An eye on the head: the development and evolution of craniofacial muscles

Ramkumar Sambasivan1,2,*, Shigeru Kuratani3,‡ and Shahragim Tajbakhsh1,2,*,†

Summary

Skeletal muscles exert diverse functions, enabling both crushing with great force and movement with exquisite precision. A remarkably distinct repertoire of genes and ontological features characterise this tissue, and recent evidence has shown that skeletal muscles of the head, the craniofacial muscles, are evolutionarily, morphologically and molecularly distinct from those of the trunk. Here, we review the molecular basis of craniofacial muscle development and discuss how this process is different to trunk and limb muscle development. Through evolutionary comparisons of primitive chordates (such as amphioxus) and jawless vertebrates (such as lampreys) with jawed vertebrates, we also provide some clues as to how this dichotomy arose.

Key words: Craniofacial muscle development, Cranial mesoderm, Genetic regulation, Head muscle evolution, Tbx, Pitx

Introduction

The skeletal muscles of the head, known as craniofacial muscles (Fig. 1), are essential for everyday movements, including those that control facial expression, mastication and eye movements. Myofibres are the functional unit of all skeletal muscles and have a common contractile function, yet a number of observations have highlighted that an extraordinary diversity exists among different muscles, including between the groups of craniofacial muscles. Such diversity at the molecular level, in terms of the expression of genetic programmes of skeletal muscles impinge on their functional tasks they perform. Significantly, such diversity could be underlie the adaptation of these muscle groups to the varied functional tasks they perform. Such diversity at the molecular level, in terms of the expression of specific metabolic activities or contractile protein isoforms (Cheng et al., 2004; Fischer et al., 2002), is likely, but not proven, to underlie the adaptation of these muscle groups to the varied functional tasks they perform. Significantly, such diversity could be a potential basis, at least partly, for the differential involvement of muscle groups in various myopathies (see Glossary, Box 1). For example, many myopathies cause severe dysfunction of some skeletal muscles, but, intriguingly, other muscles are functionally spared. How does this diversity arise? Do the developmental and genetic programmes of skeletal muscles impinge on their differentiated phenotype and hence contribute to this diversity?

Traditionally, studies of developmental myogenesis have focussed on trunk and limb musculature; thus, less is known about the development of head muscles. However, recent studies are beginning to shed light on the mechanisms that regulate craniofacial muscle development. Notably, embryological and genetic studies have shown that different strategies establish myogenesis in different regions of the organism during development. A striking difference between cranial and trunk myogenesis, for example, is that the trunk paraxial mesoderm (see Glossary, Box 1), an embryonic tissue that generates trunk and limb muscles, is segmented into transient epithelial somites (Fig. 1), whereas the cranial mesoderm (CM, see Glossary, Box 1), which is the source of most head muscle progenitors and was spared. How does this diversity arise? Do the developmental and genetic programmes of skeletal muscles impinge on their differentiated phenotype and hence contribute to this diversity?

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Table 1. Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>Branchiomyogenic progenitors</td>
<td>Precursors of heart muscle</td>
</tr>
<tr>
<td>Cardiomyogenic progenitors</td>
<td>Precursors of heart muscle</td>
</tr>
<tr>
<td>Chondrichthysans</td>
<td>Fish with a cartilaginous skeleton, such as sharks and rays</td>
</tr>
<tr>
<td>Cranial lateral mesoderm</td>
<td>Lateral portion of the cranial mesoderm close to the pharynx (also called the pharyngeal mesoderm or cranial splanchnic mesoderm) that gives rise to the anterior heart field and most first and second pharyngeal arch-derived muscles</td>
</tr>
<tr>
<td>Cranial neural crest (CNC)</td>
<td>CNC arises from the dorsal portion of the anterior neural tube and populates the face and the pharyngeal arches. It gives rise to bones, cartilage, nerves and connective tissue, and part of the base and facial bones of the skull</td>
</tr>
<tr>
<td>Cranial paraxial (paraxial head) mesoderm</td>
<td>Bilateral mesenchymal mesoderm parallel and immediately adjacent to the neural tube/notochord; it generates a subset of extra-ocular, and other head, muscles</td>
</tr>
<tr>
<td>Cucullaris muscles</td>
<td>A group of neck muscles connecting the skull to the shoulder girdle</td>
</tr>
<tr>
<td>Cyclostomes</td>
<td>Greek for 'round mouth'. Refers to modern jawless vertebrates including lampreys and hagfishes</td>
</tr>
<tr>
<td>Epibranchial placodes</td>
<td>Cranial ectodermal thickenings that contribute to cranial ganglia, particularly cranial nerves VII, IX and X</td>
</tr>
<tr>
<td>Epidermal placodes</td>
<td>Epidermal thickenings that give rise to sensory organs and cranial ganglia</td>
</tr>
<tr>
<td>Head cavities</td>
<td>Cranial mesoderm organised as epithelial cysts in some vertebrates</td>
</tr>
<tr>
<td>Hypobranchial muscles</td>
<td>A group of neck muscles located in the floor of the pharyngeal arches</td>
</tr>
<tr>
<td>Myopathies</td>
<td>A group of muscle-wasting diseases</td>
</tr>
<tr>
<td>Outflow tract</td>
<td>Ventricular portion of the heart through which blood passes in order to enter the great arteries</td>
</tr>
<tr>
<td>Pharyngeal arches</td>
<td>Literally meaning a gill-supporting structure, present in all vertebrate embryos and give rise to structures of the face, ear and neck</td>
</tr>
<tr>
<td>Prechordal mesoderm</td>
<td>Vertebrate-specific (also known as axial) mesoderm found anterior to the notochord and ventral to the neural tube. It is displaced bilaterally during development and contributes to certain extra-ocular muscles in chick</td>
</tr>
<tr>
<td>Trunk paraxial mesoderm</td>
<td>Mesoderm that lies parallel to and either side of the trunk embryonic body axis. It segments into epithelial somites that harbour dermis, skeletal muscle, brown fat, smooth muscle, bone and endothelial progenitors</td>
</tr>
</tbody>
</table>

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In evolutionary terms, the head of vertebrates is thought to be a novel structure (Gans and Northcutt, 1983; Northcutt and Gans, 1983) and some head muscles are also a vertebrate novelty. The major part of the head evolved from a non-vertebrate chordate ancestor with its anteroposterior (AP) positional landmarks preserved (Yu et al., 2007). Simultaneously, new cellular components, such as cranial neural crest and epidermal placodes (see Glossary, Box 1) evolved as additions to the ‘real head’ during this evolutionary transition from protochordates to vertebrates (Gans and Northcutt, 1983; Northcutt and Gans, 1983). Therefore, muscles might also have originated multiple times as independent events (Gans and Northcutt, 1983) and, consequently, head muscles could have arisen independently of trunk muscles.

The evolutionarily novel vertebrate head muscles, which include extra-ocular muscles (EOMs), jaw muscles and facial muscles (Fig. 1), are what we refer to collectively as craniofacial muscles and are the focus of this review. They all derive from the CM, an embryonic tissue that is also unique to vertebrates, the evolutionary origins of which are still unclear. This review first highlights our current understanding of the organisation of the CM, focussing on new insights into the molecular basis of craniofacial development and how this process is different to trunk and limb muscle development. With the help of recent reports on the genetics of head muscle development, notably that distinct gene networks govern the onset of cranial myogenesis, we also discuss the evolution of craniofacial muscles. We then discuss the development and evolution of muscles in the neck, in light of the possible dichotomy (between the trunk and cranial mesoderm) of their origin. Finally, we discuss the link between cardiac muscle fate and craniofacial muscle development, a link that sets craniofacial muscle development apart from trunk muscle development.

Cranial mesoderm organisation
To understand craniofacial muscle development, the issue of how the CM is organised first needs to be discussed. Most muscles of the head derive from the CM, which also gives rise to other cell types (Fig. 2), including cardiomyocytes (Kelly et al., 2001; Mjaatvedt et al., 2001; Waldo et al., 2001), the posterior part of the neurocranium (the portion of the skull that encases the brain), and angiogenic cells (Couly et al., 1992; Couly et al., 1993; Evans and Noden, 2006; Hacker and Guthrie, 1998; Noden, 1983b). Fate-mapping studies in quail-chick chimeras and lineage-tracing studies that have used dye or retroviral labelling in chick (Couly et al., 1992; Couly et al., 1993; Evans and Noden, 2006; Hacker and Guthrie, 1998; Noden, 1983b) and mouse embryos (Gage et al., 2005; Trainor and Tam, 1995) have identified regions of the CM that are myogenic. Some of these studies identified specific regions of the CM as being a source of founder cells for distinct muscle groups in the head (see Noden and Francis-West, 2006).

Developmental history of cranial mesoderm
One potential source of confusion about the CM is its lack of clear anatomical boundaries or defined molecular markers to distinguish between the different mesoderm populations in the head. By contrast, somitic and lateral plate mesoderm (LPM) in the trunk are delineated morphologically, as well as by gene expression profiles, as distinct physical entities (Goulding et al., 1994; Palmeirim et al., 1997; Tajbakhsh and Buckingham, 2000) (Fig. 2). At an open neural plate stage [Hamburger-Hamilton stage (HH)7-8 (1-3 somites) (Hamburger and Hamilton, 1992) in chick, around embryonic day (E)8.0 in mouse], position and boundaries set by other tissues, as well as marker gene

historically considered to be morphologically segmented, does not appear to be overtly segmented in vertebrates (Wedin, 1949; Kuratani, 2008b; Kuratani et al., 1999). Indeed, the organisation of the CM is currently delineated by molecular markers rather than by overt anatomical boundaries (Bothe and Dietrich, 2006). Another peculiarity of cranial myogenesis is its link to heart development; the CM generates cardiomyogenic progenitors (see Glossary, Box 1) as well as craniofacial muscles. This is an issue of considerable interest, as it provides clues to understanding the evolution of craniofacial muscles. We then discuss the development and evolution of muscles in the neck, in light of the possible dichotomy (between the trunk and cranial mesoderm) of their origin. Finally, we discuss the link between cardiac muscle fate and craniofacial muscle development, a link that sets craniofacial muscle development apart from trunk muscle development.
expression, delineate the CM into prechordal, cranial paraxial and cranial lateral mesoderm (see Glossary, Box 1). When the neural tube closes dorsally and the endoderm ventrally (HH8-10 in chick and E8.5-9.0 in mouse), the position and boundaries of the tissues alter. Prechordal mesoderm (PCM, see Glossary, Box 1; Fig. 2), which develops in an axial position beneath the neural plate and is located anterior to the notochord, is subsequently displaced caudally and laterally during development (accompanying the lateral growth of the anterior neural plate). It then integrates with the remaining CM, which is located anterior to the somites (Fig. 2). Thus, the CM at an early stage does not include the PCM, yet at later stages it does. Furthermore, the initial mediolateral organisation of the CM is largely transformed into a dorsoventral pattern. Moreover, for the mesoderm above and below the pharynx, a new mediolateral divide emerges by means of marker gene expression and cell fate choices. Where a particular cell population is located, and at which stage of development this localisation occurs, is yet to be established. Later, when pharyngeal arches (PAs, see Glossary, Box 1) form, a new system of organisation is established.

The mediolateral and AP compartmentalisation of the CM, in terms of fate, has been proposed based on studies of avian embryos. Lineage tracing studies have shown that the medial-posterior region of the CM is destined to contribute to part of the neurocranium and the medial-anterior region to the extrinsic eye muscles, whereas the lateral region contributes to the PA derivatives, including the craniofacial muscles (Couly et al., 1992; Evans and Noden, 2006; Noden and Francis-West, 2006). These studies also showed that the PCM is displaced bilaterally during development and that it contributes to a subset of EOMs. However, the extent of this contribution and its requirement for the development of this set of muscles is not known. The restricted expression of genes such as *Pitx2, Alx4, Msc, Twist* and *Tbx1* transcription factors (see below) reveal that molecular differences exist along the mediolateral and AP axes of the CM (Bothe and Dietrich, 2006).

**CM organisation: evolutionary considerations**

Although the CM does not appear to be partitioned morphologically, an obvious question is whether or not the CM is regionalised. Regionalisation of the CM into ‘medial’ and ‘lateral’ components has been previously suggested (Couly et al., 1992; Evans and Noden, 2006), and previous interpretations of comparative embryology have divided the CM along the AP axis into head cavities (see Glossary, Box 1) (see Kuratani, 2003). The CM has also been divided into dorsal and ventral compartments, representing the somatic (paraxial) and visceral (pharyngeal arch) mesoderm as cephalic counterparts of somites and lateral plate, respectively (Goodrich, 1930; Jarvik, 1980; van Wijhe, 1882). Whether the CM is segmented has been extensively debated [for discussion of both sides of the argument, see reviews by Wedin (Wedin, 1949), Holland (Holland et al., 2008) and Kuratani (Kuratani, 2008b)]. Views on the segmentation of the vertebrate
head were stimulated by the discovery of head cavities in the late nineteenth and early twentieth centuries. A comparative zoological view is that segments or somites in bilaterians originate from reiterating mesodermal epithelial cysts, or myogenic coeloms. Thus, the evolutionary origin of the vertebrate head mesoderm has been dealt with in the same context. One typical theory of head mesoderm evolution proposes that the three pairs of coeloms found in deuterostome larvae originated from four gut diverticula (outpouchings) in cnidarians, with the posterior pair of cavities being secondarily subdivided to generate somites (Starck, 1978). Thus, the vertebrate paraxial mesoderm was classified into three groups: premandibular mesoderm, more posterior head mesoderm, and somites.

In some basal gnathostomes (jawed vertebrates), the CM is organised into a successive series of these epithelial cysts (Balfour, 1878; Goodrich, 1930; Kuratani, 2005; van Wijhe, 1882). In many chordichnithans (see Glossary, Box 1), three pairs of cavities are observed and are called, from anterior to posterior, the premandibular, mandibular and hyoid cavities; they are ventrally associated with PA mesoderm. Of these, the premandibular cavity was assumed to generate four of the EOMs innervated by the oculomotor nerve, the mandibular cavity contributes to superior oblique muscle innervated by the trochlear nerve, and the hyoid cavity contributes to the lateral rectus innervated by the abducens. Thus, each head cavity is thought to produce EOMs, based on histological observations. In other gnathostome lineages (such as amniotes), head cavities are absent and EOMs appear to arise from mesenchymal CM, as well as from the prechordal plate (Coulby et al., 1992; Jacob et al., 1984; Noden et al., 1999; Wachtler et al., 1984). Currently, divergent views persist regarding the evolution of head cavities: (1) head cavities are homologous to some rostral somites in the amphioxus, a group of non-vertebrate chordates (Beaster-Jones et al., 2006; Holland, 2000); (2) head cavities are serially homologous to the somites of the trunk (Jacobson, 1988); or (3) head cavities are entirely different from somites, and have a secondary origin in the lineage of either gnathostomes or vertebrates (Kuratani, 2003). To complicate matters further, the presence of head cavities in the lamprey, a jawless vertebrate, is itself contested (Wedin, 1949; Beaster-Jones et al., 2006; Holland et al., 2008; Kuratani, 2003; Kuratani, 2008b; Kuratani et al., 1999; Neal, 1918).

Studies of gastrulating chick embryos provide another view on the issue of CM segmentation. Here, the apparently unsegmented CM experiences two pulses of expression of the Notch pathway segmentation clock gene, hairy1 (Dubrulle and Pourquie, 2002; Jouve et al., 2002). In the PSM, each pulse of this gene corresponds directly to the formation of a somite (Maroto and Pourquie, 2001). This finding indicates that: (1) the CM is not, as suggested previously (Jacobson, 1988; Jacobson, 1993), segmented into six cephalic somitomeres that are equivalent to somites, given that only two pulses of hairy1 are observed (Jouve et al., 2002); (2) the PCM and the rest of the CM represent two segments, even though the PCM is not initially bilateral and is considered by some to be axial mesoderm owing to its initial position (Seifert et al., 1993); or, (3) the cyclic expression of hairy1 in the CM does not define segments in the same fashion as it does in the PSM (Dubrulle and Pourquie, 2002).

**Regionalisation based on anatomical landmarks**

One way of assessing regional differences in the CM, especially along the AP axis, is to consider adjacent anatomical landmarks, such as the neural tube (Fig. 3). The organisation of the CM becomes more apparent when domains of cranial neural crest are also taken into account. Neural crest cells and the cranial nerves that arise from the neural tube harbour positional information along the AP axis (Fig. 3A,B). In addition, a close interaction occurs between neural crest cells and the mesodermal progenitors of the PAs and EOMs, with the neural crest playing a critical role in skeletal muscle cell migration, displacement and patterning (Ericsson et al., 2004; Grammatopoulos et al., 2000; Köntges and Lumsden, 1996; Matt et al., 2008; Noden, 1983a; Noden and Trainor, 2005; Olsson et al., 2001; Rinon et al., 2007; Schilling and Kimmel, 1997). Although muscle cell fate is not overtly affected when neural crest cells are genetically perturbed, a role for neural crest cells in regulating these muscle stem cells has not been ruled out (Rinon et al., 2007; von Scheven et al., 2006a).

The position-based associations between the cranial nerves (CN) and skeletal muscles are most evident between the so-called branchiomeric nerves (the nerves associated with the PAs, called CN V, VII, IX and X) and arch-derived muscles (Fig. 3C). The patterning of these nerves is guided by neural crest cells and epibranchial placodes (see Glossary, Box 1), which are distributed in a specific fashion with respect to the segmentation of the PAs. This results in a typical topographical association between the nerves and the arch-derived muscles (Cordes, 2001). Moreover, it is the crest-derived ectomesenchymal cells in the PAs that provide the connective tissues to these muscles (Coulby et al., 1993; Evans and Noden, 2006; Köntges and Lumsden, 1996; Noden and Francis-West, 2006). Thus, it is generally agreed that regional differences exist in the CM, but the link between the molecular profile and the future cell fate of the derivatives of CM is unknown. Furthermore, the initial overlap in the expression of skeletal myogenic and cardiomyogenic markers in CM has made it difficult to demarcate boundaries in this mesoderm (Grifone et al., 2007; Tzahor, 2009).

In essence, there is a lack of apparent morphological partitioning of the CM on any of the axes, thus distinguishing it from the somitic trunk mesoderm. Therefore, at least two possibilities can be suggested for the origin of the embryonic tissue that generates the evolutionarily recent craniofacial muscles. First, the CM could be a modified form of the visceral mesoderm (which arises from primitive gut) (see Kelly, 2010). This supposition agrees with the hypothesis that the CM is segmented in the form of the head cavities, which were reported to exist in early vertebrates, and that the head cavities: (1) head cavities are homologous to the so-called branchiomeric nerves (the nerves associated with the PAs, called CN V, VII, IX and X) and arch-derived muscles (Fig. 3C). The patterning of these nerves is guided by neural crest cells and epibranchial placodes (see Glossary, Box 1), which are distributed in a specific fashion with respect to the segmentation of the PAs. This results in a typical topographical association between the nerves and the arch-derived muscles (Cordes, 2001). Moreover, it is the crest-derived ectomesenchymal cells in the PAs that provide the connective tissues to these muscles (Coulby et al., 1993; Evans and Noden, 2006; Köntges and Lumsden, 1996; Noden and Francis-West, 2006). Thus, it is generally agreed that regional differences exist in the CM, but the link between the molecular profile and the future cell fate of the derivatives of CM is unknown. Furthermore, the initial overlap in the expression of skeletal myogenic and cardiomyogenic markers in CM has made it difficult to demarcate boundaries in this mesoderm (Grifone et al., 2007; Tzahor, 2009).

**Genetic networks distinguish cranial and somitic myogenesis**

Head muscles are the only musculature to develop in mice carrying mutations in certain myogenic genes, whereas other mutations cause severe developmental abnormalities in head muscles but not in trunk muscles (Table 1). Furthermore, recent findings show that distinct genetic regulatory cascades operate within individual craniofacial muscle groups, the EOMs and the PA-derived jaw and facial muscles. This highlights several interesting points that might reflect on putative subdivisions within the CM. Below, we discuss findings from mouse genetic studies that have revealed divergence in the craniofacial and trunk muscle developmental programmes.
The myogenic regulatory factors

The core myogenic network consists of the basic helix-loop-helix (bHLH) myogenic regulatory factors [MRFs; Myf5, Mrf4 (Myf6 – Mouse Genome Informatics), Myod (Myod1 – Mouse Genome Informatics) and myogenin], which determine muscle identity and/or promote muscle differentiation (Sambasivan and Tajbakhsh, 2007; Weintraub et al., 1991). These factors have been ‘redeployed’ during evolution to build the new head muscles. However, their roles differ in EOM versus PA skeletal muscle founder cells. For example, in the mouse, EOM development depends on either Myf5 or Mrf4, and EOMs require both for robust myogenesis; interestingly, in their absence, Myod expression is severely compromised in EOM progenitors (Sambasivan et al., 2009). This is also the case for the epaxial muscles in the tail, which are missing in Myf5;Mraf double mutant mice (Kassar-Duchossoy et al., 2004). Intriguingly, although Myf5 plays a critical role in regulating PA muscle progenitors as well (Sambasivan et al., 2009), the genetic hierarchy upstream of the MRFs that specify PA and EOM muscle progenitors is distinct.

Although MRFs act as a gateway into myogenesis in all muscles, there are intriguing combinations of individual MRFs that function in different muscle groups within different vertebrates. In the mouse, Myf5 and Mrf4 act genetically upstream of Myod in EOMs, as they do in the somite-derived muscle groups. In zebrafish, myf5 has been reported to be required for head muscle development (Chen and Tsai, 2002; Lin et al., 2006). However, in another zebrafish study, myod (myod1 – Zebrafish Information Network), but not myf5, was proposed to be necessary for cranial myogenesis (Hinits et al., 2009). This is a notable inter-species difference, given that Myf5 is the key player in cranial myogenesis in mouse (Sambasivan et al., 2009). As for Mrf4, in the absence of Myf5 and Myod, it drives robust differentiation of mouse...
Table 1. Null mutants that affect head, trunk/neck and limb muscle myogenesis

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Family</th>
<th>Expression/role</th>
<th>Head muscle</th>
<th>Trunk/neck muscle</th>
<th>Limb muscle</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitx2*</td>
<td>Family: Homeodomain.</td>
<td>Expressed in various embryonic tissues. Role in L/R asymmetry and specification of cell fates.</td>
<td>Pitx2 null: EOM progenitors mislocalized; deficit of 1st PA-derived muscles.</td>
<td>Pitx2 null: Hypaxial myotome and body wall muscles deformed/absent.</td>
<td>Pitx2 null: Limb muscle anlagen deformed.</td>
<td>(Gage et al., 1999; L’Honoré et al., 2010; Kitamura et al., 1999; Shih et al., 2007)</td>
</tr>
<tr>
<td>Tbx1*</td>
<td>Family: T box.</td>
<td>Expressed early in development. Speculated to have a role in regional specification of distinct subset of cell populations.</td>
<td>Tbx1 null: Random unilateral loss of 1st PA muscles; loss of 2nd PA muscles*.</td>
<td>Tbx1 null: Loss of circularris neck muscles and muscles of larynx†.</td>
<td>Tbx1 null: No apparent limb muscle deficiency.</td>
<td>(Kelly et al., 2004;‘Papaioannou and Silver, 1998; Sambasivan et al., 2009; Theis et al., 2010)</td>
</tr>
<tr>
<td>Msc,Tcf21*</td>
<td>Family: bHLH.</td>
<td>Thought to be transcriptional repressor that delay commitment to muscle lineage.</td>
<td>Msc:Tcf21 double null: No temporalis, masseter and ptterygodamus (1st PA subset); other muscles of 1st PA are present. All zebrafish cranial muscles lost in loss of functions.</td>
<td>Msc:Tcf21 double null: No apparent synergy on trunk myogenesis.</td>
<td>Msc:Tcf21 double null: No limb musculature deficit reported.</td>
<td>(Lee et al., 2002; Lu et al., 2002)</td>
</tr>
<tr>
<td>Pax7*</td>
<td>Family: Paired box.</td>
<td>TF upstream of MRFs; also expressed in CNC and developing CNS.</td>
<td>Pax7 null: Loss of postnatal muscle stem/progenitor cells; N/A in adult.</td>
<td>Pax7 null: Loss of postnatal muscle stem/progenitor cells; not required in adult.</td>
<td>Pax7 null: Loss of postnatal muscle stem/progenitor cells; not required in adult.</td>
<td>(Kuang et al., 2006; Lepper et al., 2009; Gutasarna et al., 2004; Relaix et al., 2006; Seale et al., 2000) (R.S. and S.T., unpublished)</td>
</tr>
<tr>
<td>Pax3</td>
<td>Family: Paired box.</td>
<td>TF upstream of MRFs; also expressed in CNC and developing CNS.</td>
<td>Pax3 null: Head muscles present.</td>
<td>Pax3 null: Deficits in trunk, diaphragm and tongue muscles.</td>
<td>Pax3 null: Limb muscles missing.</td>
<td>(Bober et al., 1994; Franz et al., 1993; Goulding et al., 1994; Tajbakhsh et al., 1997)</td>
</tr>
<tr>
<td>Six1, Six4</td>
<td>Family: Homeodomain.</td>
<td>Expressed in various embryonic tissues. Specify cell fates.</td>
<td>N/A</td>
<td>Six1:Six4 double null: Severe trunk muscle deficit; no limb muscles.</td>
<td>Six1:Six4 double null: Limb muscles missing.</td>
<td>(Grifone et al., 2005)</td>
</tr>
<tr>
<td>Eya1, Eya2</td>
<td>Family: Eya domain.</td>
<td>Expressed in various embryonic tissues. Specify cell fates.</td>
<td>N/A</td>
<td>Eya1,Eya2 double null: Absence of hypaxial somite derived muscles.</td>
<td>Eya1,Eya2 double null: Limb muscles missing.</td>
<td>(Grifone et al., 2007)</td>
</tr>
<tr>
<td>Myf5, Mrf4, Myod</td>
<td>Family: bHLH.</td>
<td>Muscle cell fate determination factors; Mrf4 and Myod also regulate differentiation.</td>
<td>Myf5 (Mrf4):Myod null: No myoblasts or differentiated cells throughout embryo.</td>
<td>Myf5 (Mrf4):Myod null: No myogenesis in the embryo.</td>
<td>Myf5 (Mrf4):Myod null: No myogenesis in the embryo.</td>
<td>(Kassar-Duchossoy et al., 2004; Rudnicki et al., 1993; Tajbakhsh et al., 1997) (R.S. and S.T., unpublished)</td>
</tr>
<tr>
<td>Myogenin</td>
<td>Family: bHLH.</td>
<td>Muscle differentiation factor.</td>
<td>N/A</td>
<td>Myogenin null: Severe deficiency in prenatal but not adult muscle differentiation.</td>
<td>Myogenin null: Severe deficiency in prenatal but not adult muscle differentiation.</td>
<td>(Hasty et al., 1993; Knapp et al., 2006; Meadows et al., 2008; Nabeshima et al., 1993)</td>
</tr>
</tbody>
</table>

**Table Notes:**

- **bHLH:** basic helix-loop-helix; CNC, cranial neural crest; CNS, central nervous system; EOM, extra-ocular muscles; L/R, left/right; MRF, myogenic regulatory factor; N/A, data not available; PA, pharyngeal arch; TF, transcription factor.
- *Expression not restricted to muscle progenitors.*
- †Possible non-cell autonomous role.
embryonic somitic progenitors, but in this double mutant it fails to support the differentiation of EOMs or first PA-derived muscles (Kassar-Duchossoy et al., 2004). This could be due to differences in the somitic and cranial myogenic cell populations, a different threshold level of an MRF needed to initiate myogenesis, or the lower potency of Mrf4 as a transcription factor compared with Myod and Myf5 (Bergstrom and Tapscott, 2001; Weintraub et al., 1991). In zebrafish somites, Mrf4 does not support myogenesis when myf5 and myod are compromised, but it has the potential to do so when supplied ectopically (Hinits et al., 2009; Schnapp et al., 2009). However, Mrf4 is not expressed during early head muscle development (Hinits et al., 2009).

These differences in MRF functions across phylogeny imply that these transcription factors have acquired new roles during evolution. The ancestral MRF gene is presumed to have given rise to the four family members in vertebrates (Atchley et al., 1994). Myf5 and Mrf4 are genetically linked and separated by 9 kb, suggesting that this is the most recent duplication event. In addition, there is compelling evidence to suggest that the functions of each of the MRFs are evolutionarily conserved. For example, *Xenopus* and human Myf5 are highly conserved outside the bHLH region, yet human Myf5 and Myod are divergent outside the bHLH region. This suggests that, throughout evolution, the MRF paralogues have diverged functionally whereas the orthologues have not drifted significantly. In this context, why different MRFs are used in different locations in the head, as is seen in epaxial and hypaxial trunk myogenesis (Tajbakhsh and Buckingham, 2000), remains unclear. Analysis of the combinatorial factors acting on enhancer sequences of each MRF for different organs should provide further insights into this issue.

Pax3 and Pax7

In somitic muscle founders, *Pax3* and *Pax7*, paired-homeodomain genes, act genetically upstream of *Myod* (Tajbakhsh et al., 1997), and they are likely to activate *Myod* expression by binding to its regulatory sequences (Hu et al., 2008). *Pax3* expression is absent in head muscle stem/progenitors (Hacker and Guthrie, 1998; Sambasivan et al., 2009; Tajbakhsh et al., 1997). Furthermore, in *Pax3*:Myf5:Mrf4 mutant embryos (in which embryonic Mrf4 expression is perturbed by cis disruption of the Myf5 locus) trunk muscles fail to form whereas most head muscles develop (Tajbakhsh et al., 1997).

Pitx2 and Tbx1

Pituitary homeobox 2 (*Pitx2*) is a bicoid-related homeodomain transcription factor (Gage et al., 1999; Kitamura et al., 1999) expressed widely in the head of mouse embryos, including within the CM and the pharyngeal ectoderm. It plays a key role in specifying EOMs, which are missing in *Pitx2*-null mice, and it is a key regulator of PA muscle development, (Gage et al., 1999; Kitamura et al., 1999; Shih et al., 2007). Furthermore, in *Pax3*:Myf5:Mrf4 mutant embryos (in which embryonic Mrf4 expression is perturbed by cis disruption of the Myf5 locus) trunk muscles fail to form whereas most head muscles develop (Tajbakhsh et al., 1997).

The T-box gene *Tbx1* is critical for PA muscle development but is not required for trunk myogenesis (Kelly et al., 2004). Unlike *Pitx2*, this transcription factor specifies PA, but not EOM, muscle founder cells (Kelly et al., 2004; Sambasivan et al., 2009). In *Tbx1*-null mouse mutants, first PA myogenesis is impaired, and the posterior arches themselves are absent, highlighting the additional role that *Tbx1* has in ectoderm and endoderm in PA development. Interestingly, a random monolateral ablation of skeletal muscles is often observed in *Tbx1*-null mutants, implicating an all-or-nothing mechanism for the generation of muscle founder cells (Grifone and Kelly, 2007; Kelly et al., 2004). In the progenitors downstream of these founder stem cells, cell fate is assured by cooperation between *Tbx1* and *Myf5*, as PA muscles do not form in these double mutants (Sambasivan et al., 2009). A common feature of PA and EOM founder cells is the epistatic relationship that exists between the MRFs: *Myod* acts genetically downstream of *Tbx1* and *Myf5* in the PA, and downstream of *Myf5* and *Mrf4* in the EOMs.

*Musculin* and Tcf21

*Msc* (musculin; also known as *MyoR*), which is expressed in head and body muscles, and *Tcf21* (also known as capsulin), are bHLH transcription factors. They are considered to act as repressors although this has not been shown in vivo. They play key roles in the PA, but are not expressed in the EOM founder cells. Mouse embryos with mutations in both these genes fail to develop a subset of first arch-derived jaw muscles (the masseter, pterygoid and temporalis muscles) (Lu et al., 2002). As with *Pitx2* and *Tbx1* mutants, why only a subset of muscles (Table 1) are affected by *Msc* and *Tcf21* deletion remains a mystery. The link between these transcription factors and the MRFs has not been clearly established.

Six family and Eya domain factors

The homeodomain factors *Six1*, *Six4*, *Meox1* and *Meox2*, as well as the Eya domain factors *Eya1* and *Eya2* play critical regulatory roles upstream of *Pax3* in the somitic mesoderm (Table 1). Six, Eya and Pax factors, along with Dachshund family members, act in a network to govern organogenesis in multiple contexts (Relaix and
Buckingham, 1999). However, the role of these factors in the CM, if any, remains to be explored. Critical roles of Six and Eya factors in trunk myogenesis have been demonstrated (Grifone et al., 2007; Grifone et al., 2005). Although Meox1 and Meox2 have roles in somite formation and differentiation, intriguingly, subsets of proximal and distal forelimb muscles are missing in Meox2 mutants, and overall fore- and hindlimb muscle masses are reduced (Mankoo et al., 1999; Mankoo et al., 2003). In fact, Six1 has been implicated in head muscle development in zebrafish (Lin et al., 2009). In spite of this complexity in the gene regulatory network upstream of the MRFs, one could speculate that two or more distinct regulatory cascades at the level of mesoderm specification might have evolved to regulate the MRFs and to orchestrate myogenesis. This scenario might well be true for other lineage programmes in the head versus the trunk.

Taken together, these genetic studies reveal that, although the MRFs have been redeploled to make head muscles, the upstream network that regulates MRF expression seems to have evolved independently in the head.

Temporal changes in myogenic genetic networks
Myogenesis occurs in embryonic, foetal and postnatal phases that are distinguishable by the myogenic cell populations involved and by the gene expression patterns observed during these phases (see Tajbakhsh, 2009). The upstream gene regulatory networks discussed above operate in the embryonic phase of myogenesis. This phase initiates the anlagen, which is built upon during the successive waves of myogenesis. A number of reports, however, suggest that the regulatory programmes that operate in the somite-derived muscle progenitors are different during the embryonic, foetal, juvenile and adult phases of myogenesis (Biresi et al., 2007; Messina et al., 2010; Stockdale, 1992; Tajbakhsh, 2009). A key finding that underscores this view was the discovery that the transcription factor Nfix1 (nuclear factor 1) is a critical regulator of foetal, but not embryonic, myogenesis in mice (Messina et al., 2010). Although nuclear factor protein family members regulate cell fate choices in multiple tissues by acting as repressors or activators, the role of Nfix1 in this context is to repress the embryonic, while promoting the foetal, muscle programme (Messina et al., 2010). How this novel regulator integrates with the early embryonic transcription factors mentioned above, and in different locations, remains to be explored.

Although the genetic hierarchies that act upstream of the MRFs in somitic and cranial mesoderm are distinct, the emergence of a more muscle-lineage restricted, Pax7-expressing stem/progenitor population in both the head and the trunk at mid-embryogenesis, always after the establishment of muscle anlage, appears to be common in both cases. This second wave of stem/progenitors in the head and the trunk assures muscle growth until adulthood in the mouse (Kassar-Duchossoy et al., 2005; Relaxi et al., 2005). Notably, the ontological molecular signature that characterises embryonic muscle founder cells in different locations is partially retained in the adult stem/progenitors as part of a ‘molecular memory’ (Harel et al., 2009; Sambasivan et al., 2009), hinting at a persistent role for these regulators in the distinct locations.

In summary, a comparison of the distinct regulatory strategies that make muscles in the CM to those that make muscles in the trunk reinforces the view that the CM-derived muscle progenitors are evolutionarily distinct from the somitic muscle progenitor pool. An assessment of the key molecular players that are divergent between head and trunk myogenic programmes in an evolutionary context is pertinent in this context.

Evolutionary origins of cranial muscle
The distinct organisation of CM and the divergent gene networks employed by the muscle progenitors in CM to make head muscles provide clues to the evolutionary origins of craniofacial muscles. This section analyses comparative morphology, as well as the expression of key genes, discussed above, in select model organisms in order to understand vertebrate head muscle evolution.

It is likely that the ancestor to the vertebrates looked more like the extant amphioxus than the more closely related tunicate (a urochordate), as tunicates do not have somites (Yu et al., 2007). In the amphioxus, the unsegmented CM is not present, raising the possibility that some of the anterior somites (of visceral mesoderm origin) in the amphioxus-like ancestor lost their segmental configuration, resulting in the non-segmented head mesoderm of vertebrates (Holland et al., 2008). Although overt branchial arch muscles are absent in the adult amphioxus, a distinction between the branchiomyogenic (see Glossary, Box 1) and somitomeric muscles might, nevertheless, exist (Ruppert, 1997). This is because the so-called pterygial muscles, which develop more ventrally in the amphioxus pharynx (Fig. 4A), are innervated by the peripheral nerves that resemble the branchiomyogenic nerves in vertebrates (Fritzsche and Northcutt, 1993). This implies that, even before the origin of the vertebrate-specific CM, the PA mesoderm-like cell lineages might have been present in the vertebrate ancestor and that during evolution they differentiated into muscles that could be innervated by specific sets of peripheral nerves distinct from those innervating the myotomes in the trunk. Furthermore, AmphiTbx1/10 (a single gene that is the forerunner of the vertebrate Tbx1 and Tbx10 genes) is expressed in the PA mesoderm of amphioxus (Mahadevan et al., 2004). Taken together, these findings suggest that the cell population and the genetic machinery that distinguish head muscles from those of somitic mesoderm origin might have existed, in part, long before the evolution of the vertebrate head. Concordantly, in the basal jawless vertebrate lamprey, Tbx1/10 is expressed in the mesodermal core of the PAs (Sauka-Spengler et al., 2002; Tiecke et al., 2007), as well as in the labial and velar (mandibular arch-derived muscles) muscle progenitors. Furthermore, although Caenorhabditis elegans tbx-2 is not closely related to murine Tbx1 (Larroux et al., 2008), it was shown that tbx-2 is required for the development of the C. elegans pharyngeal muscles, but only those located anteriorly (Smith and Mango, 2007). It is tempting, therefore, to speculate that the ancestral Tbx1 gene might have acquired a myogenic task in the CM early in evolution.

Whole-genome comparative data have shown that, unlike the Pax genes, for which a PaxB-like gene founded the Pax class before the separation of sponges and eumetazoans (all major animal groups), the T-box transcription factors T (Brachyury), Tbx4/5 (precursor of the paralogous group Tbx4 and Tbx5) and Tbx1/15/20 were present in the last common ancestor of metazoans (Larroux et al., 2008). With respect to the MRFs, a single ancestor was proposed to be conserved from Drosophila to jellyfish to tunicates (Atchley et al., 1994), undergoing two rounds of duplication events to yield the current MRFs in vertebrates. As for Pitx2, the AmphiPitx gene is not expressed in the expected region in the developing larval head (Yasui et al., 2000), but the lamprey Pitx (orthologue of Pitx1 or Pitx2) is expressed in the pre-mandibular mesoderm that apparently gives rise to EOMs (Boorman and Shimeld, 2002; Kusakabe and Kuratani, 2007). However, the development of the lamprey EOMs is not yet well understood, therefore, further investigation of lamprey craniomandibular development could lead to a better understanding of EOM ontogeny in vertebrates.
As mentioned previously, the Pax3 and Pax7 genes have been associated with the myogenic development of the vertebrate somitic mesoderm (Buckingham and Relaix, 2007; Sambasivan and Tajbakhsh, 2007). In the early stages of vertebrate evolution, Pax3 and Pax7 were involved in dorsal specification of the neural tube and the acquisition of neural crest, as well as in the compartmentalisation of myotomes. A Pax3/7-positive layer of cells in some bony fish embryos and even in lamprey embryos appears to be the homologue of the epithelial cells of the dermomyotome present in amniotes (Kusakabe and Kuratani, 2007) and in zebrafish (Hollway et al., 2007; Stellabotte et al., 2007). In amphioxus too, AmphiPax3/7 (ancestor of Pax3 and Pax7) could play a role in myogenic development, as it is expressed in the anterior and posterior somites (Holland et al., 1999). If the hypothesis that the anterior somites are the ancestors of vertebrate CM is true (Holland et al., 2008), the evolution of the CM would involve not only the loss of segmentation but also a radical shift in its molecular regulation, i.e. loss of the Pax3 myogenic pathway for Tbx1 and/or Pitx2 regulatory cascades. Alternatively, as suggested above, the CM might have originated as an evolutionarily novel tissue from an entirely different cell population, such as the more ventral cell lineage that expresses AmphiTbx1.

It is interesting to note that in the vertebrate gastrula, the CM population arises from the primitive streak prior to the trunk mesoderm, yet myogenic induction in the head is delayed compared with that observed in the trunk. One could speculate that this lag in differentiation might correlate with the later phylogenetic appearance of head muscles. Molecular data from across the phylogenetic spectrum support the notion that myogenic potential was acquired by distinct embryonic cell populations, and that, in the anterior mesoderm, this population evolved independent strategies with respect to the posterior mesoderm to accomplish MRF induction and myogenesis. Therefore, whereas the tongue and some neck muscles might have evolved secondarily as an extension of the trunk programme, the CM-derived head muscles are generally viewed to have evolved independently.

**Neck muscles: a transition zone**

The neck region provides interesting correlative information because it is a transition zone between the trunk and head. Typically, as seen in the region anterior to the otic placode in the head of many vertebrate embryos, only the ventral portion of the CM is divided segmentally into PA s whereas the dorsal paraxial portion is unsegmented. By contrast, the neck region is characterised by the presence of both somites and pharyngeal
mesoderm (Fig. 3). As a result, two different types of segmentation appear in this transition zone, as seen in the large part of the body axis in the amphioxus. Furthermore, the peripheral morphology of the cranial nerves shows the highest level of complexity in this region (Kuratani, 1997).

The neck muscles are known collectively as the hypobranchial and cucullaris muscle groups (see Glossary, Box 1). Some of these muscles arise as migratory founder cells from an anterior group of occipital somites and some from cervical somites (Birchmeier and Brohmann, 2000), although the origin of the cucullaris group has been contested (Huang et al., 2001; Kusakabe and Kuratani, 2005; Noden and Francis-West, 2006; Theis et al., 2010). Of note, ectomesenchyme derived from cranial neural crest cells contributes to connective tissues and influences the morphological patterning of muscles in the domain spanning the posterior cranium and the shoulder girdle (Huang et al., 2006; Matsuoka et al., 2005; McKenzie, 1962; Noden, 1983a; Rinon et al., 2007), such as the trapezius, the sternocleidomastoid, the infrahyoid muscle group, the floor of the oral cavity, the tongue muscles and the diaphragm in mammals. Most of these muscles also share a curious trait in that they are not associated with the body axis or the lateral body wall, as typical skeletal muscles are, but instead are associated with visceral components of the body, such as the digestive tract and the pharynx. Notably, the hypobranchial muscles do not form as a continuous growth of muscle plates, but as migrating myoblasts along the non-segmental pathway. Furthermore, they are not found in the lateral body wall as normally expected for trunk muscles. In this regard, lamprey hypobranchial muscle also shares some traits with the neck muscle in gnathostomes in that it arises from somites and attaches to branchial arch skeletons. The contribution of the cephalic crest cells to this muscle, however, is not known in this animal.

Genetic data suggest that some of the neck muscles are more similar to head muscles than to trunk muscles, as Pax3: Myf5 (Mef4)-null mouse mutants that lack somite-derived musculature retain trapezius and sternocleidomastoid (cucullaris group) muscles (Tajbakhsh et al., 1997; Theis et al., 2010), whereas Tbx1–/– mouse mutants that lack the first and second PA-derived muscles also lack the trapezius and sternocleidomastoid muscles (Kelly et al., 2004; Theis et al., 2010). Although earlier reports showed a somitic origin for cucullaris muscles (Huang et al., 2001; Noden and Francis-West, 2006), a recent study has reported that these muscles have a non-somitic origin (Theis et al., 2010) and concurred with a head muscle programme for cucullaris muscle development. A closer examination of the embryological origins of these muscles is warranted given that Pax3, which affects somite-derived muscles, does not appear to regulate some muscles of the neck, indicating that multiple origins might, in fact, define this complex transition zone.

Ancestral vertebrates do not seem to have possessed limb or neck muscles, thus cyclostomes (lampreys and hagfish, see Glossary, Box 1), which also lack typical neck and hypobranchial muscles, are often used as model organisms in which to investigate the evolutionary origins of this musculature. In the lamprey, a muscle called the ‘hypobranchial’ muscle does exist and it is innervated by the hypoglossal nerve. However, this muscle does not invade into the oral floor as it does in gnathostomes (Kuratani et al., 1999). It might represent, therefore, an evolutionary precursor for the neck muscles. Modern cyclostomes do not possess paired fins or shoulder girdles, either. As there is no information on the later contributions of cranial neural crest cells in the lamprey, it is more challenging to define a ‘neck’ domain in this animal (Kuratani, 2008a). Thus, evolution of the gnathostomes, after the split from cyclostomes, would have been initiated by the acquisition of the shoulder girdle. This, in turn, might have been associated with and/or based upon the establishment of cucullaris and hypobranchial muscles (as well as tongue, infrahyoid muscle groups and possibly the diaphragm) in the neck together with the posterior expansion of the cranial neural crest cells that function in the patterning of these muscles. This raises the question: which arose first in evolution – neck or pectoral fin muscles?

**Pax3** and **Lbx1** (ladybird homeobox 1) are expressed in migratory founder cells of the hypobranchial muscles and therefore might be necessary for their ontogeny in vertebrates (Neyt et al., 2000), although these muscles are present in Lbx1 mouse mutants (Gross et al., 2000). The presence of a primitive form of hypobranchial muscle in the lamprey suggests that the neck muscle began to differentiate before the acquisition of the paired fin muscle, even in the evolutionary state in which an epaxial/hypaxial (dorsal and ventral to the horizontal septum, respectively) distinction had not been obtained clearly in the myotomes (Kusakabe and Kuratani, 2005; Kusakabe and Kuratani, 2007; Kusakabe et al., 2011). Therefore, further molecular and comparative studies of the lamprey could provide additional insights into the developmental history of neck and fin muscle evolution in vertebrates.

### Cranial mesoderm duality: skeletal and cardiac muscle fates

A remarkable feature of the branchiomeric subset of head muscles is its relationship with cardiomyo genesis. The cranial lateral mesoderm supplies founder cells to the jaw and facial muscles, as well as to the right ventricle and outflow tract (OFT, see Glossary, Box 1) of the heart (Fig. 2). Importantly, a subset of cranial neural crest cells also contributes to heart development (Hutson and Kirby, 2007; Hutson et al., 2009). Hence, it is not clear whether cells in the cranial lateral mesoderm are bipotent, if there are distinct committed cardiomyo genic and myogenic founder cells in this compartment, or to what extent cranial muscle and heart programmes are molecularly related.

Lineage-tracing analysis and genetic studies have revealed that molecular overlaps exist between heart and head muscle development. Both Tbx1 and Pitx2, the hierarchical transcription factors in the gene regulatory networks that orchestrate myogenesis in the PA, are known to control the proliferation of cardiac founder cells, and they are required for normal OFT development (Grifone and Kelly, 2007; Tzahor, 2009). Tcf21 and Msc, which are key players in jaw myogenesis (Lu et al., 2002), are also expressed in myocardial founder cells (Robb et al., 1998; von Scheven et al., 2006b). Islet1 (Isl1) is a LIM homeodomain transcription factor and MeF2c is a MADS box transcription factor, and lineage analysis using IsilCre and Tg: MeF2c-Cre reporter mice has revealed that PA-derived muscles have a history of expression of these critical regulators of the heart programme (Abu-Issa and Kirby, 2007; Cai et al., 2003; Dong et al., 2006; Kwon et al., 2009; Mann et al., 2009; Nathan et al., 2008; Shih et al., 2007; Verzi et al., 2005). Finally, retrospective clonal lineage analysis in mouse embryos has revealed the existence of bipotent ancestral cells, either in the cranial lateral mesoderm or even earlier during development, that give rise to both arch-derived skeletal muscles and anterior heart field-derived cardiomyocytes (Lescroart et al., 2010). Interestingly, this report also shows the lineage relationships between first PA-derived muscles and the right ventricle, and the second PA-derived muscles and the OFT, indicating diversity at the
cellular level between first- and second-arch muscle progenitors. In fact, the first and second pharyngeal arches are likely to have distinct molecular programmes, as supported by the phenotypes of Tbx1-null mice (in which the first arch is present, whereas other arches are absent) (Jerome and Papaoannou, 2001) and Pitx2 mutant mice (in which only the first arch is hypoplastic) (Gage et al., 1999; Shih et al., 2007).

Nkx2.5, another key homeodomain-containing transcription factor in the cardiomyogenic programme (Harvey et al., 2002), is expressed early in arch-derived pterygoid muscle in vertebrates (Kasahara et al., 1998). Furthermore, targeting of the Nkx2.5 locus with Cre recombinase has allowed its expression in these muscles to be traced in mice (Kasahara et al., 1998; Stanley et al., 2002). In addition, in C. elegans, in which cardiac tissue is not present and the pharynx exhibits electrical activity, the Nkx2.5 homologue ceh-22 is expressed during the differentiation of pharyngeal muscle (Okkema and Fire, 1994). In the tunicate Ciona intestinalis, cells derived from NK4 (Nkx2.5 homologue)-expressing blastomeres generate the heart, as well as the muscles of the atrial siphon (the cloacal siphon in the body-wall) (Hirano and Nishida, 1997; Stolfi et al., 2010). Interestingly, Islet (the Ciona homologue of Isl1) is not expressed in heart progenitors but it marks the atrial siphon muscle lineage (Stolfi et al., 2010). This report also shows that some cells of the longitudinal muscles, which arise from atrial siphon muscles upon metamorphosis, also express Tbx1/10 (the gene ancestral to Tbx1 and Tbx10).

Notably, the promiscuous expression of molecular markers in cardiomyogenic and skeletal myogenic founder cells also extends to their differentiated progeny. For example, human and rabbit masticatory muscles retain cardiac α-myosin heavy chain expression (Bredman et al., 1991), as do EOMs in rabbits (Rushbrook et al., 1994). However, cardiac α-myosin heavy chain is not expressed in the jaw muscles of the rat (Kawai et al., 2009). Other evidence that cranial paraxial mesoderm retains a kinship with cranial lateral mesoderm comes from manipulations in the chick, in which ectopic bone morphogenetic protein 4, Bmp4, induces cardiac markers and suppresses skeletal muscle markers in CM (Tzahor et al., 2003). Strikingly, this is also the case with jaw, but not limb, muscle satellite cells, in which cardiac markers (e.g. Isl1 and Tbx20) are activated upon Bmp4 induction (Harel et al., 2009).

Thus, the hierarchical transcription factors that regulate muscle development in PAs are expressed in cardiac progenitors and vice versa. The overlap of hierarchical gene expression in founder cells of the cardiac and head muscle lineages raises the following question: what mechanisms define these alternate myogenic fates? Canonical Wnt signalling, which promotes somitic muscle development, is inhibitory to both cardiac and branchiomieric muscle differentiation (Cosu and Borello, 1999; Münsterberg et al., 1995; Nathan et al., 2008; Stern and Hauschka, 1995; Tajbakhsh et al., 1998; Tzahor et al., 2003; Tzahor and Lassar, 2001). By contrast, the BMP pathway, a negative influence on trunk muscle development, also acts negatively on head myogenesis (Pourquié et al., 1996; Tiros-Finkel et al., 2006; Tzahor et al., 2003; von Scheven et al., 2006a) but it promotes cardiomyogenic differentiation (Dyer and Kirby, 2009; Tiros-Finkel et al., 2006; von Scheven et al., 2006a). Furthermore, sonic hedgehog (Shh), which promotes trunk muscle development (Borycki et al., 1999), has been shown to act as a negative cue for branchiomieric myogenesis (Tzahor et al., 2003; von Scheven et al., 2006a). By contrast, Shh has been implicated in regulating Tbx1 expression in the PAs (Garg et al., 2001). The role of Shh in cardiomyogenic differentiation is not clear; however, it is critically required for progenitor cell proliferation and heart tube morphogenesis (Dyer and Kirby, 2009; Washington Smoak et al., 2005). Another influential signalling molecule, fibroblast growth factor 8 (Fgf8), inhibits skeletal myogenic differentiation (von Scheven et al., 2006a), but promotes the cardiogenic programme (Dyer and Kirby, 2009). However, BMP-mediated inhibition of Fgf signalling has been shown to promote cardiomyocyte differentiation (Tiros-Finkel et al., 2010).

In essence, the genes that are critical for head myogenesis, such as Tbx1, Pitx2, Tcf21 and Msc, and that distinguish it from the trunk muscle programme, are shared with the cardiogenesis developmental programme. This feature further underscores the theme highlighted in this review: that the head muscles are evolutionarily distinct from trunk muscles, and that the head myogenic gene network probably arose independently of the trunk muscle regulatory programme.

Conclusions

The convergence of developmental and evolutionary biology with comparative genomic data has filled in some of the gaps in our knowledge about the CM and its derivatives. A major challenge, however, is to determine when and how the CM appeared in the ancestor of vertebrates as a trait specific to vertebrates, how it became regionalised and when this coincided with the emergence of founder stem cells for skeletal and cardiac muscle, the skull bones and endothelium. An intriguing thought, from an evo-devo perspective, is whether CM patterning and differentiation mechanisms could be viewed as a modification of the myotome developmental mechanism, which is putatively ancestral to the CM. In this regard, distinguishing between the paraxial and lateral components of the CM is a fundamental issue. At present, morphological criteria are not sufficient to distinguish the cranial prechordal, paraxial and lateral components of the CM, and gene expression analyses have not yet identified specific markers for each. When this becomes possible, genetic lineage studies using an inducible Cre recombinase with reporter mice might clarify some of these issues. Another question is whether or not there is indeed a 'paraxial' and 'lateral' mesoderm, as is distinguished in the trunk, and, if so, to what extent these tissues are limited in fate potential. This remains a matter of debate. The overlap in expression of cardiac and skeletal myogenic transcription factors in each suggests that these fates are not as distinct as originally thought. Repressive signatures might also delay cell fate acquisition and allow proliferation until specific cell lineages arrive at their proper destination. Identifying novel regulators and elucidating the crosstalk between the factors that have been shown to be critical, including Tbx1, Pitx2 and the MRFs, will also be major immediate goals.

In the context of the evolution of the CM, some major shifts in thinking and classification have been proposed in the last five years, based on studies of tunicates, amphioxus and lampreys, all of which have provided informative clues as to how the CM evolved. For a true comparative analysis, it is important to consider the acquisition, as well as the loss, of traits; however, this adds to the complexity of these studies. Nevertheless, neural crest, cranial nerves and the evolution of the neck muscles provide landmarks for understanding how CM organisation and cell fate occur. Gene expression profiles of critical transcription factors in the ancestral model organisms have also been insightful in determining the developmental state of anterior mesoderm. Transplantation and cell lineage approaches in avian and mouse models have been arguably
the most informative in unravelling the complexities of CM fates. Applying these types of experimental approaches to cyclostomes and gnathostomes should provide the missing pieces of the puzzle.

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