et al. (2007) recently described the CR and carapace development of the turtle, *Pelodiscus sinensis*.

They report that the chelonian ribs and CR are entirely primaxial, and that the CR lies immediately dorsal to the lateral somitic frontier. The primaxial domain does not displace the frontier as the ribs grow laterally and remain dorsal relative to the limb girdles. (Fig. 5B). This is in contrast to mouse and chick development, in which the lateral somitic frontier is displaced ventrally as the primaxial domain expands with the developing rib cage. The drastic difference in the position of the frontier in turtles relative to mice and chicks led Nagashima et al. (2007, p 2226) to suggest that, “The uniqueness of the turtle can be seen, at least in part, in the arrest of the lateral somitic frontier.”

The lateral somitic frontier also recently has been used in a new hypothesis for the loss of axial regionalization in limbless squamates. Squamata is a large tetrapod group that includes lizards and snakes. There are over 8,000 squamate species and limblessness has evolved over 30 times within the group (Gans, '75; Greer, '91). In limbed squamates, like other tetrapods, the vertebral column has morphological boundaries between the neck, trunk, and tail (Hoffstetter and Gasc, '69). These boundaries are typically associated with other anatomical features. For example, the neck–trunk transition is at the same anterior–posterior position as the pectoral girdle, it marks the posterior extent of the ventral vertebral hypapophyses, and it corresponds with the position of the heart (Hoffstetter and Gasc, '69).

The evolutionary loss of limbs in squamates is usually associated with the loss of regional differentiation of the axial system (Hoffstetter and Gasc, '69; Cohn and Tickle, '99; Wiens and Slingluff, 2001; Sanger and Gibson-Brown, 2004). This has led to a long-standing debate over whether snakes evolved by initially elongating the cervical region at the expense of the thoracic region or vice versa. Caldwell (2000) uses morphological characters such as the ventral vertebral hypapophyses on the cervical vertebrae to argue that snakes evolved as an expansion of the neck. Alternatively, the ribs, hypaxial muscles, and pleuroperitoneal cavity extend anteriorly to the cranio-vertebral boundary in certain snakes and have been used to argue that the serpentine body plan is a result of the expansion of the thorax (Cundall and Greene, 2000). Cohn and Tickle ('99) use molecular data to support the hypothesis that snakes evolved as an expansion of the thorax. Hox genes specify the axial pattern along the vertebrate axis, and the anterior expression limits of Hox genes are correlated to changes in vertebral identity in taxa that have differing numbers of vertebrae (Burke et al., '95). In pythons, the expression domains of Hox C6 and Hox C8, which are restricted to the thorax in chicks and mice, are reported to extend anteriorly to the first vertebra suggesting the snake body results from the expansion of the thorax (Cohn and Tickle, '99).

There has been little resolution to this debate largely because of the morphological variation among the independent lineages of limbless squamates (Hoffstetter and Gasc, '69; Renous, '85; Tsuihiji et al., 2006). Tsuihiji et al. (2007, and personal communication) recently conducted an anatomical survey of limbless squamates, and categorized musculoskeletal elements typically associated with the neck–trunk boundary as primaxial or abaxial. Tsuihiji et al. (2007, and personal communication) suggest that the loss of registration of several structures at the neck–trunk boundary, previously described by Hoffstetter and Gasc ('69), correlates with the classification of primaxial and abaxial elements. In many snakes and certain limbless lizards, such as *Amphisbaena alba*, several primaxial structures including the vertebral hypapophyses and the axial cranio-vertebral muscles (i.e. m. spinalis capitis, m. recutus capitis anterior) extend further posteriorly than those elements in limbed squamates. Similarly, abaxial musculoskeletal elements including several abdominal muscles (i.e. m. obliquus internus pars dorsalis, m. transversus ventralis, m. rectus abdominis, and the m. obliquus externus) extend further anteriorly than their homologs in limbed squamates. Accordingly, Tsuihiji et al. (2007, and personal communication) propose that the dissociation of these anatomical structures results from the developmental decoupling of the primaxial and abaxial domains. Given this new hypothesis,

squamate neck and trunk characteristics may have some freedom to evolve independently by accumulated changes in regulatory networks that function in either primaxial or abaxial domains. As a result, snakes may have expanded portions of both their neck and trunk, and, therefore, possess traits of both cervical and thoracic regions at the same axial level. The modular nature of the primaxial and abaxial patterning domains could also account for the range of neck and trunk characters that differ across independent lineages of limbless squamates.

DISCUSSION

In the early 1980s Hans Meinhardt proposed a boundary model to explain the aspects of embryonic pattern formation (Meinhardt, '83a, '83b). This simple and elegant model states that borders between differentially determined cells initiate global organization by initiating the generation of additional boundaries; "Boundaries create new positional information, and its interpretation leads to new boundaries suggest[ing] a chain of relatively simple molecular interactions which could provide the basis for the reliable generation of structures during embryonic development." (Meinhardt, '83b, p 334). The lateral somitic frontier is a cryptic developmental boundary that marks the border between two distinct lineage domains initially determined by gastrulation movements, and later by the lineage of the mesenchyme that generates the connective tissue. We suggest that variation in the vertebrate body plan evolved, in part, by developmental changes occurring at the boundary between the primaxial and abaxial domains. We view the limbs as a particularly spectacular outcome of developmental changes at the frontier. There are also significant, if less dramatic ontogenetic changes in other regions of the body, which are responsible for the differing morphology in the neck, trunk, and tail. We argue that these developmental events can be traced to differences in the primaxial and abaxial developmental environments, including differences in how; for example, the Hox code is read and interpreted at the frontier. Decoupling the information networks in primaxial and abaxial environments and altering the behavior of mesodermal cells at the lateral somitic frontier could facilitate the evolution of novel vertebrate morphologies.

As described above, there are several muscles and bones that incorporate the frontier during development and these structures may offer further insight into the evolutionary history of the vertebrate musculoskeletal system. Differences in the position of the frontier in homologous structures may reflect developmental changes in the patterning that facilitated the evolution of novel morphologies. For example, in mice the latissimus dorsi and spinotrapezius are two large muscles with primaxial and abaxial components (Fig. 4). Lateral plate-derived connective tissue invests a subset of the cells contributing to these muscles, the remainder are primaxial. One hypothesis for the incorporation of the frontier in the latissimus dorsi and spinotrapezius is that primitively these muscles were entirely primaxial and associated with the axial musculoskeletal system. During the expansion of the lateral plate and the evolution of the appendicular system, the lateral plate may have recruited putative latissimus and spinotrapezius cells to the limb, thereby altering the insertion points and functions of these muscles. This hypothesis would predict that basal vertebrates, like the lamprey, would not have any abaxial component to the body wall muscles of the trunk. However, in basal fish, the cucullaris, the putative homolog to the tetrapod trapezius (Edgeworth, '35; Miyake et al., '92; Anderson, 2008) would have a distal, abaxial component. A comparative analysis of the frontier in the axial and appendicular musculature of modern fishes and amphibians could test this hypothesis and allow us to reconstruct the developmental evolution of the vertebrate appendicular musculature.

Similarly, the incorporation of the lateral somitic frontier in the development of the mouse scapula may reflect the evolutionary history of the vertebrate pectoral girdle. The first

limbed vertebrates may have developed a musculoskeletal anchor within the primaxial domain in order to support the new, abaxial appendage. This anchor would have been the first skeletal manifestation of the vertebrate pectoral girdle. In this scenario, the small primaxial portion along the vertebral margin in the mouse scapula would represent the remnant of this primitive pectoral anchor. Further outgroup comparisons of the mouse scapula to that of diapsids, amphibians, actinopterygians, and especially chondrichthyans can determine the evolutionary history of both the primaxial and abaxial portions of the vertebrate scapula.

Recent work by Nagashima et al. (2007) and Tsuihiji et al. (2007, and personal communication) on the chelonian carapace and limbless squamates, respectively, are the first forays into understanding the evolution of novel body plans using the conceptual framework of the lateral somitic frontier. These studies, together with previous work mapping the frontier in mice and chicks (Nowicki et al., 2003; Durland et al., 2008) suggest that the evolution of unique vertebrate morphologies is correlated to developmental changes in the position of the lateral somitic frontier. To further our understanding of the role of the primaxial and abaxial domains in vertebrate evolution, we must continue the broad taxonomic analysis of the lateral somitic frontier. We must also pursue developmental studies in both model and nonmodel systems aimed at testing the independent patterning of the primaxial and abaxial domains at the molecular level. This two-part approach will elucidate the role the lateral somitic frontier plays in the development and evolution of vertebrate morphology and provide a new perspective on vertebrate evolution.

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Fig. 1. Cladogram illustrating the diversity of vertebrate morphology. Actinopterygii (*Danio rerio*), Lissamphibia (*Ambystoma tigrinum*), Mammalia (*Mus musculus*), Testudinata (*Chelydra serpentina*), Lepidosauria (*Python curtus*), Archosauria (*Gallus gallus*).

Fig. 2. Illustration of the primaxial and abaxial domains in the developing embryo. (A) Cross section through a chick embryo. The incipient frontier (arrow) is present before somitic cells have begun to migrate. (B) Cross section through embryo at the forelimb level. (C) Cross section through the thorax of an embryo. The red line marks the lateral somitic frontier.

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Fig. 3.

Visualization of the lateral somitic frontier in Prx1 Cre Z/AP mice. Modified from Durland et al. (2008). (A) E15.5 Prx1 Cre Z/AP mouse in whole mount. (B–E) Series of cross sections along the anterior–posterior axis of an E15.5 Prx1 Cre Z/AP mouse. “X” marks the dorsal point of the sagittal midline. “Y” marks the superficial label boundary, which approximates the original boundary between the somitic and lateral plate mesoderm (B). Pectoral girdle. “G” marks the dorsal most extent of the labeled domain in the pectoral girdle.

(C) Lumbar region. (D) Thorax. “T” marks the ventral most extent of the frontier in the rib cage. (E) Pelvic region. “G” marks the dorsal most extent of the pelvic girdle. Scale bar 5–1000 mm.

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Fig. 4.

Incorporation of the lateral somitic frontier in musculoskeletal elements of an E 15.5 Cre Z/AP mouse embryo. (A) Doral portion of right scapula. Arrow marks the primaxial region of the scapula. (B) Rib 1. Positively labeled connective tissue invests the distal margin of the rib (arrow).

(C) Rib 2. Connective tissue investing rib 2 is not labeled (arrow). (D) Three-dimensional reconstruction of the shoulder girdle, rib cage, and latissimus dorsi in right lateral view of an E15.5 Cre Z/AP mouse embryo. The primaxial region is pink and the abaxial region is purple. (E) Three dimensional reconstruction illustrating the primaxial and abaxial portions of the spinotrapezius muscles in the same mouse shown in D. is, infraspinatus; ls, levator scapulae; r1, first rib; r2, second rib; rm, rhomboideus major; s, scapula; ssc, subscapularis; st, spinotrapezius, Scale bar 5 500 mm.

Fig. 5.

Schematic of the morphology and development of the chelonian carapace and ribs in contrast to that of a typical amniote. (A) Relationship of the pectoral girdle and ribs in a typical amniote and a turtle (modified from Burke, '89). (B) Primaxial and abaxial domains in the embryo of a typical amniote and turtle (modified with permission from Nagashima et al. 2007). CR, carapacial ridge; c 5 dr, carapace 5 dorsal ribs; dr, dorsal ribs; lsf, lateral somitic frontier; na, neural arch; nt, neural tube; str, sternal rib.