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SPECIAL ISSUE REVIEWS–A PEER REVIEWED FORUM

Global Patterning of the Vertebrate Mesoderm

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We describe recent advances in the understanding of patterning in the vertebrate post-cranial mesoderm. Specifically, we discuss the integration of local information into global level information that results in the overall coordination along the anterioposterior axis. Experiments related to the integration of the axial and appendicular musculoskeletal systems are considered, and examples of genetic interactions between these systems are outlined. We emphasize the utility of the terms primaxial and abaxial as an aid to understanding development of the vertebrate musculoskeletal system, and hypothesize that the lateral somitic frontier is a catalyst for evolutionary change. *Developmental Dynamics 236:2371–2381, 2007.*
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INTRODUCTION

The musculoskeletal system of vertebrates arises from two populations of embryonic mesoderm, the somites and lateral plate (Fig. 1A). Like the development of every anatomical system, morphogenesis transforms an apparently uniform cell population into a heterogeneous assemblage of cell types arranged in a highly organized pattern to produce functional adult morphology. During this process embryonic cells are specified by local signals to form particular cell types. The locally specified cells are also organized into global arrangements to establish functionally coordinated structures at specific locations of the body. Global patterning insures the species-specific topographical relations of body structures. For example, in the cervical region, muscle and skeletal precursors are patterned to form cervical, rather than thoracic, structures. We use the term “global patterning” to describe this phenomenology. The processes of specification and patterning are often difficult to distinguish from each other, yet developmental success is no doubt dependent on a subtle interplay between these types of information. The nature of global information is not well defined, but our working assumption is that the specific, accumulative combination of developmental signals in a cell’s environment results in global patterning. The aim of this review is to describe current knowledge and highlight persistent questions of how patterning of the musculoskeletal system is achieved by integration of local information and global patterning in the paraxial somitic mesoderm and the somatic layer of lateral plate mesoderm.

Somites are paired, serially homologous epithelial balls located on either side of the neural tube, along the anteroposterior (AP) axis. Somites are the origin of the postcranial axial skeleton (i.e., vertebrae and ribs), the connective tissue and tendons associated with axial muscles, as well as all the striated muscles in the body. Based on eventual cell fate, somites are regionalized into the dorsal/lateral dermomyotome and the ventral/medial sclerotome. The dermomyotome is the source of cells that form the dorsal dermis and all skeletal muscle precursors; sclerotome gives rise to axial skeletal elements. The dorsomedial lip of the dermomyotome gives rise to adult epaxial muscles, which lie dorsal to the transverse process of the vertebrae, while the ventrolateral lip gives rise to hypaxial muscles, which are ventral to the transverse process (Ordahl and Le Douarin, 1992; Ordahl et al., 2000).

Other than muscles and skeletal el-

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ements, somites also give rise to dorsal dermis and vascular precursors (Wilting et al., 1995; Pardanaud et al., 1996; Pardanaud and Dieterlen-Lievre, 1999; Olivera-Martinez et al., 2000) that migrate extensively and assemble vessels at their target sites (Noden, 1989; Poole and Coffin, 1989; Ambler et al., 2001). The endothelial precursors may originate from the same progenitor cell population as myogenic precursors, and locally available signals at their target sites are likely to play a role in determining their cell type (Kardon et al., 2002). Christ et al. have provided an excellent comprehensive review of somitic derivities in amniotes to this issue (pages 2382–2396).

The lateral plate mesoderm consists of dorsal somatic and ventral splanchnic layers, which flank the paraxial mesoderm as two mesenchymal sheets separated by the coelom (Fig. 1A). The splanchnic layer surrounds the endodermal gut tube and forms smooth muscle and connective tissue of the digestive organs. Epithelial–mesenchymal interactions between splanchnic mesenchyme and epithelial endoderm are essential in the induction and patterning of the digestive tract and organs such as the liver and pancreas (Wells and Melton, 1999; Grapin-Botton and Melton, 2000; Roberts, 2000). It is also the source of cardiogenic mesoderm and endothelial and hematopoietic precursors (Pardanaud and Dieterlen-Lievre, 1993; Pardanaud et al., 1996). The somatic layer of lateral plate mesoderm (LP) is the source of the appendicular skeleton; connective tissue, and tendons of the limb and body wall; and the sternum. The intermediate mesoderm is located between paraxial mesoderm and LP, and gives rise to nephric structures. The intermediate mesoderm will not be discussed here.

During gastrulation in avian embryos, the paraxial and lateral mesoderm are formed as cells invaginate through the primitive streak. Cells that come to lie medially along the AP axis are specified as somitic mesoderm, and the cells in more lateral areas become the LP mesoderm (Selleck and Stern, 1991; Schoenwolf et al., 1992). LP and surface ectoderm on both sides of the AP axis are engaged in forming the lateral body folds, lifting the embryo off the yolk and eventually meeting at the ventral midline. This establishes the closed body wall of an embryo, and more lateral tissues become extra embryonic (Pardanaud et al., 1996).

The amniote body plan includes anatomically and functionally distinct regions: occipital, cervical, thoracic, lumbar, sacral, and caudal. Each of these regions is identified by the specific morphology of vertebrae and relative location along the AP axis. Furthermore, in tetrapods the appendicular structures are always located at specific axial regions regardless of the total number of vertebrae in the organism, i.e., forelimbs originate at the cervical to thoracic transition while hind limbs lie at the lumbar to sacral transition. An important aspect of morphology along the AP axis can be traced to dramatic differences in the degree and mode of migration of somite cells away from the dorsal midline. We would like to emphasize that local specification alone cannot generate the regionally distinct and globally coordinated structures that characterize the AP axis. An additional layer of information, orchestrating the time and place of local factors, is necessary in order to establish functionally coordinated structures at the appropriate axial levels.

MESODERM DOMAINS AND THE DEFINITION OF THE LATERAL SOMITIC FRONTIER

Recent studies exploring the interface between somitic and LP mesoderm have defined two discrete domains in the developing body wall based on cell lineage (Nowicki et al., 2003). The *primaxial* domain is composed exclusively of somitic cells (Fig. 1B). Cells that form the *abaxial* domain include a subset of somitic cells that migrate away from the axis, infiltrating and mixing with LP cells. This terminology is not equivalent to and is not intended to replace the traditionally used epaxial/hypaxial domains, which are classically defined by adult criteria. Epaxial and hypaxial muscles are innervated by the dorsal or ventral rami of the spinal nerves, respectively (Sporle, 2001). Additionally, epaxial muscles originate from the medial half of the somite, and hypaxial muscles originate from the lateral somite (Cheng et al., 2004; Ahmed et al., 2006). The primaxial domain includes all the epaxial muscles plus the hypaxial ventral vertebral and intercostals muscles. The abaxial domain includes the remainder of the hypaxial muscles. The boundary between primaxial and abaxial domains is a dynamic interface that originally separates the somitic and lateral plate mesoderm populations. We call this interface the lateral somitic frontier.

In addition to the descriptive nature of this terminology, we hypothesize that the lateral somitic frontier is the site of significant signal exchange and resulting changes in cell behaviors that result in patterning along the dorsoventral/mediolateral axis of the body. The behavior of cells at the lateral somitic frontier at a particular axial level has clearly changed during the evolution of vertebrates and is not the same across taxa. Accordingly, evidence from a variety of studies indicates that primaxial and abaxial domains represent independent patterning environments (Burke and Nowicki, 2003).

LOCAL PATTERNING OF THE MESODERM

Lateral Plate Mesoderm

After gastrulation, mesoderm is subdivided into pre-somitic mesoderm and LP. Bmp4 secreted by the ectoderm acts as a lateralizer of mesoderm and specifies the LP in a gradient dependent manner. PSM transplanted to the LP conforms to the LP, and over-expression of Bmp4 in PSM blocks somitogenesis (Tonegawa et al., 1997). Noggin expression in the paraxial mesoderm antagonizes Bmp4 (Tonegawa and Takahashi, 1998), and can transform prospective LP into somitic mesoderm (Streit and Stern, 1999). Once in place, the LP expresses Bmp4, which at this time in development acts as a lateralizing signal to the somites (Pourquie et al., 1995, 1996). The LP has received far less attention than its prolific medial neighbor, the paraxial mesoderm. Using scanning electron microscopy,

Meier (1980) concluded that the LP is segmented by undulations rather than distinct boundaries, and these subtle segments correspond to the paraxial segments.

A number of genes show expression patterns localized in the LP mesoderm. *Prx1, cytokeratin*, and *Irx1* are all expressed uniformly in the LP across all axial levels (Charlebois et al., 1990; Funayama et al., 1999; Logan et al., 2002). In contrast, some of the T-box transcription factors (*Tbx*) exhibit more regionalized expression patterns. *Tbx5* is expressed only in the LP at forelimb/fin level in mice, chick, and zebrafish while *Tbx4* is expressed almost exclusively in the hind limb/fin region (Gibson-Brown et al., 1996; Isaac et al., 1998; Logan et al., 1998; Ohuchi et al., 1998; Ruvinsky et al., 2000). These data support level-specific patterning roles for *Tbx4* and *Tbx5*. However, recent data demonstrate that *Tbx4* and *Tbx5* are required for limb bud initiation, but are not required for limb identity (Minguillon et al., 2005; Hasson et al., 2007; Naiche and Papaioannou, 2007). Instead, the *Pitx1* transcription factor, which is exclusive to the hindlimb (Logan et al., 1998), appears to confer hindlimb identity (Minguillon et al., 2005).

Somitic Mesoderm

Somites arise sequentially by the process of segmentation from the anterior end of the unsegmented presomitic mesoderm (called segmental plate in chick). Cooke and Zeeman (1976) proposed the "clock and wave front" model to explain the temporal and spatial control of somitogenesis. Recent studies have found support for aspects of this model by uncovering a molecular oscillatory mechanism, now known as the segmentation "clock." Palmeirim et al. (1997) found that the chick *Hairy* gene is expressed in pulses that are coincident with segmentation. The oscillation of *Hairy* and other *Notch* pathway components allows presomitic mesoderm cells to establish segmental boundaries in a temporally and spatially controlled manner during somitogenesis (Aulehla and Herrmann, 2004).

The "wave" component of the segmentation clock model is thought to consist of an Fgf gradient (Dubrulle et al., 2001; Sawada et al., 2001). Fgf8 is expressed in caudal presomitic mesoderm, and its concentration declines in a posterior to anterior gradient. When the Fgf8 concentration drops below a certain threshold, presomitic mesoderm cells become competent to form a segmentation boundary (Dubrulle et al., 2001; Sawada et al., 2001). Because of the periodicity of the clock, cells can make boundaries only once during a clock cycle, and somites are formed in a spatially controlled manner, with the posterior somite boundary corresponding to an FGF8 threshold (Dubrulle et al., 2001).

After segmentation, somites mature under the influence of local signals. These are reviewed extensively elsewhere in this issue and we will concentrate our discussion on factors with demonstrated links to global patterns. Somite maturation into different regions is associated with the expression of multiple marker genes in specific spatio-temporal patterns (Dietrich, 1999; Stockdale et al., 2000; Sporle, 2001). For example, *Pax3* is first expressed throughout the somite, but its expression is later restricted to the dermomyotome (Goulding et al., 1993, 1994; Williams and Ordahl, 1994). *Pax1* is expressed later than *Pax3*, but is restricted to the sclerotome (Brand-Saberi et al., 1993; Pourquie et al., 1993; Muller et al., 1996).

The dermomyotome develops into muscle under the control of muscle regulatory factors. Extensive studies of muscle regulatory factors reveal that their complex and dynamic expression is driven by highly modular cis-regulatory regions (Summerbell and Rigby, 2000; Buckingham, 2001). For example, the expression of *Myf5* is regulated by multiple enhancers, each of which drives *Myf5* expression in distinct subpopulations of muscle progenitors, such as the ventral body wall, limb, and branchial arches (Hadchouel et al., 2000; Summerbell et al., 2000; Bajard et al., 2006; Chen et al., 2007). These enhancers also control *Myf5* expression in a temporally specific manner. Different enhancers are involved in distinct developmental events, such as early myotome commitment in epaxial dermomyotome, or later *Myf5* expression in the hypaxial dermomyotome (Teboul et al., 2002).

Regional Gene Expression Along the AP Axis

The majority of the known signals involved in local somite maturation and differentiation are present along the entire AP axis and reflect no regionalization. There are some notable exceptions to this uniformity. The secreted protein scatter factor/hepatocyte growth factor (Sf/hgf) is expressed by the LP solely at occipital, cervical, and limb levels in amniotes (Myokai et al., 1995; Thery et al., 1995; Heymann et al., 1996; Yang et al., 1996). Through its receptor Met, Sf/hgf de-epithelialize, and is necessary for myoblast migration into the limb bud (Bladt et al., 1995). These cells have been termed migratory muscle precursors by Dietrich (1999). Unlike its ligand, Met is expressed at the medial and lateral dermomyotome lips uniformly along the entire AP axis of the paraxial mesoderm (Sonnenberg et al., 1993). Interestingly, the reverse is true in zebrafish. In this taxon, Met is localized to fin level somite cells that are induced to migrate, and Sf/hgf is expressed along the entire AP axis (Haines et al., 2004). An implication of this change is that only a subset of Zebrafish somitic cells may be competent to respond to Sf/hgf. These developmental differences represent evolutionary change in the lineages leading from the last common ancestor of ray finned fishes and tetrapods.

The homeobox-containing transcription factor *Lbx1* also has regionalized expression, and is seen in migratory muscle precursors only at occipital, cervical, and limb levels in mouse and chick, complementary to Sf/hgf expression in the LP (Jagla et al., 1995; Dietrich et al., 1998; Mennerich et al., 1998; Neyt et al., 2000). When *Lbx1* is disabled in mouse, an extensive amount of limb muscle fails to form, though tongue and diaphragm muscles are not severely affected, nor is *Met* expression (Brohmann et al., 2000; Gross et al., 2000). Limb level lateral plate is sufficient to induce *Lbx1* in both the lateral and medial dermomyotome (Alvares et al., 2003). In *Pax3*−/− (*splotch*) mutants,

Lbx1 is absent from the ventrolateral dermomyotome, though it is expressed in other tissues. Delamination of the dermomyotome does take place in *splotch* mutants, but migration of myoblasts is aberrant, leading to deficiencies of specific limb muscles, particularly the distal limb muscles (Mennerich et al., 1998). Such data suggest that *Lbx1* is involved in the interpretation of migratory cues.

Xenopus also shows some variation in the expression of *Lbx1*. In contrast to its expression in model amniotes, *Lbx1* is expressed in ventrolateral dermomyotome of all *Xenopus* trunk somites, including somites that produce body wall muscles (Martin and Harland, 2006). These authors show that *Lbx1* functions not specifically to promote migration, but rather to repress muscle differentiation by inhibiting *MyoD* in *Xenopus* and allowing for increased proliferation and migration (Martin and Harland, 2006).

Consistent with the evolutionary divergence mentioned above, in zebrafish *Lbx1* is expressed in the ventral lateral dermomyotome in the fin bud. In zebrafish, muscle precursors migrate as individual cells at fin levels, but in the chondrycthian *Scyliorhinus canicula* (Dogfish), they expand as epithelial extensions of the dermomyotome (Neyt et al., 2000). The latter mode of migration is seen in somite cell populations that form intercostal muscles in all vertebrates and is considered more primitive (Goodrich, 1930). It has been hypothesized that the mesenchymal mode of migration at fin/limb levels evolved later in vertebrate evolution, before the last common ancestor of Sarcopterigians and Actinopterigians (Haines and Currie, 2001; see also Cole and Currie, this issue, pages 2421–2431).

In *Xenopus*, Shh from the neural tube and notochord induces myogenesis, and inhibits *Pax3* and *Lbx1* (Martin et al., 2007). Over-expression of Shh in the ventral-lateral somite leads to a loss of migratory muscle precursors in *Xenopus*. The hypaxial abdominal rectus and other hypaxial body wall muscles are shown to arise from migratory muscle precursors using the typical tetrapod gene expression network (Martin and Harland, 2001). Zebrafish also have migratory muscle precursors; however, only limb bud level somites are capable of producing them (Haines et al., 2004). Again, these differences represent evolutionary change in global, not local patterning.

HOX GENES AND GLOBAL PATTERNING

The morphological changes along the AP axis are reflected in the expression patterns of *Hox* genes in the mesoderm, and a correlation between gene expression and morphology exists, which is consistent between species (Burke et al., 1995). These highly conserved homeobox-containing transcription factors influence global segmental patterning in vertebrates and arthropods (see a review by Wellik in this issue, pages 2454–2463). *Hox* genes show a remarkable characteristic called colinearity, wherein the order of their physical locations on a chromosome is recapitulated by their expression patterns along the AP axis (Duboule and Dolle, 1989; Graham et al., 1989). Missexpression data indicate that the most 5′ gene holds the most influence on the morphology of a specific level. This phenomenon is known as “posterior prevalence” (Duboule and Morata, 1994). Genome level organization of *Hox* is clearly instrumental for global body patterning.

Colinear expression is initiated before gastrulation. Using explants from gastrulating mice, Forlani et al. (2003) demonstrated that *Hox* genes are expressed colinearly in the posterior primitive streak. In chick, it has recently been shown that the timing of cell ingression is tied to *Hox* expression (Iimura and Pourquie, 2006). Ectopic expression of more posterior level *Hox* genes will cause epiblast cells to delay ingression until the endogenous posterior level *Hox* expressing cells ingress. Epiblast cells misexpressing *Hox* genes always end up at the AP level appropriate for the misexpressed gene (Iimura and Pourquie, 2006). These data further demonstrate that *Hox* genes are involved in setting up global patterning from very early stages in development.

Deletions of *Hox* genes in vertebrates are often associated with homeotic mutations of vertebrae (Krumlauf, 1994; de la Cruz et al., 1999; Branford et al., 2000). In mouse, *Hox* expression can be altered by heat shock, which also results in homeotic shifts of the axial skeleton (Li et al., 1997). The discovery of homeotic shifts lead to the concept of a “*Hox* code,” proposed by Kessel and Gruss (1991). This idea suggested that a combination of Hox proteins functioning at a specific AP axial level provides positional information to the structures at that level. It also allows for some level of functional redundancy between some Hox proteins (Branford et al., 2000). For example, in mouse *Hoxa3* can substitute for *Hoxd3* and vice versa (Greer et al., 2000), and *Hoxa1* can substitute for *Hoxb1* and vice versa (Tvrdik and Capecchi, 2006; Iimura and Pourquie, 2007).

The disruption generated by a single knockout is often more dramatic than the loss of an entire cluster. Large regional deletions do not necessarily show severe, compound effects (Medina-Martinez et al., 2000; Suemori and Noguchi, 2000; Spitz et al., 2001). This finding does not support a strict combinatorial *Hox* code model as would be predicted by the effects of single or small-scale *Hox* gene mutations. Crawford (2003) proposed a model in which *Hox* expression acts more like a “metronome” than a coded readout, conveying positional information from temporal colinearity. In this view, mutation of a single *Hox* gene causes asynchrony of the colinearity between all the clusters. The loss of an entire cluster has little effect on the other clusters since no “stuttering” or asynchrony is generated. In Crawford’s (2003) model, *Hox* genes regulate global pattern without representing a rigid code that specifies particular axial structures.

Aspects of both models seem to apply to the phenotypes generated when full paralogue groups are disrupted. Wellik and Capecchi (2003) generated mice with deletions of full paralogues 10 or 11. These paralogue knockouts impact entire AP regions, not only individual vertebrae. Absence of all members of paralogue 10 results in the loss of the lumbar region and complete transformation of lumbar vertebrae into the thoracic phenotype. Absence of all members of paralogue 11 eliminates the sacral region, transforming sacral vertebrae into a lum-

bar phenotype. This implies that both the spatial position of the paralogues, as well as the combination of genes between clusters, have regional impact.

Regulation and Effectors of *Hox* Expression

Given the dramatic relationship between *Hox* genes and body patterning, many studies have sought to uncover regulatory mechanisms of these genes. As such, a number of upstream factors have been identified that control the refinement of *Hox* expression boundaries. The examples provided below arise primarily from neural tube development literature; however, the mesoderm develops directly adjacent to the neural tube and some of the same molecules have been shown to effect the mesoderm (e.g., the role of Fgf8 in segmentation). We will not attempt to describe all of the *Hox* regulators. Instead we will discuss several key factors involved. Fgf8 and retinoic acid (RA) are the best documented *Hox* regulators. These two extra-cellular signaling molecules form opposing gradients along the AP axis (Diez del Corral and Storey, 2004). High levels of RA are found anteriorly, and high levels of Fgf8 are found posteriorly. Accordingly, high levels of RA have been shown to activate anterior level *Hox* genes, and high levels of Fgf8 activate posterior level paralogues (Bel-Vialar et al., 2002).

RA regulates *Hox* gene expression through retinoic acid response elements (RAREs) found in *Hox* regulatory regions (Marshall et al., 1996; Wada et al., 2006). As mentioned, anterior *Hox* members are generally more sensitive to RA. Also, distinct RAREs can drive *Hox* gene expression in a spatially specific manner. For example, RAREs regulate the expression of *Hoxb1* in both the foregut and neural epithelium (Huang et al., 1998, 2002) and the post-otic level of the neural crest (Wada et al., 2006). Bel-Vialar et al. (2002) showed that specifically *Hoxb1* through *Hoxb5* could be anteriorized by addition of RA to chick, whereas *Hoxb6* through *Hoxb9* could be anteriorized by Fgf8.

Fgf8 has been shown to regulate both *Hox* genes and segmentation (Dubrulle and Pourquie, 2002). Beads soaked in Fgf8 and implanted in the presomitic mesoderm result in disrupted segmentation, such that somitic boundries are condensed anteriorly to the bead (Dubrulle et al., 2001). The shift of somite boundaries also results in the anterior shift of *Hox* gene expression, demonstrating the coupling of *Hox* gene expression with the segmentation clock (Dubrulle et al., 2001). This seems to represent an instance where local information, in the form of a threshold level of RA and Fgf8 in cells of the paraxial mesoderm, is translated into a global pattern by refinement of colinear *Hox* expression.

Tgf-βs such as Gdf11 can also regulate posterior *Hox* paralogues (Liu et al., 2001). *Gdf11* is expressed beginning at the 11 somite stage in the chick tail bud and is thought to act synergistically with FGF8 to refine posterior *Hox* paralogues in the AP axis (Liu et al., 2001). Mutation of *Gdf11* leads to posteriorization of *Hoxc* genes (McPherron et al., 1999) and over-expression of *Gdf11* causes rostral shifts along the axis of *Hoxc* genes (Liu, 2006). *Hoxd10* is also shifted when *GDF11* is overexpressed, as is axial morphology (Fig. 2). Gdf11 up-regulates *Hoxd11* and *Hoxd13* in the limb (Gamer et al., 2001).

Recently, microRNAs have been shown to regulate Hox expression (Pearson et al., 2005; Chopra and Mishra, 2006). The available data are mostly from *Drosophila*. However, in mouse *Hoxb8* can be repressed by microRNAs in the posterior trunk mesoderm (Mansfield et al., 2004; Yekta et al., 2004; Hornstein et al., 2005). So far, *Hox* regulation by microRNAs appears to only inhibit Hox activity, either by cleavage of *Hox* transcripts or prohibition of translation (Chopra and Mishra, 2006).

Over 30 *Hox* targets, known as effector or realizator genes, from a wide range of organisms have been identified (Pearson et al., 2005). These data suggest that *Hox* genes regulate a wide array of functions. For example, in *Xenopus*, Hoxb4 has been demonstrated to regulate other *Hox* genes (Hooiveld et al., 1999); other homeodomain transcription factors such as *Irx5* (Theokli et al., 2003); the small GTPase *Ras1* (Morsi El-Kadi et al., 2002); and a component of the apoptotic pathway, *Flash* (Morgan et al., 2004). It appears that *Hox* activity is extremely broad and generic. There may be many hundreds of effector genes regulating all sorts of local cellular functions like adhesion, motility, and proliferation (Pearson et al., 2005). Getting to the brass tacks of such a seemingly diffuse system is proving difficult.

What is most important from the perspective of body plans is the highly conserved genomic, "collinear" organization of these genes in bilaterians. It may be that other gene families yet to be discovered use a genomic level of organization to facilitate their function, but *Hox* genes are so far the only example. There is still much to be learned from the elegant colinear organization of the *Hox* genes, not only about morphogenesis but also about the large-scale organization of the genome itself.

INTEGRATION OF THE AXIAL AND APPENDICULAR MUSCULOSKELETAL SYSTEMS

Much of what we know about global patterning in the mesoderm has been determined through classical perturbation experiments. Surprisingly, local populations within each somite are not committed until after segmentation (Dockter and Ordahl, 2000), but global axial identity (i.e., morphology of the individual vertebrae) is autonomously patterned prior to segmentation. When the segmental plate is transplanted heterotopically to a different axial level, the morphology of the vertebrae maintains the morphology of its original location (Kieny et al., 1972). This has been confirmed for *Hox* expression as well (Nowicki and Burke, 2000; Fomenou et al., 2005). Misexpression experiments of *Hoxa10* either prior to or during segmentation have suggested that *Hox* expression is much more relevant to morphology prior to segmentation (Carapuco et al., 2005).

How are the axial and appendicular musculoskeletal systems coordinated, such that they can be so reproducibly integrated in various vertebrate species? The faithfulness with which

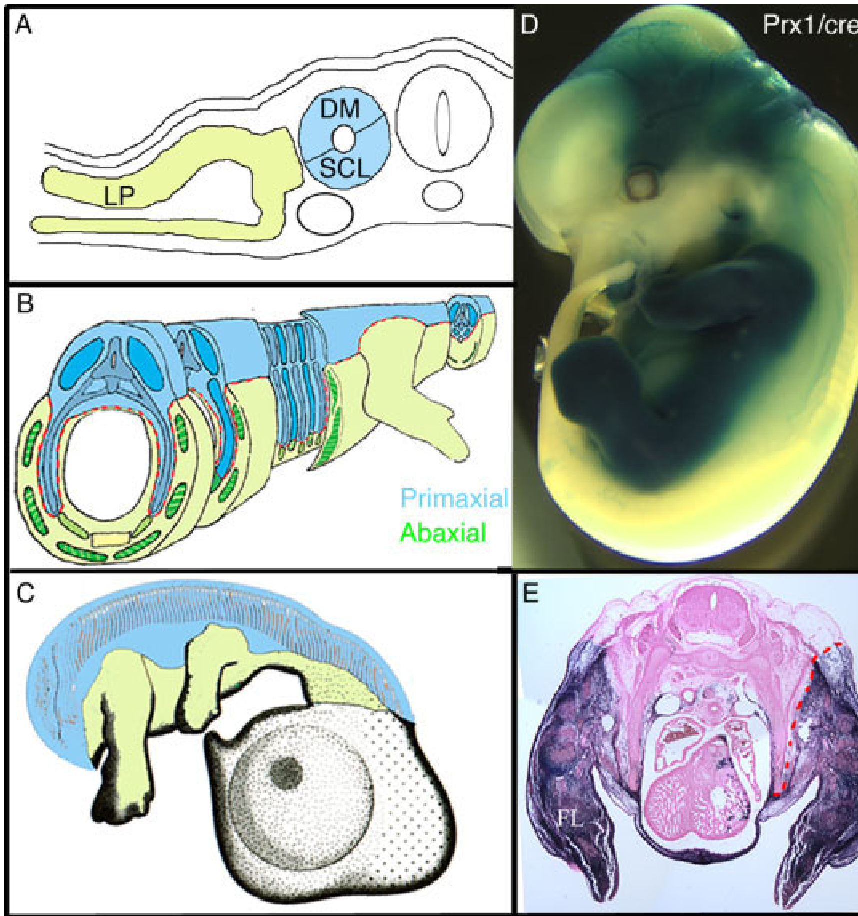

Fig. 1.

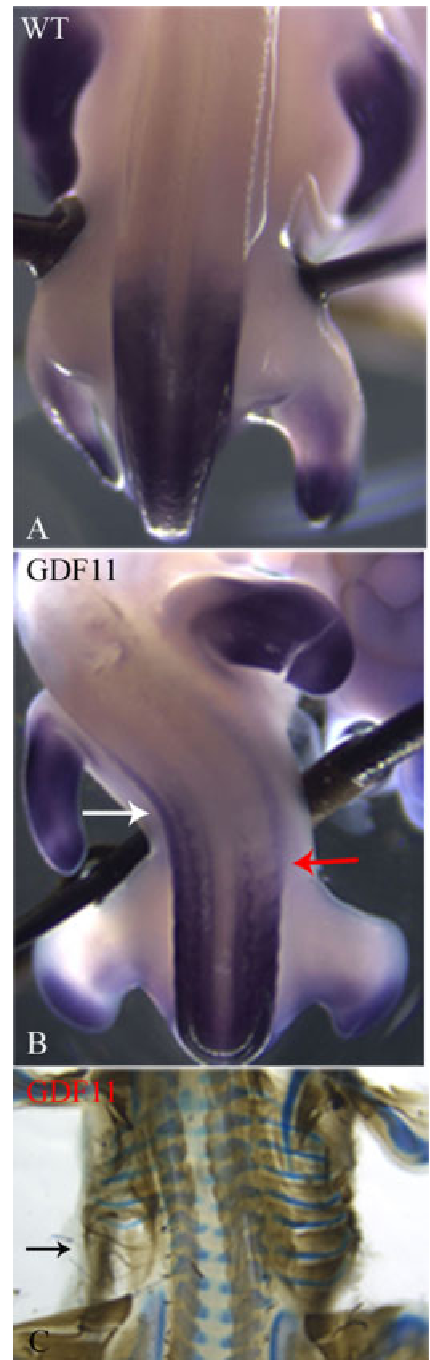

Fig. 2.

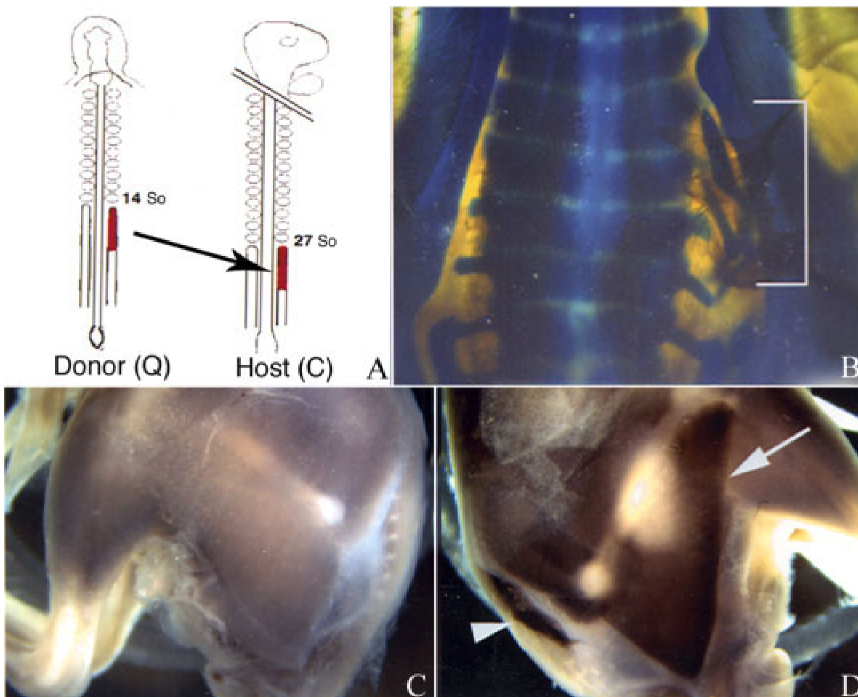

Fig. 3.

limbs and body are coordinated suggests that there is considerable information exchange between the axial and appendicular systems. The nature of this information remains unknown, but is probably represented by local signaling factors coordinated on a global scale. Morphological and *Hox* gene expression studies suggest that the primaxial and abaxial domains reflect two different patterning environ-

ments in the embryo (Burke and Nowicki, 2003), and that there is significant information exchange across the lateral somitic frontier, such as the lateralization of somitic mesoderm by Bmp4 expressed by the LP (Pourquie et al., 1995, 1996). The consistent correlation of limb position and axial Hox code in vertebrates with different numbers of vertebrae is strong curcumstantial evidence that the axial Hox code determines limb position and other morphological transitions along the AP axis (Burke et al., 1995; Wellik and Capecchi, 2003).

As mentioned, both morphology and *Hox* gene expression exhibit autonomous patterning when transplanted segmental plate cells differentiate in the primaxial domain (Kieny et al., 1972; Nowicki and Burke, 2000). Later in development, as the two mesodermal populations mix, evidence indicates that information inherent to the LP may be able to override the endogenous pattern of axial tissue (Gumpel-Pinot, 1984). However, when the graft-derived cells migrate across the lateral somitic frontier and mix with LP cells, they appear to conform to the patterning of the host (Fig. 3). For example, when lumbar to thoracic level somitic transplants are performed, ectopic lumbar body wall muscles form in the primaxial domain of the host, while the abaxial pectoralis muscle is chimeric (Murakami and Nakamura, 1991). When paraxial mesoderm is transplanted from forelimb to hindlimb levels, the somitic cells contribute to the hindlimb, and the abaxial leg muscles form according to their normal morphology, despite the fact that the cells originated from a different axial level (Fig. 3). Furthermore, cells migrating from the somitic mesoderm into the limb are not specified as specific muscles or even muscle types. Rather, they appear to obtain this information from the limb mesoderm (Kardon et al., 2002).

There are also experimental data showing that axial mesoderm can influence gene expression in the LP and vice versa. For instance, wing level paraxial mesoderm transplanted to the hind limb level paraxial mesoderm just after gastrulation can induce forelimb level *Tbx* expression (*Tbx5)* in the LP (Saito et al., 2006). Alvares et al. (2003) demonstrated that *Lbx1* expression is dependent on *Hox* expression in the paraxial mesoderm. They also showed that the LP can override the Hox code in the ventrolateral somite. When limb level LP is transplanted along the axis, it is able to induce *Lbx1* in all somites. This is another example of information exchanged across the lateral somitic frontier.

There are numerous examples of gene regulation and interactions that appear to differ between the primaxial and abaxial domains. Many mutations in patterning genes result in phenotypes that differ between the domains (reviewed in Burke and Nowicki, 2003). Recent examples of differential regulation include *Pax3*, where an identified 5′ regulatory element drives expression specifically in the leading edge of the ventrolateral dermomyotome (Brown et al., 2005). Six1 binds this specific enhancer at the lateral somitic frontier, and *Six1*/*Six4* mutant embryos lack ventral body wall and limb muscle (Grifone et al., 2005, 2007). Brown et al. (2005) call this regulatory element a "hypaxial enhancer"; however, it does not drive *Pax3* in all of the hypaxial derivatives, only in the subset giving rise to the ventral body wall, limbs, diaphragm, and tongue. These are the cells at the lateral somitic frontier, and the enhancer might be better described as "abaxial," since intercostal muscles are not involved. Pax3 itself also binds specific enhancer elements, including a 145–base pair enhancer for *Myf5* that drives this gene specifically in the ventral body wall and limb muscle cells (i.e., cells of the abaxial domain) (Bajard et al., 2006).

These studies suggest that the abaxial and primaxial domains represent different patterning regions. It is likely that the LP-derived connective tissue acts as a mediator of patterning in the abaxial domain. A similar phenomenon has been observed in the craniofacial region where mesoderm invades neural crest–derived connective tissue (Noden, 1983, 1986; Evans and Noden, 2006). The neural crest: mesoderm interface is similar to the

Fig. 1. Vertebrate mesoderm development and primaxial and abaxial domains describe modified from Nowicki et al., 2003). **A:** Schematic of a cross-section through a segmented chick. Dorsal is to the top. Mesoderm is color coded: Blue = somitic mesoderm, which is composed of dermomyotome (DM) and sclerotome (SCL); Yellow = lateral plate mesoderm; LP = somatic layer of the lateral plate mesoderm. **B:** Schematic of a cross-section through an embryonic day 9 (E9) chick, with the primaxial and abaxial domains distinguished by color. The primaxial domain (blue) consists of somitic cells that differentiate in the somitic environment. The abaxial domain (green) consists of somitic cells that differentiate in the lateral plate. **C:** Whole mount schematic of an E9 chick with the primaxial and abaxial domains color coded as in B. **D:** Whole mount E13.5 mouse in which *Prx1* expressed by the LP drives an alkaline-phosphatase reporter via cre recombinase, causing the LP to label blue. **E:** Cross section through an E13.5 *Prx1* reporter mouse at the second rib level, counterstained with eosin. The blue distinguishes cells of the LP, and represents the abaxial domain. FL, forelimb; red dotted line, lateral somitic frontier.

Fig. 2. Overexpression of GDF11 in the neural tube shifts *Hoxd10* expression and axial morphology. **A:** In situ hybridization showing wildtype *Hoxd10* expression in H&H stage 25+ chick (E4), dorsal view. **B:** In situ hybridization for *Hoxd10* in St. 25+ chick (E4) in which GDF11 was overexpressed in the left side of the neural tube via electroporation at H&H stage 11 (13 somites). *Hoxd10* extends more anteriorly on the electroporated (left) side of the embryo, marked by the white arrow. The red arrow marks the normal level of *Hoxd10* expression (right side, not electroporated). **C:** Dorsal view of a day 10 chick that was electroporated at H&H stage 9 (8 somites). Muscle was labeled with MF20 antibody and nerve with 3A10 antibody (brown). The skeleton was stained with alcian blue for cartilage (blue) and cleared in glycerol:KOH. The axial morphology is shifted anteriorly on the electroporated side (left). Black arrow points to area of anteriorized lumbar morphology. Electroporations conducted by J. P. Liu, University of Virgina.

Fig. 3. Heterotopic transplants reveal differences in patterning behavior between the primaxial and the abaxial domains. **A:** Schematic of transplants shown in B–D. Segmental plate from a 14-somite donor quail was transplanted to the segmental plate of a 27-somite chick host. **B:** Dorsal view of the pelvic region of an E10 chimera cleared and stained for cartilage. Bracket indicates ectopic ribs in the lumbar region. **C,D:** E10 chimera, unoperated side (C) and operated sides (D), lateral views. The chimera was stained for muscle, (light brown, MF20 antibody, DAB secondary) and quail donor cells (black, QcPN antibody, DAB secondary with NiCl). Unoperated side (C) shows normal muscles. Operated side (D): Arrowhead indicates ectopic, donor level shoulder muscle in the primaxial domain. Arrow points to donor (forelimb level) -derived cells contributing normally to host hindlimb level muscle in the abaxial domain.

paraxial:LP interface in this patterning aspect. Both interfaces are initially smooth, but become irregular as development proceeds (Evans and Noden, 2006; Noden and Francis-West, 2006).

We suggest that the terms primaxial and abaxial are often more appropriate than epaxial and hypaxial for describing embryonic phenomena. In work on *Xenopus* (e.g., Martin and Harland, 2001, 2006; Martin et al., 2007) and zebrafish (Haines et al., 2004), the effects of disruption of migratory muscle precursors using morpholinos are described as affecting “hypaxial” muscle. This is not incorrect. However, the great bulk of hypaxial muscle, that which is primaxial, is unaffected by these perturbations. Thus, describing the effects as abaxial would be more precise.

CONCLUSIONS

Here we have reviewed current data and our thinking regarding how the vertebrate mesoderm is patterned on the global scale. The exact mechanisms are not clearly defined, though it can be assumed that the mixture of local signaling factors is orchestrated into a global pattern. In the paraxial mesoderm, *Hox* gene expression correlates with morphology and takes part in global patterning. Though much is known about *Hox* regulation and some *Hox* effector genes have been identified, there is still a gap in knowledge between gene expression data and how genes enforce specific morphology. It is becoming increasingly obvious that the power of *Hox* genes depends largely on their orchestrated colinear expression.

A conceptual framework for thinking about the role of developmental control genes has been proposed by Davidson and Erwin (2006). These authors have outlined several gene regulatory networks (GRNs) that regulate aspects of development. They propose that GRNs are hierarchically organized, with the top, or central GRNs orchestrating lower, or peripheral GRNs. Small differences between the central GRNs are hypothesized to be responsible for macro-evolutionary events, such as the phylum level body plan generation characteristic of the Cambrian explosion. Changes to the peripheral GRNs are, therefore, responsible for progressively smaller scale evolutionary events, with the most peripheral GRNs likely responsible for species-level differences (Davidson and Erwin, 2006). A computational approach to defining GRNs was recently conducted in *Caenorhabditis elegans*. Gunsalus et al. (2005) combined gene expression data, protein interaction data, and phenotype data to generate gene groupings that likely represent GRNs.

The recognition of discrete GRNs is a major advance in understanding genetic regulation. However, to link this molecular level of understanding to the generation of form during development requires understanding the cellular and tissue level environment in which the genes take their action. In terms of mesodermal patterning, we have previously proposed terminology that describes two discrete domains that interact during development. Specifically, the connective tissue environment is the site of global patterning information derived either from the somites in the primaxial domain or the lateral plate in the abaxial domain. Evidence suggests that each domain influences the other at the lateral somitic frontier, and we hypothesize that this divide provides a switch point for steps in the GRN hierarchy. As such, the frontier is also the site of evolutionary change of gene regulatory networks, and therefore morphology. Furthermore, the discrete primaxial and abaxial domains likely allow evolutionary change and organismal stability to occur simultaneously, providing an operational avenue for evolutionary change.

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