Heart and craniofacial muscle development: A new developmental theme of distinct myogenic fields

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ABSTRACT

Head muscle development has been studied less intensively than myogenesis in the trunk, although this situation is gradually changing, as embryological and genetic insights accumulate. This review focuses on novel studies of the origins, composition and evolution of distinct craniofacial muscles. Cellular and molecular parallels are drawn between cardiac and branchiomeric muscle developmental programs, both of which utilize multiple lineages with distinct developmental histories, and argue for the tissues’ common evolutionary origin. In addition, there is increasing evidence that the specification of skeletal muscles in the head appears to be distinct from that operating in the trunk: considerable variation among the different craniofacial muscle groups is seen, in a manner resembling myogenic specification in lower organisms.

Introduction

There are approximately 60 distinct skeletal muscles in the vertebrate head that control food intake, facial expression and eye movement. These muscles develop in a manner that is tightly coordinated with other craniofacial tissues. In recent years, interest in this unique group of skeletal muscles (reviewed in Bothe et al., 2007; Grifone and Kelly, 2007; Noden and Francis-West, 2006; Noden and Trainor, 2005; Sambasivan and Tajbakhsh, 2007) has significantly increased, with the accumulation of new lineage tracing, molecular profiling and gene targeting studies.

The head musculature is known to originate from the cranial paraxial mesoderm (CPM) located anterior to the somites. Unlike the paraxial mesoderm in the trunk, the cranial paraxial mesoderm lacks any overt signs of segmentation. Together with cranial neural crest cells, CPM cells move into the branchial (pharyngeal) arches, paired thickenings around the pharynx that eventually give rise to the facial structures. The head muscles themselves are generally classified according to their anatomical location within the head: for example, the six extraocular muscles (EOM) move and rotate the eye in a highly coordinated manner; branchiomeric muscles control jaw movement and facial expression, as well as pharyngeal and laryngeal function. Muscles in the neck and tongue are derived from myoblasts originating in the most anterior set of somites (reviewed in Noden and Francis-West, 2006).

The regulation of head muscle patterning and differentiation by signals from adjacent tissues has been extensively explored in recent years (Rinon et al., 2007; Tzahor et al., 2003; von Scheven et al., 2006), as well as in earlier studies reviewed in Noden and Trainor (2005). Though it appears that cranial neural crest cells play multiple distinct roles in the patterning and differentiation of muscles during craniofacial development, a detailed examination of these issues is beyond the scope of this review.

Regionalization of the head mesoderm

In vertebrates, head mesoderm precursors undergo gastrulation in the primitive streak prior to the somites, precursors of trunk paraxial mesoderm (Psychyosos and Stern, 1996). At later stages, the loosely connected (mesenchymal) CPM is positioned along both sides of the neural tube and notochord, whereas the lateral splanchnic mesoderm (SpM), lying adjacent to the CPM, is maintained in an epithelial shape. Subsequently, during embryonic ventral folding, the lateral SpM is located on the ventral side of the embryo, beneath the floor of the pharynx.

The exact molecular nature of the CPM and SpM, and the border separating them, remains unclear. Recent gene expression analyses in avian models have begun to reveal the molecular milieu of head muscle progenitors in Str. 8 (Nathan et al., 2008), Str. 10 (Bothe and Dietrich, 2006), Str. 16 (Tirosh-Finkel et al., 2006) and Str. 20 and 24 (Dastjerdi et al., 2007) chick embryos. Pitx2, Tcf21 (capsulin), Msc (MyoR), Twist, Alx4, and Tbx1 genes were shown to be expressed in the head mesoderm: Alx4, Tbx1, Cyp26C1, and Twist were expressed in the CPM, whereas Pitx2 and MyoR labeled more lateral areas (presumably SpM) (Bothe and Dietrich, 2006). In Str. 8 chick embryos, the SpM was found to express a set of second heart field markers, Isl1, Nkx2.5, Fgf10, and Tbx20 (Nathan et al., 2008), in agreement with the cardiogenic potential of these cells (Fig. 1).

It has been shown in the chick that there is considerable overlap in the expression of head muscle markers e.g., Myf5, Tcf21 (capsulin), Msc...
(MyoR), Tbx1, Pitx2] and cardiac lineage markers (e.g., Islet1 and Nkx2.5; Bothe and Dietrich, 2006; Nathan et al., 2008; Tirosh-Finkel et al., 2006; also reviewed in Grifone and Kelly, 2007) in the CPM and SpM. Taken together, these studies have begun to delineate the molecular regionalization of the head mesoderm (Figs. 1A–D). Importantly, there seems to be no clear border between CPM and SpM cells, reflecting a dynamic continuum in these fields along the medial-lateral/dorsal-ventral axes.

Branchiomeric muscles are derived from both CPM and SpM cells

Previous fate-mapping studies in chick, mouse, and zebrafish models have defined the migratory pathways by which CPM cells fill the myogenic core within the branchial arches (Coulby et al., 1992; Hacker and Guthrie, 1998; Noden, 1983; Schilling and Kimmel, 1994; Trainor et al., 1994). Nathan et al. recently extended these analyses,
showing that CPM cells mainly contribute to the proximal region of the myogenic core in the 1st branchial arch, while SpM cells contribute to its distal region (Figs. 1C–F). Subsequently, CPM-derived myoblasts in the 1st branchial arch contribute to the mandibular adductor complex (equivalent to the masseter in mammals), whereas SpM-derived distal arch myoblasts give rise to lower (e.g., intermandibular) jaw muscles (Marcucio and Noden, 1999; Nathan et al., 2008; Noden et al., 1999) (Figs. 11, J). Furthermore, gene expression analyses in the chick uncovered a distinct molecular signature for CPM- and SpM-derived branchiomeric muscles. For example, Isl1 is expressed in the SpM-derived intermandibular anlagen, and its expression is correlated with delayed differentiation of this muscle (Nathan et al., 2008).

Lineage tracing experiments in mice using Mef2c AHF-Cre (Dong et al., 2006; Verzi et al., 2005) and Islet1-Cre (Nathan et al., 2008) alleles, also corroborated the contribution of SpM cells expressing the LIM homeodomain transcription factor Islet1 (Isl1+) to 1st branchial arch-derived muscles. In both chick and mouse models, Isl1+ SpM cells were shown to contribute to a set of branchiomeric muscles – the intermandibular muscle in the chick, and the myohyoideus, stylohyoideus, and digastric in the mouse – at the base of the mandible, facilitating its opening (Figs. 11, J). Isl1+ cells were also found in 2nd branchial arch-derived muscles controlling facial expression. Isl1+ cells contribute to a lesser extent to mastication muscles (masseter, pterygoideus, and temporalis) in the mouse, and to the mandibular adductor complex in the chick. In both species, tongue muscles (e.g., genioglossus) and all extraocular muscles are not derived from the Isl1 lineage (Nathan et al., 2008) (Figs. 11, J). In addition to the contribution of the Isl1 cell lineage to both cardiac and head muscle progenitors, Isl1 was recently shown to be expressed in both sites (Bothe and Dietrich, 2006; Nathan et al., 2008; Tirosh-Finkel et al., 2006).

The effects of gene targeting on craniofacial muscle phenotypes in the mouse

The bHLH transcription factors, Capsulin and MyoR, were shown to act as upstream regulators (presumably repressors) of branchiomeric muscle development. In Capsulin/MyoR double mutants, the masseter, pterygoideus, and temporalis muscles were missing, while distal 1st branchial arch muscles (e.g., anterior, digastric and myohyoideus) were not affected (Lu et al., 2002). A plausible developmental explanation for the Capsulin/MyoR double mutant phenotype is compatible with findings that branchiomeric muscles are composed of at least two myogenic lineages, CPM-derived and SpM-derived muscle cells.

In T-box transcription factor Tbx1 mutants, branchiomeric muscles (including those derived from both CPM and SpM) were frequently hypoplastic and asymmetric, whereas the EOM and tongue muscles were not affected (Kelly et al., 2004). The authors suggest that all 1st branchial arch muscles require Tbx1 for robust bilateral specification. The head muscle defects in Tbx1 mutants are likely due to an intrinsic defect in the mesoderm (Dastjerdi et al., 2007), as well as to Tbx1’s indirect function in the endoderm and ectoderm (Arnold et al., 2006). Indeed, analyses of various Tbx1 mutant embryos indicated that several Fgf family members, expressed in these adjacent tissues, were downregulated, demonstrating a role for Tbx1 and Fgf signaling during head muscle development (Hu et al., 2004; Kelly et al., 2004; Knight et al., 2008; Vitelli et al., 2002; von Schenning et al., 2006).

Tbx1 and the bicoid-related homeodomain transcription factor Pitx2 are thought to be linked to the same genetic pathway in many developmental processes, including cardiac and craniofacial muscle development (Grifone and Kelly, 2007). In both mouse and chick, Pitx2 is expressed in the head mesoderm and, subsequently, in the mesodermal core of the 1st branchial arch (Dong et al., 2006; Shih et al., 2007). In Pitx2 mutants, the EOM and mastication muscles of the 1st branchial arch are affected: SpM-derived myoblasts, marked by the Mef2c AHF-Cre lineage in the mouse (Verzi et al., 2005), were significantly reduced in Pitx2 mutant embryos (Dong et al., 2006). However, the degree to which the myohyoideus and anterior digastric muscles, derived from SpM, were affected in Pitx2-null embryos (Shih et al., 2007) is less clear. These loss-of-function studies, combined with the lineage tracing studies described above, highlight the heterogeneity in cranial muscle development. Furthermore, Tbx1 and Pitx2 constitute the first known examples of transcription factors that regulate branchiomeric muscle and cardiac outflow tract development in mice (Ai et al., 2006; Dong et al., 2006; Kelly et al., 2001; Shih et al., 2007; Xu et al., 2004; reviewed in Grifone and Kelly, 2007).

Heart and craniofacial muscle developmental programs are linked

As described herein, an increasing number of researchers have begun to explore the link between cardiac and head muscle developmental programs. Quail chick transplantation assays indicated that progenitor cells originating in the CPM can give rise to angioblasts that populate the endocardium of the outflow tract (Noden, 1991). Another study in chick embryos revealed the cardiogenic potential of the CPM in vitro, and further suggested that signals from the dorsal neural tube (e.g., Wnt1 and Wnt3a) block cardiogenesis in CPM explants (Tzahor and Lassar, 2001). A subsequent study, also in chick embryos, demonstrated both in vitro and in vivo that a subset of CPM cells contributes to both myocardial and endocardial cell populations within the cardiac outflow tract (Tirosh-Finkel et al., 2006; see also Fig. 1G). Taken together, these findings have uncovered the cardiogenic potential of the CPM.

The cardiogenic potential of the pharyngeal mesoderm (consisting of both CPM and SpM) was further revealed in chick and mouse embryos (Kelly et al., 2001; Mjaatvedt et al., 2001; Rana et al., 2007; Waldo et al., 2001). These studies raised the possibility that during tubular heart looping, the arterial pole of the heart develops by the addition of cells derived from the pharyngeal mesoderm (Figs. 1E–H). In accordance with these results, other studies involving various transgenic mouse lines demonstrated an overlap in the progenitor populations contributing to branchiomeric and cardiac muscle (Dong et al., 2006; Verzi et al., 2005) (Figs. 1F, H, J). Thus, it appears that the genetic program controlling the development of CPM into branchiomeric muscle overlaps with that controlling a subset of SpM cells that gives rise to the arterial pole of the heart.

It is by now well-established that the vertebrate heart is formed from two distinct mesoderm populations or “heart fields”, which arise from a common origin, and express both distinct and overlapping molecular markers (Fig. 1). The earliest population of cardiac progenitors, the first heart field, corresponds to the anterior lateral mesoderm and, subsequently, to the cardiac crescent. Ultimately, cells from the first heart field (Fig. 1, marked in red) contribute to the left ventricle and atria. An additional source of cardiac precursors, the second (or secondary/anterior) heart field (Fig. 1, marked in yellow), derives from mesoderm located medial to the cardiac crescent. These cells contribute primarily to the cardiac outflow tract, right ventricle and atria (Fig. 1; see also Black, 2007; Buckingham et al., 2005; Garry and Olson, 2006; Srivastava, 2006).

Heart development takes place in close apposition to the developing head. The separation between the heart and the head commences gradually, following heart-looping stages as the heart shifts caudally. The link between heart and craniofacial development was discovered more than two decades ago, when Margaret Kirby first identified the cardiac neural crest, and demonstrated its involvement in heart development (Kirby et al., 1983). Cardiac neural crest cells migrate from specific sites in the hindbrain into the cardiac outflow tract (conotruncus) and developing aortic arch arteries. The term “cardio-craniofacial morphogenetic field” reflects the intimate developmental and cellular relationship between the head, face, and heart, which is manifested in numerous cardiac and craniofacial birth defects (Hutson and Kirby, 2003).
Similarches between the second heart field, and the second myogenic field in the head

Recent discoveries in chick and mouse models of two distinct mesodermal fields (CPM and SpM), both of which contribute cells to the developing facial musculature in a specific temporal and spatial manner (Dong et al., 2006; Nathan et al., 2008), suggest that these fields are analogous to the two distinct myogenic heart fields (Fig. 1). In both cases, the second myogenic field resides in SpM cells expressing Isl1 (Fig. 1, marked in yellow). These Isl1+ cells migrate into the arterial pole of the heart, as well as into the distal myogenic core of the 1st branchial arch (Cai et al., 2003; Nathan et al., 2008). Within the heart or the lower jaw, the differentiation of Isl1+ SpM cells comprising the second heart field/second myogenic field is delayed (Nathan et al., 2008). Because the expression of myogenic differentiation markers was also found to be delayed in SpM-derived Isl1+ myoblasts, it has been suggested that Isl1 represses skeletal muscle differentiation, either directly or indirectly (Nathan et al., 2008; and data not shown). Thus, in accordance with recent reports (Cai et al., 2003; Laugwitz et al., 2005; Moretti et al., 2006; Qyang et al., 2007), Isl1 stands at a nodal point in the differentiation and lineage specification of distinct mesoderm-derived cardiovascular and skeletal muscle progenitors.

In the trunk, self-renewing satellite cell progenitors, which serve as a source of adult muscle stem cells, can be identified by the expression of Pax3 and, later, Pax7; however, they lack expression of the myogenic regulatory factors Myf5 and MyoD (Zammit et al., 2006). In the head, on the other hand, Pax3 is not expressed in muscle progenitors, and Pax7 expression ensues only after Myf5 expression (Hacker and Guthrie, 1998; Nathan et al., 2008). It is tempting to speculate that Isl1 plays a role in regulating the quiescence and self-renewal of satellite cell progenitors in distinct branchiomeric muscles, analogous to Pax3/Pax7 in trunk skeletal muscles. Ptx2, MyoR, capsulin and Tbx1 could fulfill these criteria in other head muscle anlagen.

In summary, recent lineage studies in both chick and mouse models clearly demonstrate the heterogeneous nature of cranial muscles, and enable us to draw an analogy between branchiomeric muscle and cardiac development: both arise from multiple lineages with distinct developmental histories (Fig. 1).

Evolution of distinct myogenic programs

Existing evidence that cardiac and branchiomeric muscle development programs are tightly linked suggests that these tissues share common evolutionary origins (Fig. 2). Throughout evolution, both the architecture and the cellular physiology of muscles have been remarkably conserved. Striated muscles of invertebrates strongly resemble vertebrate skeletal muscles, though the architecture of the former is somewhat less complex. Recently, it was shown that the development of striated muscles of the body wall, which enable the worm’s locomotion in a manner analogous to that of skeletal muscles in vertebrates, is dependent on the involvement of the SRF and HAND transcription factors, both of which play prominent roles in regulating smooth and cardiac muscle development in vertebrates (Baugh and Hunter, 2006; Fukushige et al., 2006). Accordingly, hh-l (CeMyoD, unc-120 (MADS-box/CFR), and hnd-1 (HAND/bHLH) redundantly control muscle specification in C. elegans, together comprising a functionally robust “muscle module”. These findings suggest that skeletal muscle likely evolved from an ancestral developmental program involving a single contractile myogenic cell type (Baugh and Hunter, 2006; Fukushige et al., 2006) (Fig. 2A).

Most strikingly, though nematodes do not possess a heart per se, their pharyngeal muscle contracts like a heart, and exhibits electrical activity similar to that of mammalian cardiomyocytes. Moreover, it has been shown that the development of the pharyngeal muscle in nematodes is regulated by the homeobox gene Nlx2.5 (ceh-22) (Harfe and Fire, 1998) and may be functionally replaced by the zebrafish Nlx2.5 (Haun et al., 1998) (Fig. 2A). Nevertheless, pharyngeal muscles are still present in ceh-22 mutants (Ogkuma and Fire, 1994), indicating that additional factors are required for pharyngeal muscle formation in nematodes. Indeed, it was recently shown that tbx-2 is critical to the development of a subset of pharyngeal muscles in nematodes (Smith and Mango, 2007), similar to the function of Tbx1 in a subset of branchiomeric muscles in the mouse (Kelly et al., 2004). Therefore, it is likely that mechanisms regulating nematode pharyngeal muscle development, and those regulating a subset of branchiomeric muscles and heart development in vertebrates, were co-opted and maintained throughout evolution.

The fact that regulatory mechanisms of myogenic progenitors have been largely conserved throughout the animal phyla indicates that early muscle evolution passed a selective bottleneck (Olson, 2006; Seipel and Schmid, 2005). It could be argued that the last common ancestor of nematodes and mammals (~700 million years ago) already contained at least two distinct contractile cell types, one cardiac and one skeletal in nature. Moreover, it appears that while cardiac, smooth, and skeletal muscle cells all arose from a common contractile ancestor, the evolutionary linkage between the pharyngeal muscles and the heart is tighter (Figs. 2A, B). Conceivably, Isl1+ SpM-derived branchiomeric muscles evolved from an ancestral myogenic program for both cardiac and skeletal muscle lineages (Figs. 2A, B).

In the fruit fly Drosophila melanogaster, the Isl1 homolog tailup encodes a LIM homeodomain transcription factor expressed in all cardioblasts and pericardial cells of the heart tube, as well as in associated hematopoietic organs and alary muscles that attach the dorsal vessel to the epidermis (Tao et al., 2007). Functional studies further demonstrated that Tailup plays a role in dorsal vessel morphogenesis (analogous to the heart in vertebrates) and lymph gland formation, and places this transcription factor directly upstream of Hand in these developmental processes (Tao et al., 2007). Identifying Tailup as an important component of the gene regulatory network controlling heart and hematopoietic formation is consistent with the evolutionary conservation of transcriptional regulators in the cardiovascular system.

The robustness of myogenesis in all animals is provided by redundancy of function and gene regulation. The myogenic regulatory network functions to irreversibly commit cells to the muscle lineage. Myogenesis in the trunk is largely characterized by hierarchical interactions of upstream regulators of myogenic specification such as Pax3, Myf5 and Mrf4, which activate myogenic cell fate and, later, the differentiation of these muscle progenitors via MyoD and Myogenin (Kassar-Duchossoy et al., 2004) (Fig. 2B). As highlighted in Fig. 2B, the specification of the muscle lineage in the head appears to be distinct from that operating in the trunk. In addition, early myogenesis in the head may resemble myogenic specification in lower organisms such as the nematode C. elegans and the fruit fly Drosophila melanogaster.

The current view concerning the specification of muscle identity in Drosophila is that a combination of transcription factors endows muscle founder cells with the capacity to execute the myogenic program that is specific to each muscle fiber. Each muscle fiber is an individual syncytium that can be distinguished by its position, shape, epidermal attachment sites, and innervation (reviewed in Baylies and Michelson, 2001). Consistent with this view, there is increasing evidence that the developmental program of the head musculature in vertebrates varies among the different muscle groups: while Pax3 and Mrf4 are not necessary for cranial myogenesis, distinct combinations of transcription factors [involving Tcf21 (capsulin), Msc (MyoR), Tbx1, Ptx2, Isl1, and Nlx2.5] act upstream of MyoD and myogenin to specify the different craniofacial muscles (Fig. 2B). Remarkably, most of these transcription factors also play prominent roles in cardiogenesis, in
agreement with the evolutionary link between branchiomeric muscles and heart development in vertebrates.

Immunohistochemical staining for the cardiac marker α myosin heavy chain revealed its presence specifically in the heart and branchiomeric muscles of both human and rabbit (Bredman et al., 1991) leading the authors to conclude that, “the ‘cardiac’ α myosin heavy chain is only found in skeletal muscles originating from the cranial part of the embryo (including the heart muscle), suggesting that its expression might be determined by the developmental history of these muscles”. This view fits well with the concept of a single developmental cardio-craniofacial field. In addition, analyses of the expression of distinct myosin heavy chain isoforms in various muscles have shown that fiber types in jaw muscles of different species are extremely divergent, while those in trunk muscles show only a minimal degree of phylogenetic plasticity (reviewed in Hoh, 2002). It could be argued that the heterogenic developmental nature of distinct branchiomeric muscles may account for the recent evolutionary adaptations of these muscles, in response to changes in diet or feeding patterns (Hoh, 2002).

By now, it is widely accepted that head and trunk myogenic programs vary considerably (reviewed in Bothe et al., 2007; Grifone and Kelly, 2007; Noden and Francis-West, 2006). These differences persist into adulthood, as reflected in the distinct genetic signatures and susceptibility to muscle myopathies of head and trunk skeletal muscles (Emery, 2002; Porter et al., 2006). This review summarized recent studies in both chick and mouse models that uncovered the heterogeneity in head muscle developmental programs, and focused on the analogy between branchiomeric muscle and cardiac development: both arise from multiple lineages with distinct developmental histories.

The challenges that lie ahead will be to precisely decipher the muscle phenotype of an individual, as well as a combination of, regulators of head myogenesis by means of loss-of-function experiments, and to explore in greater depth how these factors function in tandem to assemble distinct craniofacial muscles in a specific temporal and spatial manner. The signals that regulate the dynamic developmental processes underlying the specification, migration and differentiation of the different myogenic lineages in the head, have yet to be discovered. A number of approaches, including genetic, cellular biochemical and Evo–Devo studies should be undertaken, in order to reveal the molecular underpinnings of the link between heart and craniofacial muscle development.

**Fig. 2.** Evolutionary and molecular similarities in the mechanisms underlying heart and craniofacial muscle development. (A) Krause et al. demonstrated a “muscle module” controlling bodywall muscle specification in C. elegans. This and other studies suggested that skeletal muscles likely evolved from an ancestral developmental program involving a single contractile myogenic cell type. Moreover, a functional and molecular analogy may be drawn between the pharyngeal muscle in nematodes, and the heart in vertebrates. (B) Based on lineage analyses and genetic inactivation of several myogenic transcription factors in mice, it appears that the development of the head musculature varies among the different muscle groups (highlighted in the boxes on the right; the question mark indicates a lack of data from gene inactivation experiments). In addition, specification of the muscle lineage in the head appears to be distinct from that operating in the trunk (lower box, pink), and resembles muscle development in lower organisms such as C. elegans (A). As discussed in this review, there are evolutionary, molecular, cellular and clinical links between the developmental mechanisms of the heart and craniofacial muscles. MRFs, myogenic regulatory factors.
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