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Development of the musculoskeletal system: meeting the neighbors

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Summary

In March 2011, researchers met for the second Batsheva Seminar on Integrative Perspectives on the Development of the Musculoskeletal System. This meeting was a unique opportunity for researchers working on muscle, connective tissue, tendons, ligaments and bone to discuss the development of the musculoskeleton, recognizing that it is an integrated, functional system. The talks and discussions at this meeting highlighted that interactions between the different tissue components are crucial for musculoskeletal morphogenesis.

Key words: Bone, Connective tissue, Muscle, Musculoskeletal system, Tendon

Introduction

The musculoskeletal system is essential for structural support, locomotion and movement. It is a multicomponent system composed of muscle, muscle connective tissue, tendon, ligament and bone, innervated by nerves and vascularized by blood vessels. Thus, development of the musculoskeletal system is complex. Musculoskeletal development requires not only the specification and differentiation of these different cell types, but also their coordinated morphogenesis into an integrated functional system. In addition, development of the musculoskeletal system is complicated by the different embryological origins of the various musculoskeletal components. Furthermore, there are clear differences in the development of the axial, head and limb musculoskeleton.

Owing to the complexity of musculoskeletal development, researchers have specialized by studying the development of individual tissues or have concentrated on understanding head, axial or limb development. Reflecting this specialization, meetings on myogenesis, bone development, craniofacial morphogenesis and limb development abound. However, clearly an understanding of musculoskeletal development requires a more integrative approach, and the second Batsheva Seminar on the development of the musculoskeletal system at Ein Gedi (Israel), organized by Eldad Tzahor and Elazar Zelzer (Weizmann Institute, Rehovot, Israel), was designed to foster such integration. In addition to talks on all the component tissues, researchers presented studies on axial, head and limb musculoskeletal development. The meeting brought together researchers who use many different model vertebrate systems (zebrafish, frog, chick and mouse), as well as new, emerging systems (such as shark, duck and jerboa). Also, talks from *Drosophila* and *C. elegans* geneticists were crucial to the discussion, as many new conceptual advances have arisen from these studies. Most importantly, the talks revealed that interactions between the different components are crucial for musculoskeletal morphogenesis (Fig. 1). Another emerging theme was the importance of muscle mechanical forces in shaping cartilage and bone during development. Finally, several new studies provided insights into the evolution of the musculoskeletal system by identifying key innovations in the development of the head, neck and limb.

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Lessons on motility, fusion, nuclear position and muscle fiber type

One of the earliest developmental steps in establishing the musculoskeletal system is the formation of the presomitic mesoderm, which ultimately forms the entire axial musculoskeleton and gives rise to the limb muscles. How the presomitic mesoderm forms and elongates was the subject of Olivier Pourquie's (IGBMC, Strasbourg, France) keynote talk. Using beautiful live imaging of developing chick embryos he showed that, surprisingly, elongation of the presomitic mesoderm does not result from directed cell migration but instead is an emergent property of the collective regulation of graded, random cell motility (Benazeraf et al., 2010).

Several talks highlighted the importance of *C. elegans* and *Drosophila* genetic studies in uncovering basic cellular processes crucial for the development of the musculoskeletal system. Fusion is an essential part of the formation of multinucleated myofibers from individual myoblasts. *C. elegans* is an excellent organism in which to study cell-cell fusion as one-third of its somatic nuclei are part of a synctia, formed via fusion. Through genetic screens, Benjamin Podbilewicz's group (Technion-Israel Institute of Technology, Haifa, Israel) has identified two genes, *eff-1* and *aff-1*, that are necessary and sufficient to fuse cells (Oren-Suissa et al., 2010). These fusogens are founding members of the fusion family (FF), which are found not only in nematodes, but also in some arthropods and chordates (such as amphioxus). The ability of these fusogens to promote cell-cell fusion in cultured vertebrate kidney (BHK) cells suggests that FF fusogens might function within vertebrates (Avinoam et al., 2011). Eyal Schetjer and Benny Shilo (Weizmann Institute, Rehovot, Israel) also discussed their work on fusion, examining myoblast fusion in *Drosophila*. They showed that the *Drosophila* WASp homolog, a conserved factor that promotes Arp2/3-based actin filament nucleation, is essential for both embryonic and adult myoblast fusion. In addition, the fly SCAR/WAVE homolog, the second major Arp2/3 nucleation-promoting factor, is also required during adult myoblast fusion (Mukherjee et al., 2011).

Following the fusion of myoblasts into multinucleate myofibers, myonuclei move to a peripheral position and spread along the length of the myofiber. Improperly positioned nuclei are a hallmark of numerous muscle diseases, including centronuclear myopathies. However, the molecular mechanisms that govern myonuclear position are largely unknown and whether correct nuclear positioning is necessary for muscle function is unclear. To explore these questions, Mary Baylies' lab (Sloan-Kettering Institute, NY, USA) conducted a forward genetic screen, using live *Drosophila* embryos with fluorescently labeled myonuclei, to identify mutants with abnormal myonuclear positioning. They found that two microtubule-associated proteins are essential, evolutionary conserved regulators of myonuclear positioning. Furthermore, she

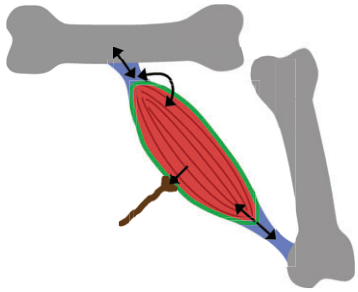

Fig. 1. Tissue interactions in musculoskeletal morphogenesis. A vertebrate muscle, showing the interactions between muscle (red), muscle connective tissue (green), tendon (blue), bone (gray) and nerve (brown) that are crucial for musculoskeletal morphogenesis.

showed that defects in myonuclear positioning impair larval motility. Determining why nuclear position is critical for muscle function will be an interesting area of future research.

Muscle and bone can arise from surprising places

Although complex, the embryological origin of the different components of the head, axial and limb musculoskeletal system has generally been well characterized. Axial and appendicular muscle arise from the somites, whereas head muscle derives from the cranial paraxial and splanchnic mesoderm. The appendicular connective tissue, tendon, ligament and bone derive from the lateral plate, whereas in the head these tissues derive from the neural crest, and in the axial musculoskeleton they originate from different regions of the somite. However, in three talks, alternate new sources for muscle and bone were described. Ketan Patel (University of Reading, UK) showed elegant experiments in chick and mouse that demonstrate that the superficial neck muscles are derived not from the somites, as previously thought, but from the occipital lateral plate mesoderm (Theis et al., 2010). This is the first time that any postcranial muscles have been shown to be derived from non-somitic cells. In the adult (and potentially in the embryo), muscle may also be derived from yet another source. Zipora Yablonka-Reuveni (University of Washington, WA, USA) used a Cre line driven by smooth muscle myosin heavy chain regulatory elements in mouse to demonstrate a unique cell lineage contribution, potentially of smooth muscle origin, to the adult muscle stem cell pool in the orbital domain of extraocular muscles. Potentially, this alternative origin of extraocular muscle stem cells explains why extraocular muscles are spared in myopathies, such as Duchenne muscular dystrophy. Finally, Bjorn Olsen (Harvard Medical School, MA, USA) presented data showing that, during disease, cartilage and bone can derive from non-canonical tissue sources. In the pathological condition fibrodysplasia ossificans progressiva (FOP), cartilage and bone ectopically form within soft tissues. Surprisingly, these heterotopic ossifications derive from endothelial cells, which via their expression of activin-like kinase 2 differentiate into chondrocytes or osteoblasts (Medici et al., 2010). Whether endothelial cells can give rise to cartilage and bone during normal skeletal development is a tantalizing, but unexplored, possibility.

Muscle interacts with tendons and nerves during development

Musculoskeletal function requires that muscle be linked to bone via tendons and be innervated by motoneurons. Interactions between muscle and tendon are crucial for their development. *Drosophila* have tendon-like cells, which transmit muscle contractile force to the cuticle. Work from Talila Volk's lab (Weizmann Institute, Rehovot, Israel) has shown that Stripe, an Egr (early growth response)-like transcription factor, is essential for the specification and differentiation of these tendon-like cells. Now, Volk's group has identified, via microarrays, new tendon-specific genes. She showed that two of these genes, *Thrombospondin* and *slowdown*, are regulated by Stripe, and that these factors are secreted from tendon cells and direct the adhesion of muscle to its corresponding cells (Gilsohn and Volk, 2010; Subramanian et al., 2007). Based on the prominent role of Stripe in *Drosophila* tendon development, Delphine Duprez's lab (Universite Pierre et Marie Curie, Paris, France) examined the role of Egr factors in vertebrate tendon development. She reported that Egr1 and Egr2 are strongly expressed in the limb tendons of chick and mouse and regulate expression of the tendon marker scleraxis, as well as that of several tendon collagens (Lejard et al., 2011). Furthermore, she showed that fibroblast growth factor (FGF), derived from muscle, is able to activate the expression of Egr genes and tendon-associated collagens.

Interactions between muscle and nerve are also required for the formation of the neuromuscular junction. Previous work in mouse from the lab of Steve Burden (Skirball Institute, NY, USA) has shown that Lrp4, an LDL receptor-related protein, binds to neurally derived agrin and stimulates MuSK, a receptor tyrosine kinase expressed in muscle, which is essential for the postsynaptic differentiation of muscle (Kim et al., 2008). At the meeting, Burden presented new data showing that Lrp4, expressed in muscle, is also necessary and sufficient for presynaptic differentiation of the motoneurons at the neuromuscular synapse. Thus, Lrp4 plays a pivotal role in presynaptic, as well as postsynaptic, differentiation at neuromuscular synapses.

Muscle is regulated by sclerotome and connective tissue during development

An important step in musculoskeletal morphogenesis is the migration of myogenic progenitors and their differentiation into anatomical muscles. Many classical quail-chick chimera studies (e.g. Chevallier et al., 1977; Jacob and Christ, 1980) demonstrated that extrinsic signals are crucial for regulating muscle migration and differentiation. Chaya Kalcheim (Hebrew University, Jerusalem, Israel) presented new data showing that sclerotome-derived Slit1 drives the directional migration and differentiation of Robo2-expressing myogenic cells in the early chick myotome (which will give rise to axial muscle). Anne Gaelle Borycki (University of Sheffield, UK) also showed that sonic hedgehog (Shh) signaling is required for the assembly of the mouse myotomal basement membrane and that this basement membrane controls the migration and differentiation of myotomal cells (Anderson et al., 2009).

In the adult, vertebrate muscle is surrounded by muscle connective tissue, which is composed of a small number of fibroblasts that produce, and are embedded in, extracellular matrix. Although muscle connective tissue is essential for adult muscle structure and function, its role in musculoskeletal morphogenesis has remained largely unexplored. Peleg Hasson (Technion, Haifa, Israel) presented work undertaken in Malcolm Logan's lab (National Institute of Medical Research, London, UK) showing that the transcription factor Tbx5 non-cell-autonomously, via the muscle connective tissue, regulates the pattern of limb muscles (Hasson et al., 2010). Interestingly, Tbx5 also non-cell-autonomously regulates the pattern of limb blood vessels. A difficulty in studying muscle connective tissue has been the lack of markers for connective tissue

fibroblasts. Gabrielle Kardon (University of Utah, UT, USA) presented data showing that the transcription factor Tcf4 (Tcf7L2) is strongly expressed in these fibroblasts. Using newly generated *Tcf4*^{GFPCre} and tamoxifen-inducible *Tcf4*^{CreERT2} Cre driver lines in mouse, she showed that during development connective tissue regulates muscle fiber type and maturation (Mathew et al., 2011) and that during regeneration connective tissue fibroblasts regulate the expansion of adult muscle stem cells. Thus, these studies demonstrate that connective tissue is an important component of the niche that regulates muscle development and regeneration.

A tendon and bone tug of war

Tendon and bone are structurally and biomechanically linked in the adult musculoskeleton. New data from Ronen Schweitzer's lab (Portland Shriners Research Center, OR, USA) show that the relationship between tendon and bone is even more intimate than previously thought. Their lineage experiments in mouse show that Sox9^{+} cells (Sox9 is a transcription factor required for skeletal development) not only give rise to cartilage, but also to the enthesis (bone-tendon junction) and the nascent distal tendons of the limb. Subsequently, these distal tendons elongate by the scleraxis-dependent recruitment of tenocytes to the forming tendons. Thus, developmentally, the distal limb tendons are derived from Sox9^{+} prechondrogenic cells, as well as from scleraxis^{+} tenocytes.

Several talks highlighted the role of mechanical forces in the formation of cartilage and bone. Stavros Thomopoulos (Washington University, MO, USA) showed in mice that mechanical loading from muscle is important for the development of the muscle insertion into bone. The absence of muscle contractile force, via paralysis by botulinum toxin, impairs the formation of bone, fibrocartilage and tendon at the developing insertion. Elazar Zelzer also demonstrated that the development of the distinctive shape of the long bones in the limb is regulated by muscle contractile force. Bones are shaped by asymmetric mineral deposition and transient cortical thickening and these processes are dependent on muscle contractions. Finally, Rich Schneider (University of California, San Francisco, CA, USA) has examined the formation of secondary cartilage in the jaw mandibular adductor muscles of birds. This secondary cartilage is present in ducks but not in quails. Using quail-duck chimeras, he demonstrated that the neural crest controls the species-specific muscle pattern, which in turn regulates the muscle mechanical environment and the formation of secondary cartilage.

Neural crest: the central mediator of the head musculoskeleton

As reviewed by Drew Noden (Cornell University, NY, USA), the neural crest is central to the development of the cranial musculoskeletal system. The neural crest gives rise to the muscle connective tissue, tendons, ligaments and bones of the head and is crucial for patterning head muscles. Neural crest cells undergo an epithelial-to-mesenchymal transition as they exit the neural tube and then migrate extensively throughout the developing head. As discussed by Dalit Sela-Donenfeld (Hebrew University, Rehovot, Israel) and by Eldad Tzahor, matrix metalloproteases and the tumor-suppressor gene *p53* are essential for these processes. Inactivation of p53 (Trp53) in the mouse embryo leads to broad defects in the head skeleton, muscle and nerves (Rinon et al., 2011). Giovanni Levi's (CNRS, Paris, France) research also highlights the importance of neural crest in the formation of the vertebrate jaw. He has shown that the transcription factors Dlx5 and Dlx6, which are expressed in the cranial neural crest, are necessary and sufficient to specify the lower jaw of mice. Furthermore, he demonstrated that the expression of Dlx genes in the neural crest non-autonomously regulates the determination, differentiation and pattern of mouse jaw muscles (Heude et al., 2011). Finally, Paul Trainor (Stowers Institute, KS, USA) described a severe human congenital craniofacial syndrome, syngnathia, in which the maxilla and mandible are fused and widespread defects in the cranial musculoskeletal system are present. New data from the Trainor lab identify a FoxC-Fgf signaling cascade that is essential for the proper formation of the jaw joint and suggest that defects in this cascade might underlie syngnathia. Whether this pathway functions specifically within the cranial neural crest will be an interesting question for future research.

Evolution of head, neck and limbs

During the course of chordate evolution, the musculoskeletal system has become increasing complex (Fig. 2). The ancestral chordate musculoskeletal system, as demonstrated in cephalochordates (e.g. amphioxus) and urochordates (e.g. tunicate larvae), consisted of an axial musculoskeleton, which both supported the body and enabled locomotion. With the evolution of agnathans (hagfishes and lampreys), a head musculoskeleton evolved and became essential for feeding. With the evolution of cartilaginous fishes (e.g. sharks), an appendicular skeleton was added in the form of paired fins, allowing for more complex modes of locomotion. In tetrapods, these paired fins became further elaborated into paired limbs. Developmental innovations were undoubtedly key to driving the remarkable evolution of the musculoskeletal system.

The evolutionary origin and elaboration of the head are strongly linked to the origin and modification of the neural crest. As illustrated by the quail-duck studies of Rich Schneider, specialization of the head musculoskeletal system (e.g. changes in the jaw to allow for different modes of feeding) is encoded in changes in the cranial neural crest. Understanding the evolutionary origin of the head musculoskeletal system requires the study of more basal vertebrates than the model systems usually studied. Hagfish would be an ideal vertebrate to study, but the breeding and rearing of hagfish have proved notoriously difficult. Thus, it was exciting to learn that Shigeru Kuratani (Riken Institute, Kobe, Japan) is now able to culture hagfish embryos, and his study of these embryos should provide many insights into the evolution of the head musculoskeleton.

Although often overlooked, the evolution of a neck is an important tetrapod innovation because it allows the head to move independently of the body. Development of a neck requires both the reduction of vertebral ribs and the enlargement of muscles to stabilize and move the neck and head. As Ketan Patel's research demonstrates, the developmental origin of the neck muscles is unique. These muscles arise from the occipital lateral plate, deploy the head myogenic program, and are patterned by connective tissue derived from the cranial neural crest. Such developmental innovations were likely to have been crucial for the evolution of these muscles and the neck.

The evolution of paired appendages was an important locomotor innovation. Expression of the transcription factors Tbx5 and Tbx4 in the developing fore- and hindlimbs, respectively, has suggested that these genes might be essential for initiating limb outgrowth and determining fore- and hindlimb identity. Using a series of mouse transgenes and targeted alleles, Malcolm Logan's lab has shown that Tbx5 and Tbx4 are crucial for limb outgrowth, but do not play a role in establishing limb identity. Furthermore, his lab has shown that it is probably the vertebrate acquisition of regulatory sequences that drive the expression of Tbx5 and Tbx4 in the lateral plate that was the important developmental innovation which enabled the

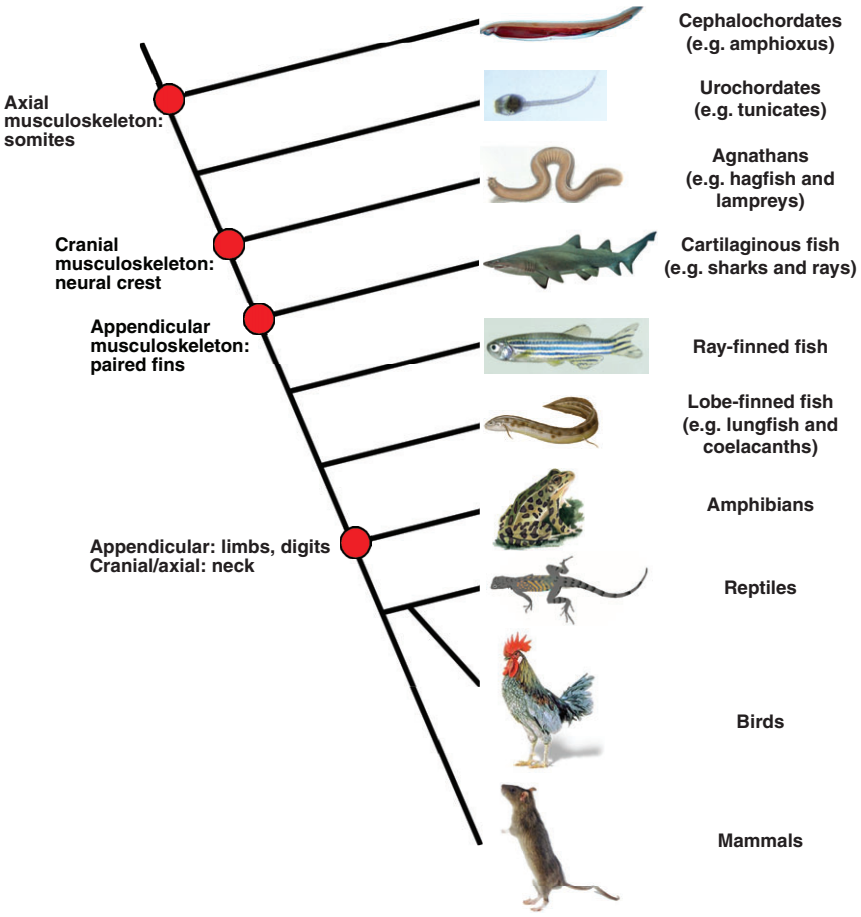

Fig. 2. Evolutionary origin of the axial, cranial and appendicular musculoskeleton. Red dots indicate the origin of components of the axial, cranial and appendicular musculoskeleton (Coates, 1994; Gans and Northcutt, 1983; Liem et al., 2001; Theis et al., 2010). Phylogeny is according to Bourlat et al. and Meyer and Zardoya (Bourlat et al., 2006; Meyer and Zardoya, 2003).

evolution of paired appendages (Minguillon et al., 2009). With the evolution of tetrapods, the hindlimbs became increasingly important for locomotion. By analyzing different fish groups (including sharks, paddlefish and lung fish), Peter Currie's group (ARMI, Melbourne, Australia) has identified a stepwise modification of a primitive to a more derived mode of pelvic fin muscle formation. This derived mode of hindlimb muscle formation enabled the development of load-bearing hindlimbs.

Within tetrapods, modification of limb bone lengths has been a common feature. Several approaches to understanding the mechanisms underlying limb elongation were presented. Kim Cooper and Cliff Tabin (Harvard Medical School, MA, USA) have focused on the development of an unusual rodent, the jerboa, which has greatly elongated hindlimbs. Kathryn Kavanagh (University of Massachusetts, MA, USA) with Cliff Tabin has used a combination of experimental and comparative embryology to evaluate the developmental origins of variation in the size proportions of phalanges, whereas Uri Alon (Weizmann Institute, Rehovot, Israel) has used this phalanges system to demonstrate the use of mathematical reasoning, based on the Pareto front principle of optimization, as a general explanation of the limited morphological variations in nature.

Conclusions

In summary, research on musculoskeletal development is moving in an exciting direction, with many new studies examining muscle, connective tissue, tendon and bone in an integrative way. Such integrative studies reveal that molecular and cellular interactions between the different tissues are crucial for the development of the musculoskeletal system. In addition, this research is providing important insights into the etiology of human musculoskeletal diseases and into the developmental innovations that have been key to the complex evolution of the musculoskeletal system.

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Competing interests statement

The author declares no competing financial interests.

References

Anderson, C., Thorsteinsdottir, S. and Borycki, A. G. (2009). Sonic hedgehog-dependent synthesis of laminin alpha1 controls basement membrane assembly in the myotome. *Development* **136**, 3495-3504.

Avinoam, O., Fridman, K., Valansi, C., Abutbul, I., Zeev-Ben-Mordehai, T., Maurer, U. E., Sapir, A., Danino, D., Grunewald, K., White, J. M. et al. (2011). Conserved eukaryotic fusogens can fuse viral envelopes to cells. *Science* **332**, 589-592.

Benazeraf, B., Francois, P., Baker, R. E., Denans, N., Little, C. D. and Pourquie, O. (2010). A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature* **466**, 248-252.

Bourlat, S. J., Juliusdottir, T., Lowe, C. J., Freeman, R., Aronowicz, J., Kirschner, M., Lander, E. S., Thorndyke, M., Nakano, H., Kohn, A. B. et al. (2006). Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. *Nature* **444**, 85-88.

Chevallier, A., Kieny, M. and Mauger, A. (1977). Limb-somite relationship: origin of the limb musculature. *J. Embryol. Exp. Morphol.* **41**, 245-258.

Coates, M. I. (1994). The origin of vertebrate limbs. *Development* **Suppl.**, 169-180.

Gans, C. and Northcutt, R. G. (1983). Neural crest and the origin of vertebrates: a new head. *Science* **220**, 268-273.

Gilsohn, E. and Volk, T. (2010). Slowdown promotes muscle integrity by modulating integrin-mediated adhesion at the myotendinous junction. *Development* **137**, 785-794.
Hasson, P., DeLaurier, A., Bennett, M., Grigorieva, E., Naiche, L. A., Papaioannou, V. E., Mohun, T. J. and Logan, M. P. (2010). Tbx4 and tbx5 acting in connective tissue are required for limb muscle and tendon patterning. *Dev. Cell* **18**, 148-156.
Heude, E., Bouhali, K., Kurihara, Y., Kurihara, H., Couly, G., Janvier, P. and Levi, G. (2011). Jaw muscularization requires Dlx expression by cranial neural crest cells. *Proc. Natl. Acad. Sci. USA* **107**, 11441-11446.
Jacob, H. J. and Christ, B. (1980). On the formation of muscle pattern in the chick limb. In *Teratology of the Limbs* (ed. H. J. Merker, H. Nau and D. Neubert), pp. 89-97. Berlin: Walter de Gruyter and Co.
Kim, N., Stiegler, A. L., Cameron, T. O., Hallock, P. T., Gomez, A. M., Huang, J. H., Hubbard, S. R., Dustin, M. L. and Burden, S. J. (2008). Lrp4 is a receptor for Agrin and forms a complex with MuSK. *Cell* **135**, 334-342.
Lejard, V., Blais, F., Guerquin, M. J., Bonnet, A., Bonnin, M. A., Havis, E., Malbouyres, M., Bidaud, C. B., Maro, G., Gilardi-Hebenstreit, P. et al. (2011). EGR1 and EGR2 involvement in vertebrate tendon differentiation. *J. Biol. Chem.* **286**, 5855-5867.
Liem, K. F., Bemis, W. E., Walker, W. F. and Grande, L. (2001). *Functional Anatomy of the Vertebrates* (3rd edn). Belmont, CA: Brooks/Cole.
Mathew, S. J., Hansen, J. M., Merrell, A. J., Murphy, M. M., Lawson, J. A., Hutcheson, D. A., Hansen, M. S., Angus-Hill, M. and Kardon, G. (2011). Connective tissue fibroblasts and Tcf4 regulate myogenesis. *Development* **138**, 371-384.
Medici, D., Shore, E. M., Lounev, V. Y., Kaplan, F. S., Kalluri, R. and Olsen, B. R. (2010). Conversion of vascular endothelial cells into multipotent stem-like cells. *Nat. Med.* **16**, 1400-1406.
Meyer, A. and Zardoya, R. (2003). Recent advances in the (molecular) phylogeny of vertebrates. *Annu. Rev. Ecol. Evol. System.* **34**, 311-338.
Minguillon, C., Gibson-Brown, J. J. and Logan, M. P. (2009). Tbx4/5 gene duplication and the origin of vertebrate paired appendages. *Proc. Natl. Acad. Sci. USA* **106**, 21726-21730.
Mukherjee, P., Gildor, B., Shilo, B.-Z., VijayRaghavan, K. and Schejter, E. D. (2011). The actin nucleator WASp is required for myoblast fusion during adult Drosophila myogenesis. *Development* **138**, 2347-2357.
Oren-Suissa, M., Hall, D. H., Treinin, M., Shemer, G. and Podbilewicz, B. (2010). The fusogen EFF-1 controls sculpting of mechanosensory dendrites. *Science* **328**, 1285-1288.
Rinon, A., Molchadsky, A., Nathan, E., Yovel, G., Rotter, V., Sarig, R. and Tzahor, E. (2011). p53 coordinates cranial neural crest cell growth and epithelial-mesenchymal transition/delamination processes. *Development* **138**, 1827-1838.
Subramanian, A., Wayburn, B., Bunch, T. and Volk, T. (2007). Thrombospondin-mediated adhesion is essential for the formation of the myotendinous junction in Drosophila. *Development* **134**, 1269-1278.
Theis, S., Patel, K., Valasek, P., Otto, A., Pu, Q., Harel, I., Tzahor, E., Tajbakhsh, S., Christ, B. and Huang, R. (2010). The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. *Development* **137**, 2961-2971.