Published in final edited form as:

Curr Osteoporos Rep. 2014 December ; 12(4): 420–427. doi:10.1007/s11914-014-0241-0.

Hox Genes and Limb Musculoskeletal Development

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Abstract

In the musculoskeletal system, muscle, tendon and bone tissues develop in a spatially and temporally coordinated manner, and integrate into a cohesive functional unit by forming specific connections unique to each region of the musculoskeletal system. The mechanisms of these patterning and integration events are an area of great interest in musculoskeletal biology. *Hox* genes are a family of important developmental regulators and play critical roles in skeletal patterning throughout the axial and appendicular skeleton. Unexpectedly, *Hox* genes are not expressed in the differentiated cartilage or other skeletal cells, but rather are highly expressed in the tightly associated stromal connective tissues as well as regionally expressed in tendons and muscle connective tissue. Recent work has revealed a previously unappreciated role for *Hox* in patterning all the musculoskeletal tissues of the limb. These observations suggest that integration of the musculoskeletal system is regulated, at least in part, by *Hox* function in the stromal connective tissue. This review will outline our current understanding of *Hox* function in patterning and integrating the musculoskeletal tissues.

Introduction

How tissues are patterned then integrated with one another during development is an important and highly complex question in developmental biology. The interaction between cells of different embryonic origins and between different tissues within the organism is essential for proper development and function. The musculoskeletal system is an example of a highly complex set of structures that integrate cell types from several distinct embryonic origins. The musculoskeletal system is composed of three basic components: muscle, tendon, and bone, which undergo substantial changes to achieve their final form. One family of highly conserved developmental regulators that have been shown to be important for the integration and patterning of the musculoskeletal tissues is the *Hox* genes. This review will

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Conflict of Interest

KM Pineault and DM Wellik both declare no conflicts of interest.

Human and Animal Rights and Informed Consent

All studies by KM Pineault and DM Wellik involving animal research were performed after approval by the appropriate institutional review boards.

provide an overview of the role of *Hox* genes in musculoskeletal development with an emphasis on how *Hox* regulates musculoskeletal development and patterning in the limb.

Hox Function and Skeletal Patterning

Hox genes are a family of highly conserved homeodomain-containing transcription factors that were first described in the fruit fly, *Drosophila*. These genes instruct what has been referred to as positional identity along the anterior to posterior (AP) body axis. The collinear arrangement of these genes along the chromosome reflects their spatial and temporal expression within the organism (Fig 1) [1–6].

The linked *Hox* cluster underwent duplications during vertebrate evolution. This resulted in a total of 39 *Hox* genes in all mammalian species, arranged on four chromosomal clusters (Fig 1) [2, 7]. The collinear arrangement of the *Hox* genes has been maintained. The genes in each cluster are further subdivided into 13 paralogous groups based on sequence similarity and position within the cluster [8]. Members of each paralogous group share similar expression domains, and significant functional redundancy has been retained among paralogs. In many cases assessing *Hox* gene function has required combining loss-of-function mutations in multiple members within the paralogous groups [6, 9–23].

Along the AP axis of the body, *Hox* genes are expressed in collinear, but overlapping domains within the somites and input from multiple *Hox* paralogous groups are responsible for establishing correct positional identity of the vertebra (reviewed in detail [24, 25]). This combinatorial code of *Hox* expression along the AP axis results in vertebrae morphology being determined by patterning information from multiple *Hox* genes, usually two or more paralogous groups. When loss of an entire paralogous group occurs, the region that normally receives this input is patterned by the remaining *Hox* genes in the region. In the majority of cases this leads to anterior homeotic transformations in which the vertebrae assume a more anterior morphology [6, 13–15].

In addition to the role of *Hox* genes in patterning the axial skeleton, the posterior most *Hox* paralogs (*Hox9-13*) pattern the limb skeleton along the proximodistal (PD) axis. The vertebrate limb can be divided into three segments: the proximal stylopod (humerus/femur), the medial zeugopod (radius and ulna/tibia and fibula), and the distal autopod (hand/foot bones). In contrast to the anterior homeotic transformations observed in the axial skeleton, loss of *Hox* paralogous groups in the limb results in a complete loss of patterning information within a specific limb segment. For example, loss of *Hox10* paralogous genes results in severe stylopod mis-patterning, loss of *Hox11* paralogous genes results in severe zeugopod mis-patterning, and loss of *Hox13* paralogous genes results in a complete loss of autopod skeletal elements [6, 10–12, 26]. The difference between axial and limb patterning is due to the non-overlapping function of *Hox* paralogous groups within the limb. The posterior *HoxA* and *HoxD* clusters are expressed in both the forelimb and the hindlimb, while the *HoxC* cluster is only expressed in the hindlimb [6, 10, 27]. With loss of function for all posterior *HoxA* and *HoxD* genes in the limb, there are severely truncated skeletal elements [28]. Additionally, posterior *Shh* expression is not initiated or maintained

demonstrating a combined requirement for *Hox* function in early limb AP patterning as well as PD patterning [28].

Roles for two other paralogous groups, *Hox5* and *Hox9*, have recently been described in patterning the AP axis of the forelimb. With complete loss of *Hox9* genes (*Hoxa9* $-/-$; *Hoxb9* $-/-$, *Hoxc9* $-/-$, *Hoxd9* $-/-$), *Shh* expression is not initiated, disrupting AP patterning within the developing limb bud [29]. *Hox9* promotes posterior *Hand2* expression resulting in the inhibition of the hedgehog pathway inhibitor Gli3, allowing for induction of *Shh* expression in the posterior limb bud. *Gli3* expression is not repressed when *Hand2* is lost in the posterior limb bud. The overexpression of Gli3 results in a complete loss of *Shh* expression and no AP axis is established. This results in only one skeletal element in each limb segment, identical to the phenotype resulting from loss of *Shh* function [29]. Loss-of-function mutations in the *Hox5* paralogous group (*Hoxa5* $-/-$, *Hoxb5* $-/-$, *Hoxc5* $-/-$) results in loss of repression of anterior *Shh* expression in the anterior limb bud leading to anterior patterning defects in the limb [30]. In this case, *Hox5* has been reported to interact with *Plzf* and functions to restrict *Shh* to the posterior limb bud [30].

Vertebrate Limb Musculoskeletal Development

Musculoskeletal skeletal development is a complex process whereby bone, tendon and muscle tissues must be appropriately patterned and precisely connected for physiologically relevant movement. An excellent model for studying musculoskeletal system development is the vertebrate limb. During limb development, bone, tendon, and muscle precursors differentiate and are coordinately patterned and integrated into a functional unit. There is significant knowledge on the developmental regulation of bone, tendon, and muscle tissue individually (reviewed in detail [31–33]) however, our understanding on how these tissues integrate into a cohesive unit is less clear. Work in recent years has begun to tease apart the mechanisms behind these coordinated patterning events, and it is clear that there is substantial communication between these tissues as limb pattern is established.

The limb musculoskeletal system is composed of mesodermal tissue that is derived from two distinct embryonic compartments (Fig II). The lateral plate mesoderm gives rise to the limb bud itself and the cartilage and tendon precursors arise within the lateral plate mesoderm of the limb bud [34, 35]. In contrast, muscle precursors differentiate from the dermomyotomal compartment of the axial somites adjacent to the limb field and migrate into the limb bud to form the limb musculature [36–39]

In the mouse, the forelimb bud emerges at embryonic day 9 (E9) from the lateral plate mesoderm on each side of the embryo. The skeletal pattern of the limb arises as *Sox9* positive cartilage precursors condense centrally within the limb bud mesenchyme from proximal (stylopod) to distal (autopod) beginning around E11.5 [40]. Concurrently, muscle precursor cells delaminate from the ventrolateral dermomyotome of the somites adjacent to the limb bud and migrate into the limb as dorsal and ventral masses, where they continue to proliferate, aggregate, and differentiate into muscle tissue [36]. Finally, tendon primordia arise in the dorsal and ventral limb mesenchyme directly from the lateral plate mesoderm then align between the muscle masses and the skeletal elements (Fig III) [41, 42]. The dorsal

and ventral muscle bundles segregate into individual anatomical groups as muscle connective tissue cells align along future sites of splitting and initiate cell death [41, 43, 44]. This process generally proceeds in a proximal to distal and dorsal to ventral fashion along the limb axis. Bone, tendon, and muscle precursors undergo concurrent differentiation and patterning in a highly coordinated fashion, however there is a significant lack of understanding on the mechanisms behind these highly coordinated patterning events.

Early patterning events are tissue autonomous

Muscle precursors themselves have no intrinsic or pre-determined patterning information. Classical chick-quail graft experiments, where somites outside the normal limb region are grafted into the axial region adjacent to the limb, demonstrated that muscles from any somite are capable of migrating into the limb and forming normal limb musculature [36, 37]. More complex single-cell lineage analysis has demonstrated that transplanted somitic muscle precursors are not biased to form specific anatomical muscles [45]. If tendon primordia are surgically removed, muscle progenitors still migrate into the limb bud mesenchyme and are able to undergo differentiation demonstrating that the differentiation of muscle tissue is autonomous and does not require signals from the tendon [41]. Markers of early tendon progenitors have been described relatively recently, but it is not clear if they mark the earliest tendon progenitors [42]. Therefore, there has been no genetic ablation of all limb tendon progenitors. The generation of a tendon-less limb mouse will be necessary to test whether early muscle patterning events occur in the complete absence of tendon progenitors.

Various muscle-less limb models have demonstrated that early patterning of the connective tissue and skeletal elements do not require the presence of muscle. Initiation of tendon and muscle connective tissue pattern occurs normally in muscle-less limb models in both mouse and chick [41, 42, 46, 47]. The Sox9⁺ skeletal elements are also normally patterned in the absence of muscle.

Muscle and tendon/muscle connective tissue interactions are required for later patterning events

After the initial pre-patterning events of the tendon and the muscle connective tissue occur, integration of the muscle, bone, and tendon requires interaction between tissues. While early specification and patterning of tendon and muscle connective tissue is normal in muscle-less limbs, tendon progenitors are progressively lost in a proximal to distal fashion [41]. After migrating into the limb, patterning of the dorsal and ventral muscle masses requires interactions with the tendon. If tendon primordia are surgically removed from the limb, proper muscle patterning is disrupted and aberrant muscles form [41]. The formation of aberrant muscle in the absence of tendon demonstrates a role for tendon in negatively regulating muscle formation in specific regions of the limb [41].

There is growing evidence that the connective tissue elements of the limb, tendons and muscle connective tissue, have critical roles in patterning the muscle. The T-box transcription factors *Tbx4* and *Tbx5* are broadly expressed in the limb bud mesenchyme including the muscle connective tissue and tendon precursors [48]. Loss of *Tbx5* gene function results in ectopic splitting of the dorsal and ventral limb muscle masses and

disruption of the muscle connective tissue organization. Additionally, levels of β -Catenin and N-cadherin were reduced. These data suggest that the organization of connective tissue and catenin/cadherin mediated cell adhesion are required for connective tissue-mediated muscle patterning.

The transcription factor *Tcf4*, a member of the Tcf/Lef family which acts downstream of *Wnt* signaling, has been identified as a marker of muscle connective tissue cells [49]. Overexpression of *Tcf4* results in the formation of ectopic muscle and loss of *Tcf4* disrupts muscle fiber type switching suggesting that the muscle connective tissue directs differentiation of muscle precursors [49, 50]. There is also evidence that *Fgf4* signals from muscle at sites of tendon attachment function to maintain expression of tendon specific genes [47]. Together these data demonstrate a critical requirement for muscle-tendon interactions for maintenance and patterning of both tissues.

Muscle and tendon interactions with bone are necessary for patterning bone ridges

Attachment of muscle to the bone through tendon is important in patterning the skeletal elements at sites of tendon attachment. It has long been appreciated that mechanical forces generated in muscle and transmitted to the bone through tendon attachments influence the final skeletal morphology [51–53]. In *Scx* null mutants, tendon condensation and differentiation is disrupted and consequently the deltoid tuberosity does not form on the humerus demonstrating a requirement for proper tendon formation in the development of this bone process [54]. Recent data provide evidence that bone ridge cells originate from a pool of *Scx*⁺*Sox9*⁺ progenitor cells [55–57]. Bone ridge patterning appears to be a biphasic process of initiation and growth stages, and signals from the tendon are critical to initiate formation of these skeletal structures [58]. Additionally, there is also a well-described role for muscle loading in regulating proper tendon structure [57, 59, 60]. These studies highlight the critical requirement for muscle, tendon and bone interactions in regulating bone morphology. What determines the sites of tendon-bone attachment is still a largely unanswered question in the field.

Establishment of Regional Patterning

Despite significant data on the requirement for crosstalk between muscle, tendon, and bone during musculoskeletal patterning, the mechanisms responsible for these processes are still under active exploration. A critical component in patterning the musculoskeletal system is the incorporation of regional differences in patterning along the AP axis of the vertebrae and the PD axis of the limb. The same tissue components, muscle, tendon and bone, are present throughout the limb, but substantial morphological differences and unique connections occur within these tissues along the axial and limb axes. While there is significant information on the development of muscle, tendon, and bone, how these tissues form precise connections and become patterned and integrated into a functional unit is largely unknown. Work published recently from our laboratory suggests that this regional patterning information is provided, at least in part, by the master developmental regulators, the *Hox* genes.

Hox genes expression is initiated very early in limb bud development. The posterior *Hox* genes are expressed in a nested and highly overlapping pattern within the limb bud. As the

limb develops and grows in length, the *Hox* paralogous groups become progressively restricted to their appropriate functional domains such that *Hox9* and *Hox10* are expressed in the stylopod, *Hox11* is expressed in the zeugopod, and *Hox13* is expressed in the autopod (Fig III) [6, 10–12, 26, 61]. This restricted expression pattern is then maintained throughout embryonic development [61].

The regionalized expression and function of *Hox* genes along the limb axis makes them an attractive candidate for directing regionalized patterning and integration of the musculoskeletal tissues. A *Hoxa11eGFP* knock-in mouse model was utilized to examine *Hox11* expression in detail within the developing limb. Characterization of *Hoxa11eGFP* expression in whole mount and in longitudinal limb sections shows broad expression throughout the distal limb bud mesenchyme early in development. This expression rapidly becomes restricted to the presumptive zeugopod [61]. Interestingly, *Hoxa11eGFP*⁺ cells are largely excluded from the *Sox9*⁺ chondrogenic precursor population at all stages of development [61]. Careful examination of *Hoxa11eGFP* expression shows *Hoxa11eGFP*⁺ cells in the outer perichondrial layer surrounding the presumptive zeugopod condensations (Fig IVA–B). Analysis with markers for muscle and tendon revealed *Hoxa11eGFP* expression within the muscle connective tissue and all cells of the tendon. Throughout limb development, *Hoxa11eGFP*⁺ cells are always excluded from muscle and bone and remain expressed in the stromal connective tissues (Fig IV A, C) [61]. *Hoxa13GFP* expression has also been reported to be expressed in the perichondrium and excluded from condensing cartilaginous elements, however detailed co-expression analysis with muscle and tendon markers has not yet been examined [62].

The cartilage elements destined to become the limb skeletal elements still condense in *Hox* loss-of-function mutants but they are not elaborated to form the appropriate anatomical bones [26]. The observation that *Hox* is not expressed in the skeletal elements but in the perichondrial stromal layer surrounding the elements significantly changes how the skeletal phenotypes observed in these mutants are interpreted. These data strongly suggest that signals from the perichondrial stromal cells have an important role in determining the morphology and patterning of the skeletal elements.

Hoxa11eGFP expression in the connective tissues of the limb was a surprising finding and prompted analysis of the muscle and tendon phenotypes in *Hox11* mutants. A potential role for *Hox* in mediating muscle or tendon development had not been previously investigated in detail. In addition to the well-described regional skeletal phenotypes observed in *Hox* paralogous loss-of-function mutants, severe mis-patterning of the zeugopod muscles and tendons is observed in *Hox11* loss-of-function mutants [61]. Early in development, tendon progenitors are specified in *Hox11* mutants and appear similar to controls, suggesting that *Hox* functions in patterning and integration but does not function in specification of connective tissue. In *Hox11* mutants, the migration of muscle precursors into the limb occurs normally and dorsal and ventral muscle masses are present. However, the subsequent splitting of the masses into individual muscle bundles is abnormal and loss or fusion of various muscle groups is observed [61]. Muscle differentiation, however, still occurs as mature muscle cells are observed in these mutants. The muscle-specific phenotypes in *Hox11* mutants suggest a critical role for *Hox* in connective tissue-mediated patterning of

the muscle but not in directing muscle migration or differentiation. These data provide strong evidence that *Hox* function in the connective tissue stromal cells is critical for regionally patterning all components of the musculoskeletal system.

Development and patterning of the skeletal elements remains normal in *Hox11* compound mutants where one of the four functional alleles of forelimb *Hox11* (either *Hoxa11* or *Hoxd11*) is still expressed [6, 10, 26, 61]. *Hox11* compound mutants allow us to address the question of whether the muscle and tendon phenotypes observed in *Hox11* double mutant animals represent independent roles for *Hox11* in patterning these tissues, or whether the phenotypes are a consequence of the severely disrupted skeletal patterning. In *Hox11* compound mutants, skeletal patterning is preserved but regional mis-patterning of the muscles and tendons is still observed, demonstrating that the muscle and tendon phenotype is independent of skeletal pattern [61]. These analyses also demonstrate that skeletal patterning and muscle/tendon patterning can be uncoupled. Analysis of limb muscle and tendon phenotypes in other posterior *Hox* gene paralogous mutants has not been investigated in detail, but we hypothesize that similar muscle and tendon phenotypes will be observed.

Perspectives and Future Directions

These observations raise very important questions of how *Hox* genes function in the musculoskeletal system and how they contribute to integrating the patterning of muscles, tendons and bones. Many studies highlight the importance of the connective tissues in patterning the limb, and the expression of *Hoxa11:eGFP* in the stromal connective tissues of the limb is consistent with an important role for *Hox* in these stromal cells in directing the pattern of musculoskeletal tissues [41, 48–50, 58, 61]. The continuum of *Hoxa11:eGFP* expression from the muscle connective tissue, into the tendon, and then the perichondrium surrounding the bone suggests a role for *Hox11* in integrating patterning information between these tissues. The connective tissue extracellular matrix is made up of largely the same components throughout the limb and identification of region and tissue specific markers is important for understanding how these tissues direct regional patterning. Expression of *Hoxa11:eGFP* provides a unique tool to specifically identify these connective tissue elements for analysis within the zeugopod. Generation of fluorescent alleles and conditional loss of function experiments for other *Hox* genes will be beneficial in addressing these questions. Our recent work strongly suggests that connective tissue cells possess regional identity information and play a critical role in establishing proper region-specific pattern of the musculoskeletal tissues [61].

Musculoskeletal injury and disease can have a devastating impact on a patient's quality of life and there is a great need for novel and improved treatments and therapies. As master developmental regulators, understanding and exploiting the *Hox* signaling program could be invaluable for the development of usable stem cell therapies designed to treat musculoskeletal injury and disease. Our observations suggest that different *Hox* programs may need to be activated to direct appropriate stem cell activity for use in repair at specific axial or proximal/distal levels.

References

Papers of particular interest, published recently, have been highlighted as:

•• Of major importance

• Of importance

1. Duboule D. Patterning in the vertebrate limb. *Current opinion in genetics & development*. 1991; 1(2):211–6. [http://dx.doi.org/10.1016/S0959-437X\(05\)80072-3](http://dx.doi.org/10.1016/S0959-437X(05)80072-3). [PubMed: 1688004]
2. Krumlauf R. Hox genes in vertebrate development. *Cell*. 1994; 78(2):191–201. [PubMed: 7913880]
3. Lewis EB. Genes and Developmental Pathways. *American Zoologist*. 1963; 3(1):33–56.10.2307/3881152
4. Lewis EB. A gene complex controlling segmentation in *Drosophila*. *Nature*. 1978; 276(5688):565–70. [PubMed: 103000]
5. Lewis EB. Regulation of the genes of the bithorax complex in *Drosophila*. *Cold Spring Harbor symposia on quantitative biology*. 1985; 50:155–64.
6. Wellik DM, Capecchi MR. Hox10 and Hox11 genes are required to globally pattern the mammalian skeleton. *Science*. 2003; 301(5631):363–7.10.1126/science.1085672 [PubMed: 12869760]
7. Scott MP. Vertebrate homeobox gene nomenclature. *Cell*. 1992; 71(4):551–3. [PubMed: 1358459]
8. Pearson JC, Lemons D, McGinnis W. Modulating Hox gene functions during animal body patterning. *Nature reviews Genetics*. 2005; 6(12):893–904.10.1038/nrg1726
9. Condie BG, Capecchi MR. Mice with targeted disruptions in the paralogous genes *hoxa-3* and *hoxd-3* reveal synergistic interactions. *Nature*. 1994; 370(6487):304–7.10.1038/370304a0 [PubMed: 7913519]
10. Davis AP, Witte DP, Hsieh-Li HM, Potter SS, Capecchi MR. Absence of radius and ulna in mice lacking *hoxa-11* and *hoxd-11*. *Nature*. 1995; 375(6534):791–5.10.1038/375791a0 [PubMed: 7596412]
11. Fromental-Ramain C, Warot X, Lakkaraju S, Favier B, Haack H, Birling C, et al. Specific and redundant functions of the paralogous *Hoxa-9* and *Hoxd-9* genes in forelimb and axial skeleton patterning. *Development*. 1996; 122(2):461–72. [PubMed: 8625797]
12. Fromental-Ramain C, Warot X, Messadecq N, LeMeur M, Dolle P, Chambon P. *Hoxa-13* and *Hoxd-13* play a crucial role in the patterning of the limb autopod. *Development*. 1996; 122(10):2997–3011. [PubMed: 8898214]
13. Horan GS, Ramirez-Solis R, Featherstone MS, Wolgemuth DJ, Bradley A, Behringer RR. Compound mutants for the paralogous *hoxa-4*, *hoxb-4*, and *hoxd-4* genes show more complete homeotic transformations and a dose-dependent increase in the number of vertebrae transformed. *Genes & development*. 1995; 9(13):1667–77. [PubMed: 7628700]
14. McIntyre DC, Rakshit S, Yallowitz AR, Loken L, Jeannotte L, Capecchi MR, et al. Hox patterning of the vertebrate rib cage. *Development*. 2007; 134(16):2981–9.10.1242/dev.007567 [PubMed: 17626057]
15. van den Akker E, Fromental-Ramain C, de Graaff W, Le Mouellic H, Brulet P, Chambon P, et al. Axial skeletal patterning in mice lacking all paralogous group 8 Hox genes. *Development*. 2001; 128(10):1911–21. [PubMed: 11311170]
16. Wellik DM, Hawkes PJ, Capecchi MR. Hox11 paralogous genes are essential for metanephric kidney induction. *Genes & development*. 2002; 16(11):1423–32.10.1101/gad.993302 [PubMed: 12050119]
17. Chen F, Capecchi MR. Targeted mutations in *hoxa-9* and *hoxb-9* reveal synergistic interactions. *Dev Biol*. 1997; 181(2):186–96.10.1006/dbio.1996.8440 [PubMed: 9013929]
18. Chen F, Capecchi MR. Paralogous mouse Hox genes, *Hoxa9*, *Hoxb9*, and *Hoxd9*, function together to control development of the mammary gland in response to pregnancy. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(2):541–6. [PubMed: 9892669]

19. Chen F, Greer J, Capecchi MR. Analysis of Hoxa7/Hoxb7 mutants suggests periodicity in the generation of the different sets of vertebrae. *Mechanisms of development*. 1998; 77(1):49–57. [PubMed: 9784603]
20. Gavalas A, Trainor P, Ariza-McNaughton L, Krumlauf R. Synergy between Hoxa1 and Hoxb1: the relationship between arch patterning and the generation of cranial neural crest. *Development*. 2001; 128(15):3017–27. [PubMed: 11532923]
21. Manley NR, Capecchi MR. Hox group 3 paralogs regulate the development and migration of the thymus, thyroid, and parathyroid glands. *Dev Biol*. 1998; 195(1):1–15.10.1006/dbio.1997.8827 [PubMed: 9520319]
22. Studer M, Gavalas A, Marshall H, Ariza-McNaughton L, Rijli FM, Chambon P, et al. Genetic interactions between Hoxa1 and Hoxb1 reveal new roles in regulation of early hindbrain patterning. *Development*. 1998; 125(6):1025–36. [PubMed: 9463349]
23. Wahba GM, Hostikka SL, Carpenter EM. The paralogous Hox genes Hoxa10 and Hoxd10 interact to pattern the mouse hindlimb peripheral nervous system and skeleton. *Dev Biol*. 2001; 231(1):87–102.10.1006/dbio.2000.0130 [PubMed: 11180954]
24. Wellik DM. Hox patterning of the vertebrate axial skeleton. *Developmental dynamics: an official publication of the American Association of Anatomists*. 2007; 236(9):2454–63.10.1002/dvdy.21286 [PubMed: 17685480]
25. Wellik DM. Hox genes and vertebrate axial pattern. *Current topics in developmental biology*. 2009; 88:257–78.10.1016/s0070-2153(09)88009-5 [PubMed: 19651308]
26. Boulet AM, Capecchi MR. Multiple roles of Hoxa11 and Hoxd11 in the formation of the mammalian forelimb zeugopod. *Development*. 2004; 131(2):299–309.10.1242/dev.00936 [PubMed: 14668414]
27. Hostikka SL, Capecchi MR. The mouse Hoxc11 gene: genomic structure and expression pattern. *Mechanisms of development*. 1998; 70(1–2):133–45. [PubMed: 9510030]
28. Kmita M, Tarchini B, Zakany J, Logan M, Tabin CJ, Duboule D. Early developmental arrest of mammalian limbs lacking HoxA/HoxD gene function. *Nature*. 2005; 435(7045):1113–6.10.1038/nature03648 [PubMed: 15973411]
29. Xu B, Wellik DM. Axial Hox9 activity establishes the posterior field in the developing forelimb. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(12):4888–91.10.1073/pnas.1018161108 [PubMed: 21383175]
30. Xu B, Hrycaj SM, McIntyre DC, Baker NC, Takeuchi JK, Jeannotte L, et al. Hox5 interacts with Plzf to restrict Shh expression in the developing forelimb. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110(48):19438–43.10.1073/pnas.1315075110 [PubMed: 24218595]
31. Karsenty G, Kronenberg HM, Settembre C. Genetic control of bone formation. *Annual review of cell and developmental biology*. 2009; 25:629–48.10.1146/annurev.cellbio.042308.113308
32. Schweitzer R, Zelzer E, Volk T. Connecting muscles to tendons: tendons and musculoskeletal development in flies and vertebrates. *Development*. 2010; 137(17):2807–17.10.1242/dev.047498 [PubMed: 20699295]
33. Murphy M, Kardon G. Origin of vertebrate limb muscle: the role of progenitor and myoblast populations. *Current topics in developmental biology*. 2011; 96:1–32.10.1016/B978-0-12-385940-2.00001-2 [PubMed: 21621065]
34. Kieny M, Chevallier A. Autonomy of tendon development in the embryonic chick wing. *Journal of embryology and experimental morphology*. 1979; 49:153–65. [PubMed: 448266]
35. Shellswell G, Wolpert L. The pattern of muscle and tendon development in the chick wing. *Vertebrate limb and somite morphogenesis*. 1977:71–86.
36. Chevallier A, Kieny M, Mauger A. Limb-somite relationship: origin of the limb musculature. *Journal of embryology and experimental morphology*. 1977; 41(1):245–58. [PubMed: 591873]
37. Christ B, Jacob HJ, Jacob M. Experimental analysis of the origin of the wing musculature in avian embryos. *Anatomy and embryology*. 1977; 150(2):171–86. [PubMed: 857700]
38. Ordahl CP, Le Douarin NM. Two myogenic lineages within the developing somite. *Development*. 1992; 114(2):339–53. [PubMed: 1591996]

39. Wachtler F, Christ B, Jacob HJ. On the determination of mesodermal tissues in the avian embryonic wing bud. *Anatomy and embryology*. 1981; 161(3):283–9. [PubMed: 7187823]
40. Thorogood PV, Hinchliffe JR. An analysis of the condensation process during chondrogenesis in the embryonic chick hind limb. *Journal of embryology and experimental morphology*. 1975; 33(3): 581–606. [PubMed: 1176861]
41. Kardon G. Muscle and tendon morphogenesis in the avian hind limb. *Development*. 1998; 125(20): 4019–32. [PubMed: 9735363]
42. Schweitzer R, Chyung JH, Murtaugh LC, Brent AE, Rosen V, Olson EN, et al. Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. *Development*. 2001; 128(19):3855–66. [PubMed: 11585810]
43. Schroeter S, Tosney KW. Spatial and temporal patterns of muscle cleavage in the chick thigh and their value as criteria for homology. *The American journal of anatomy*. 1991; 191(4):325–50.10.1002/aja.1001910402 [PubMed: 1951134]
44. Schroeter S, Tosney KW. Ultrastructural and morphometric analysis of the separation of two thigh muscles in the chick. *The American journal of anatomy*. 1991; 191(4):351–68.10.1002/aja.1001910403 [PubMed: 1951135]
45. Kardon G, Campbell JK, Tabin CJ. Local extrinsic signals determine muscle and endothelial cell fate and patterning in the vertebrate limb. *Developmental cell*. 2002; 3(4):533–45. [PubMed: 12408805]
46. Brent AE, Braun T, Tabin CJ. Genetic analysis of interactions between the somitic muscle, cartilage and tendon cell lineages during mouse development. *Development*. 2005; 132(3):515–28.10.1242/dev.01605 [PubMed: 15634692]
47. Edom-Vovard F, Schuler B, Bonnin M-A, Teillet M-A, Duprez D. Fgf4 Positively Regulates scleraxis and Tenascin Expression in Chick Limb Tendons. *Developmental biology*. 2002; 247(2): 351–66.10.1006/dbio.2002.0707 [PubMed: 12086472]
48. Hasson P, DeLaurier A, Bennett M, Grigorieva E, Naiche LA, Papaioannou VE, et al. Tbx4 and tbx5 acting in connective tissue are required for limb muscle and tendon patterning. *Dev Cell*. 2010; 18(1):148–56.10.1016/j.devcel.2009.11.013 [PubMed: 20152185]
49. Kardon G, Harfe BD, Tabin CJ. A Tcf4-positive mesodermal population provides a prepattern for vertebrate limb muscle patterning. *Developmental cell*. 2003; 5(6):937–44. [PubMed: 14667415]
50. Mathew SJ, Hansen JM, Merrell AJ, Murphy MM, Lawson JA, Hutcheson DA, et al. Connective tissue fibroblasts and Tcf4 regulate myogenesis. *Development*. 2011; 138(2):371–84.10.1242/dev.057463 [PubMed: 21177349]
51. Hall BK, Herring SW. Paralysis and growth of the musculoskeletal system in the embryonic chick. *Journal of morphology*. 1990; 206(1):45–56.10.1002/jmor.1052060105 [PubMed: 2246789]
52. Hosseini A, Hogg DA. The effects of paralysis on skeletal development in the chick embryo. II. Effects on histogenesis of the tibia. *Journal of anatomy*. 1991; 177:169–78. [PubMed: 1769891]
53. Sharir A, Stern T, Rot C, Shahar R, Zelzer E. Muscle force regulates bone shaping for optimal load-bearing capacity during embryogenesis. *Development*. 2011; 138(15):3247–59.10.1242/dev.063768 [PubMed: 21750035]
54. Murchison ND, Price BA, Conner DA, Keene DR, Olson EN, Tabin CJ, et al. Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons. *Development*. 2007; 134(14):2697–708.10.1242/dev.001933 [PubMed: 17567668]
55. Akiyama H, Kim JE, Nakashima K, Balmes G, Iwai N, Deng JM, et al. Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(41):14665–70.10.1073/pnas.0504750102 [PubMed: 16203988]
56. Blitz E, Sharir A, Akiyama H, Zelzer E. Tendon-bone attachment unit is formed modularly by a distinct pool of Scx- and Sox9-positive progenitors. *Development*. 2013; 140(13):2680–90.10.1242/dev.093906 [PubMed: 23720048]
57. Sugimoto Y, Takimoto A, Akiyama H, Kist R, Scherer G, Nakamura T, et al. Scx+/Sox9+ progenitors contribute to the establishment of the junction between cartilage and tendon/ligament. *Development*. 2013; 140(11):2280–8.10.1242/dev.096354 [PubMed: 23615282]

- 58**. Blitz E, Viukov S, Sharir A, Shwartz Y, Galloway JL, Pryce BA, et al. Bone ridge patterning during musculoskeletal assembly is mediated through SCX regulation of Bmp4 at the tendon-skeleton junction. *Dev Cell*. 2009; 17(6):861–73. This work highlights the critical interaction between bone and tendon during the integration of these two tissues during development as well as key regulators of these processes. 10.1016/j.devcel.2009.10.010 [PubMed: 20059955]
59. Ralphs JR, Benjamin M, Waggett AD, Russell DC, Messner K, Gao J. Regional differences in cell shape and gap junction expression in rat Achilles tendon: relation to fibrocartilage differentiation. *Journal of anatomy*. 1998; 193 (Pt 2):215–22. [PubMed: 9827637]
60. Thomopoulos S, Williams GR, Gimbel JA, Favata M, Soslowsky LJ. Variation of biomechanical, structural, and compositional properties along the tendon to bone insertion site. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2003; 21(3):413–9. 10.1016/S0736-0266(03)00057-3 [PubMed: 12706013]
61. Swinehart IT, Schlientz AJ, Quintanilla CA, Mortlock DP, Welik DM. Hox11 genes are required for regional patterning and integration of muscle, tendon and bone. *Development*. 2013; 140(22): 4574–82. 10.1242/dev.096693 [PubMed: 24154528]
62. Stadler HS, Higgins KM, Capecchi MR. Loss of Eph-receptor expression correlates with loss of cell adhesion and chondrogenic capacity in Hoxa13 mutant limbs. *Development*. 2001; 128(21): 4177–88. [PubMed: 11684655]

Fig. I.

Schematic of *Drosophila* and mammalian *Hox* genes clusters. Colored boxes represent linear arrangement (3' to 5') along the chromosome. Color-coding depicts orthologous relationships between *Drosophila* and mammalian *Hox* genes, and paralogous relationships within the mammalian *Hox* clusters.

Fig. II.

The limb musculoskeletal tissues are derived from two distinct mesodermal compartments. Tendon (green) and chondrocyte (red) precursors are derived from the lateral plate mesoderm within the limb bud, and tendon primordia arise adjacent to the centrally localized chondrocyte condensations. Muscle precursors (purple) delaminate from the ventral somite adjacent to the limb and migrate into the limb bud as dorsal and ventral muscle masses.

Fig. III.

Posterior *Hox* paralogous genes are progressively restricted to specific limb regions during development. In the early limb bud, *Hox9-13* paralogs are expressed in an overlapping and nested pattern. As the limb develops, *Hox* paralogs are progressively restricted resulting in *Hox9/10* (yellow) expressed proximally (stylopod), *Hox11* (green) expressed medially (zeugopod), and *Hox13* (blue) expressed distally (autopod).

Fig. IV.

Hox11 is expressed in the connective tissue (stromal) compartment of the forelimb. (A) *Hoxa11eGFP* expression is restricted to the zeugopod and excluded from bone and muscle cells. *Hox11* is expressed in the outer perichondrium surrounding the bones, in the muscle connective tissue, and in the tendons. (B) Longitudinal section of E14.5 forelimb showing expression of *Hox11eGFP* (green) surrounding the *Sox9*⁺ (red) skeletal elements. (C) Transverse view of an individual forelimb muscle bundle at E14.5 showing expression of *Hox11eGFP* (green) in the muscle connective tissue surrounding individual muscle (red) cells. My32, myosin 32.