Cardiovascular Research (2011) **91**, 196–202
doi:10.1093/cvr/cvr116

SPOTLIGHT REVIEW

Pharyngeal mesoderm development during embryogenesis: implications for both heart and head myogenesis

Eldad Tzahor[1]* and Sylvia M. Evans[2]

[1]Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel; and [2]Skaggs School of Pharmacy, University of California—San Diego, La Jolla, CA 92093, USA

Received 7 February 2011; revised 4 April 2011; accepted 12 April 2011; online publish-ahead-of-print 15 April 2011

Abstract The pharyngeal mesoderm (PM), located in the head region of the developing embryo, recently triggered renewed interest as the major source of cells contributing to broad regions of the heart as well as to the head musculature. What exactly is PM? In this review, we describe the anatomical and molecular characteristics of this mesodermal population and its relationship to the first and second heart fields in chick and mouse embryos. The regulatory network of transcription factors and signalling molecules that regulate PM development is also discussed. In addition, we summarize recent studies into the evolutionary origins of this tissue and its multipotential contributions to both cardiac and pharyngeal muscle progenitors.

Keywords Heart • Head muscle • Pharyngeal mesoderm

This article is part of the Spotlight Issue on: Cardiac Development

1. Pharyngeal mesoderm: definition, location, and contribution

Pharyngeal mesoderm (PM) constitutes a subset of head mesoderm that surrounds the developing pharynx and is situated within the core of the pharyngeal arches (also known as branchial arches). Head mesoderm precursors undergo gastrulation in the primitive streak, prior to those of trunk mesoderm.[1,2] The PM can be divided into two subdomains: the loosely connected, mesenchymal paraxial mesoderm, located on both sides of the neural tube and notochord (*Figure 1A* and *B*), and the medial splanchnic mesoderm, which is maintained as epithelial tissue, although there seems to be no clear border between these two domains (*Figure 1A*).

The lateral splanchnic mesoderm is located on the ventral side, beneath the floor of the pharynx (*Figure 1B*). Both paraxial and splanchnic mesoderm cells converge to form the mesodermal core within the pharyngeal arches[3] (*Figure 1C* and *D*). Hence, the broad definition of the PM should include both paraxial and lateral mesoderm cells surrounding the pharynx (*Figure 1*, marked in green). Taken together, PM cells contribute to the core of the pharyngeal arches (*Figure 1D*). These cells give rise to significant parts of the heart and the pharyngeal muscles. PM cells are found in close proximity to the pharyngeal endoderm, ectoderm, and neural crest cells, which tightly influence PM development (*Figure 1D*; see below).

Studies in both chick and mouse embryos have shown that cardiac progenitor cells populating the cardiac outflow tract and right ventricle are progressively added during heart looping stages by PM cells, and are referred to as the anterior heart field.[4–6] In the mouse, the anterior heart field is a subset of the second heart field, which contributes to the outflow tract and right ventricle, and will also contribute a majority of cells to the atria. Thus a subset of PM cells constitutes the second heart field, in contrast to the more lateral splanchnic mesoderm, known as the first heart field (*Figure 1A* and *D*, pink colour), which is contiguous with the PM, differentiates earlier, and eventually populates the left ventricle (reviewed in references 7–10).

The secondary heart field in the chick, which also gives rise to the arterial pole of the heart, is also part of the anterior heart field. The secondary heart field is situated slightly caudal to the anterior heart field, and gives rise to the myocardium and smooth muscle of the distal outflow tract.[10] The cells added at the arterial and venous poles of the heart are derived from different regions of the mesoderm,[11] and whether caudal PM contributes to the venous pole of the heart is currently not clear. Collectively, the anatomical

* Corresponding author. Tel: +972 8 934 3715; fax: +972 8 934 4116, Email: eldad.tzahor@weizmann.ac.il

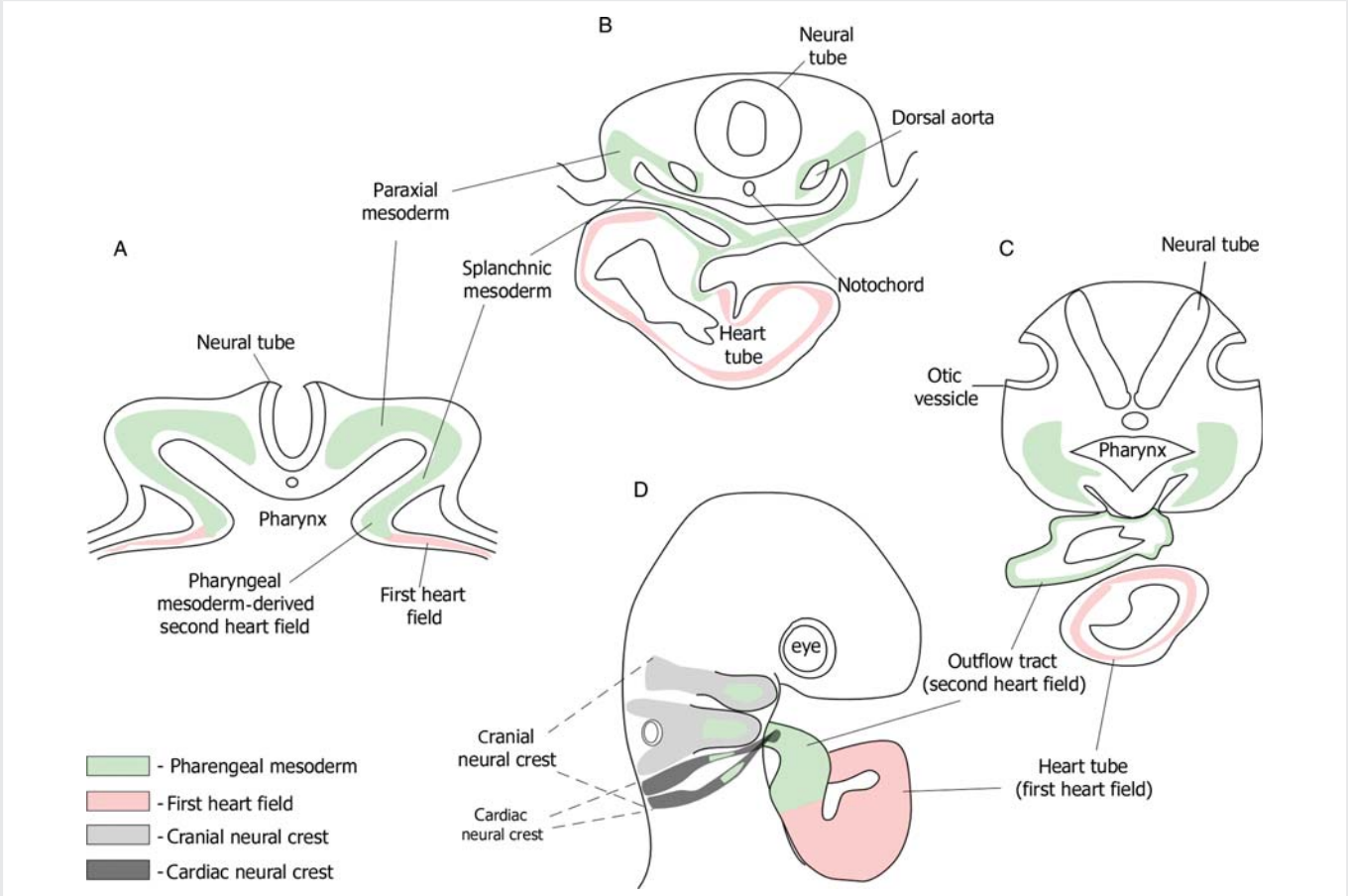

Figure 1 Pharyngeal mesoderm cells give rise to parts of the heart and the pharyngeal muscles. (*A*–*D*) Schematic illustration of the anatomy of the pharyngeal mesoderm in sections of a 1.5–3-day-old chick embryo. Pharyngeal mesoderm cells (green) in the anterior part of the embryo surround the pharynx. Later, these cells fill the mesoderm core of the pharyngeal arches, and are incorporated into the arterial pole of the heart (e.g. outflow tract). The first heart field (pink) is restricted to the lateral splanchnic mesoderm that later contributes to the linear heart tube. Second heart field cells (green) are PM cells that contribute to the arterial pole of the heart. PM cells interact and migrate together with cranial neural crest cells. Cardiac neural crest cells are part of the cranial neural crest population, migrating into the outflow tract via the posterior arches (arches 3–6).

terminology in the chick is such that the secondary heart field is a subdomain of the anterior heart field, which is a subdomain of the PM, which is part of the head mesoderm. In addition, recent studies indicate that PM progenitors contribute to both head muscles (pharyngeal muscles) and parts of the heart.[3,4,12–16] Hence, PM cells are critical part of the cardio-craniofacial field during early embryogenesis.

The skeletal myogenic potential of PM cells and their contribution to pharyngeal muscles have long been documented.[17] In contrast, the cardiogenic potential of these cells has only been revealed over the last decade (reviewed in references 7–10,18). In keeping with these findings, PM explants dissected from early chick embryos undergo cardiogenesis.[19] This study further suggested that signals from the dorsal neural tube (e.g. Wnt1 and Wnt3a) attenuate PM-derived cardiogenesis.[19] The *in vivo* cardiogenic potential of PM was further revealed in chick embryos.[3,12,20] Furthermore, considerable overlap in the expression of head muscle markers [e.g. *Myf5, Tcf21* (*capsulin*), *Msc* (*MyoR*), *Tbx1, Pitx2*] and cardiac markers such as *Islet1* and *Nkx2.5* has been documented in the PM, suggesting that these cells play a dual role in myogenesis and cardiogenesis.[3,12,21] Likewise, lineage studies in the mouse demonstrated an overlap in progenitor populations contributing to pharyngeal muscles and second heart field derivatives.[3,22–24] Thus, the genetic programme controlling pharyngeal muscles overlaps with that of the PM-derived heart.

Genetic studies in vertebrate embryos have begun to reveal the regulatory network of transcription factors expressed in the PM. The major players in this network include the transcription factors *Tbx1*, *Pitx2, Tcf21* (*capsulin*), *Msc* (*MyoR*), and Isl1 (reviewed in references 8,13–15,25). Although these reviews describe muscle phenotypes resulting from knockout, singly or in combination, of these transcription factors in mouse embryos, exactly how they function in a hierarchical regulatory network to coordinate both myogenesis and cardiogenesis is far from clear.

2. Pharyngeal muscle heterogeneity

Myogenic programmes that lead to the formation of skeletal muscles vary considerably between the body and head regions. There are ~60 distinct skeletal muscles in the vertebrate head which control food intake, facial expression, and eye movement; for example, the extraocular muscles (EOMs) move and rotate the eye in a highly coordinated manner; pharyngeal 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 located in the trunk (reviewed in reference 26). Importantly, while head mesoderm gives rise to all muscles of the head, the PM only contributes to subsets of these muscles. For example, EOMs derive from non-PM cells (e.g. prechordal mesoderm[26]) but it is not clear whether PM contributes in any way to EOMs.

Recent studies have begun to uncover an unexpected heterogeneity in head muscles with respect to their origins, genetic lineages, and transcriptional programmes, as well as their proliferative, differentiative, and regenerative properties.[24,27,28] Heterogeneity in skeletal muscles can also be seen during adulthood, as reflected in distinct genetic signatures, and susceptibilities to muscle myopathies of both head and trunk skeletal muscles.[29,30]

Lineage mapping of head musculature in the mouse revealed genetic heterogeneity.[24] This lineage map corroborates, at the cellular level, our understanding of craniofacial muscle phenotypes seen in several recent loss-of-function studies in mice. A complementary view of craniofacial muscle formation in mice, based on dissection of genetic programmes promoting myogenesis in distinct head muscles (e.g. EOMs vs. pharyngeal muscles) was demonstrated by the Tajbakhsh laboratory.[27] Pharyngeal muscle progenitors are regulated by a combination of Tbx1 and Myf5 acting upstream of MyoD, as *Tbx1:Myf5* doubled mutants lack these muscles completely, while EOMs in these mutants are spared. In contrast, EOMs are selectively lost in another genetic combination, the *Myf5:Mrf4* double mutant, although other head and trunk muscles are present in the same double mutant.[27] These observations, in addition to other mutant analyses (see below), suggest that the underlying mechanism that leads to selected myopathies may have an ontological aetiology.

Along theses same lines, Capsulin and MyoR act as upstream regulators of pharyngeal muscle development. In *Capsulin/MyoR* double mutants, the masseter, pterygoid, and temporalis muscles are missing, while lower jaw muscles (e.g. anterior digastric and mylohyoid), EOMs and tongue muscles are not affected.[31] Similarly, pharyngeal muscles in *Tbx1* mutants are frequently hypoplastic and asymmetric, whereas EOMs and tongue muscles are spared.[32] Genetic lineage analyses of head musculature[24] indicated that EOMs and tongue muscles derive from lineages distinct from those of pharyngeal muscles. In *Pitx2* mutants, the EOMs and first arch muscles are affected[22,33]; first arch myoblasts marked by the *Mef2c AHF-Cre* lineage in the mouse[16,23] were fewer in *Pitx2* mutant embryos.[22]

3. Isl1 lineage-derived PM cells contribute to a broad range of cardiovascular and skeletal muscle progenitors

The LIM-homeodomain protein Islet1 (Isl1) is expressed in and required for a broad subset of cardiac progenitors in the mouse.[34–37] Gene expression and lineage experiments in the chick have revealed that the core of the pharyngeal arch is divided along the proximal–distal axis such that paraxial mesoderm cells mainly contribute to the proximal region of the core, while the splanchnic mesoderm contributes to its distal region[3] (*Figure 2A–C*). Isl1 is expressed in the distal part of the PM, and its expression is correlated with delayed differentiation of lower jaw muscles.[3] Over-expression of Isl1 in the chick represses pharyngeal muscle differentiation.[24]

Lineage tracing experiments in the mouse using an *Isl1-Cre* line revealed a significant contribution of $Isl1^+$ cells to the mesodermal core of the pharyngeal arches,[3,24] as well as to the heart[38] (*Figure 2D*). $Isl1^+$ PM cells were shown to contribute to a subset of pharyngeal muscles, the mylohyoid, stylohyoid, and digastric, at the base of the mandible, facilitating its opening (*Figure 2E* and *F*). $Isl1^+$ cells were also found in second arch-derived muscles controlling facial expression[3,24] and, to a lesser extent, in the masseter, pterygoid and temporalis, the jaw closing muscles (*Figure 2E*, orange; *Figure 2F*, brown), indicating that this gene is not expressed in all cells of the PM. In both species, tongue muscles and EOM are not derived from the Isl1 lineage.[3,24] A similar lineage map of $Isl1^+$ PM cells was seen in adult pharyngeal muscles and their associated satellite cells.[24] These findings highlight the link between myogenesis in the early embryo, and the generation of adult muscle progenitor pools required for muscle maintenance and regeneration. Taken together, Isl1 marks a subset of PM cells, and plays an important role in the development of distinct PM-derived cardiovascular and skeletal muscle progenitors. The direct role of Isl1 in pharyngeal muscle development has yet to be resolved, as Isl1 knockout embryos die at around E10.[34]

4. Pharyngeal mesoderm evolution: from pharyngeal muscles to the heart

The architecture, function, and physiology of muscle cells have been remarkably conserved throughout evolution. Hence, all muscle cells likely evolved from an ancestral developmental programme involving a single contractile myogenic cell type.[39,40] The fact that the developmental programmes of the heart and pharyngeal muscles are tightly linked suggests that these tissues share common evolutionary origins[13,14] (*Figure 3*).

Nematodes such as the worm, *C. elegans*, are invertebrates that do not possess a heart or defined circulatory system. Instead, their pharyngeal muscle contracts like a heart, and exhibits electrical activity similar to that of mammalian cardiomyocytes (*Figure 3A*). Moreover, it has been shown that development of the pharyngeal muscle in nematodes is regulated by the homeobox gene *Nkx2.5* (ceh-22)[41] and may be functionally replaced by the zebrafish *nkx2.5* ([42], reviewed in references 13,14,43).

Tunicates belong to the *Chordata* phylum, and are considered as the 'sister group' of vertebrates.[44] The tunicate *Ciona intestinalis* is a sessile marine invertebrate. As in vertebrates, the *Ciona* heart is located ventrally and posterior to the pharynx, and anterior to the stomach; in the gastrulating embryo, its heart arises from a pair of blastomeres expressing the MesP gene. Several studies suggest significant similarities in the gene regulatory networks controlling cardiogenesis in vertebrates and tunicates.[44–46] The heart and pharyngeal muscle cells in *Ciona* (*Figure 3B*) are seemingly distinct, based on the expression of different myosin heavy chain isoforms[47]; yet both are derived from $MesP^+$ cells.

Strikingly, $Isl1^+$ PM cells in both *Ciona*[48] and vertebrates[3,24] give rise to pharyngeal muscles (termed siphon muscles in *Ciona*). These findings suggest that the last common ancestor of tunicates and vertebrates had PM cells derived from $MesP^+$ lineages that expressed Isl1, FoxF, and Nkx2.5, and had the potential to give rise to both

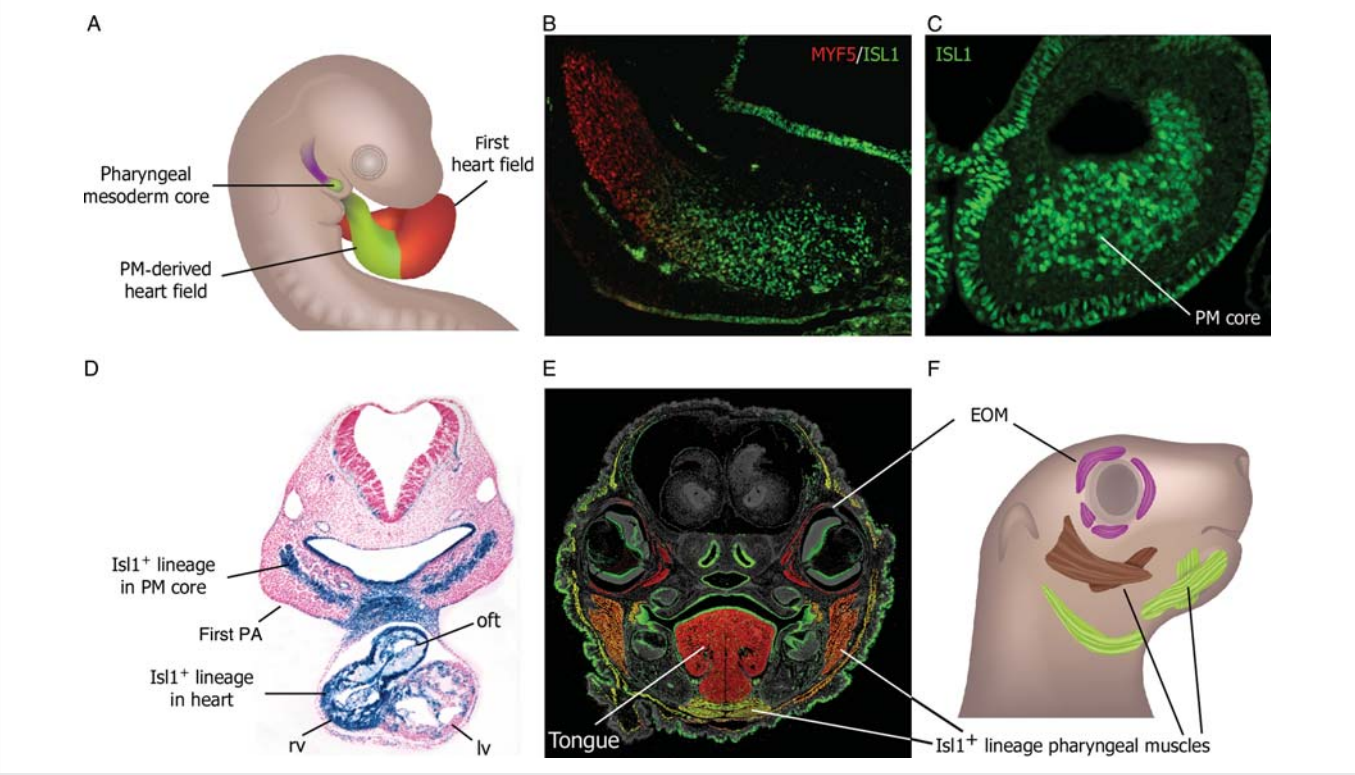

Figure 2 Isl1 marks pharyngeal mesoderm cells that populate the heart and the pharyngeal muscles. (*A–C*) Lineage studies in the chick have revealed the contribution of PM cells to the distal core of the pharyngeal arches and the heart. ISL1 protein expression is seen in this region (*B–C*), which is correlated with delayed differentiation of these myogenic progenitors ($MYF5^-$). (*D–F*) Similarly, lineage analyses in mouse *Isl1Cre; Rosa26LacZ* E10.5 embryos (*D*) demonstrate the significant contribution of these cells to the core of the first pharyngeal arch and the heart. The heterogenic contribution of the Isl1 lineage to the head musculature at E16.5 is shown (*E–F*): A section of the *Isl1Cre; Rosa26YFP* embryo stained for muscle (red) or GFP (green). $Isl1^+$ cells contribute strongly to lower jaw muscles (yellow), muscles of facial expression shown in the head periphery, and less to jaw closing muscles (orange). Extraocular muscles (EOMs) or tongue muscles are not derived from $Isl1^+$ cells. (*F*) A diagram of the embryonic head (lateral view) reveals the contribution of high (yellow) and intermediate levels (brown) of the Isl1 lineage to the head musculature.

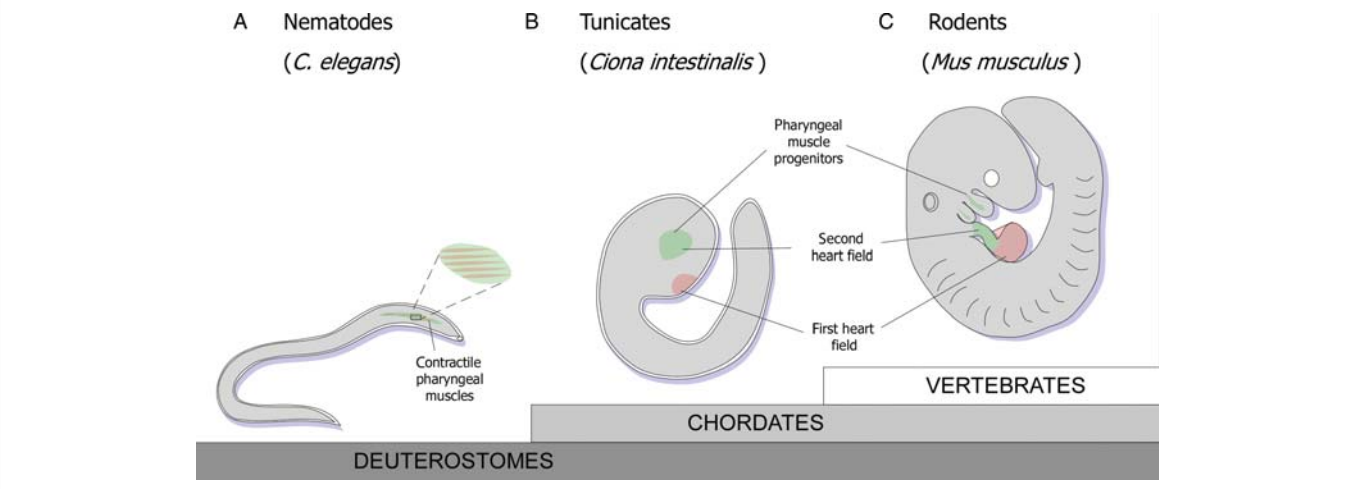

Figure 3 $Isl1^+$ PM cells evolved from an ancestral myogenic programme for both cardiac and skeletal muscle lineages. (*A*) Nematodes such as the worm *C. elegans* do not have a heart; instead, they have a contractile pharyngeal muscle that functions like the heart in vertebrates. (*B*) Tunicates (*Ciona intestinalis*) are chordates that are considered as a 'sister group' to the vertebrates. Unlike nematodes, the heart and pharyngeal muscle cells in tunicates are seemingly distinct. $Isl1^+$ cells in *Ciona* give rise to the pharyngeal muscles (termed siphon muscles), and not to the heart. (*C*) Reallocation of Isl1+ PM cells into the looping heart, which occurred during evolution from chordates to vertebrates, represents the emergence of the second heart field.

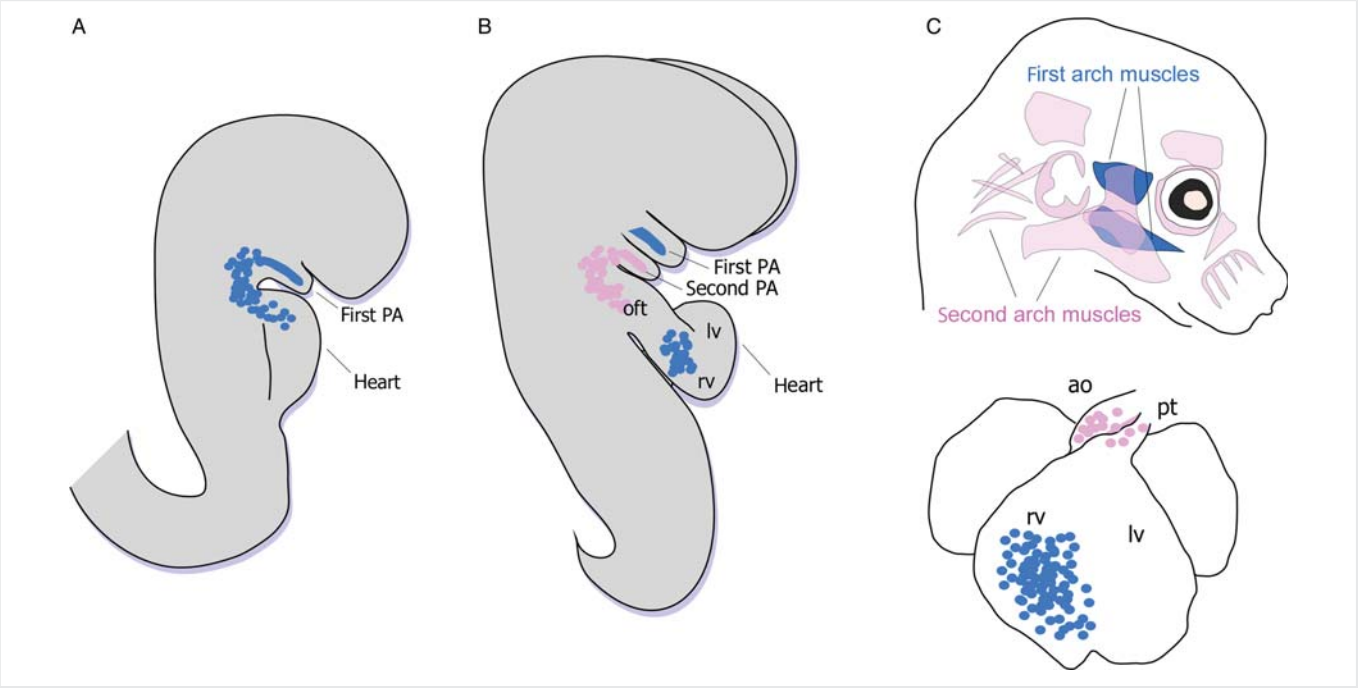

Figure 4 Clonal relationships of head and heart muscle progenitors in the mouse. Schematic representation of PM cells contributing to the core of the pharyngeal arches in the mouse embryo. (*A*) The linear heart tube at E8.0 is aligned at the level of the first pharyngeal arch; 1–2 days later, the heart undergoes looping, and shifts posteriorly. (*B*) First arch PM cells (blue) migrate to the developing heart tube to contribute to right ventricular myocardium. PM cells from the second arch (pink) migrate later (around E9.5–E10), to contribute to outflow tract myocardium (oft). (*C*) Diagram depicting the contribution of the different lineages to head muscles and heart myocardium: The first lineage (blue) contributes to masticatory muscles (temporalis and masseter) and to right ventricular (rv) myocardium. The second lineage (pink) contributes to facial expression muscles, and to myocardium at the base of the pulmonary trunk (pt) or aorta (ao). Modified after Lescroart F *et al.* Adapted with permission.[16]

heart tissue and pharyngeal muscles.[48] With the increasing complexity of the vertebrate heart and, in particular, during the heart tube elongation that occurs in vertebrates, Isl1^{+} PM cells were recruited into the looping heart to give rise to cardiomyocytes (*Figure 3C*). Hence, this study suggests that reallocation of PM cells into the looping heart represents the emergence of the second heart field in vertebrates. In addition, these findings suggest distinct evolutionary origins for the two heart fields.

5. Signalling mechanisms affecting pharyngeal mesoderm development

PM cells are maintained in a proliferative and undifferentiated state by multiple signalling mechanisms that lately have begun to be defined. Abnormal transition of proliferating PM-derived cardiac progenitors into differentiating cardiomyocytes severely affects cardiac looping and outflow tract extension, processes which are generally associated with congenital heart disease.[7,10] Many signalling pathways influence cardiac progenitor cell proliferation and differentiation (reviewed in references 8,10,49). For example, recent studies have shown that the Wnt/β-catenin pathway plays distinct roles at various stages of cardiac development by triggering the renewal and expansion of cardiac progenitors, and blocking their differentiation (reviewed in reference 50).

Bone morphogenic protein (BMP) and fibroblast growth factor (FGF) signals are initially required for cardiac specification and, later, for the differentiation of cardiac progenitors in chick and frog embryos. Loss-of-function studies in mice have underscored the essential roles of BMP and FGF signalling pathways in cardiogenesis (reviewed in references 8,49). BMP signalling affects PM cells in two phases: it is initially required to 'lock' these cells into the cardiogenic lineage (and block skeletal muscle markers);[12] while at later stages, BMP signalling promotes PM cardiac differentiation by blocking FGF signalling, to facilitate the accurate deployment of these progenitors to the looping heart.[51] Indeed, FGF signalling has been shown to play key roles in the survival and expansion of PM-derived cardiac progenitors,[52–55] where down-regulation of this pathway is both required and sufficient for the differentiation of PM-derived cardiomyocytes.[51] Hence, opposing activities of BMP and FGF signalling pathways constitute a major regulatory mechanism during cardiogenesis.[51,56] In addition, BMP–FGF crosstalk regulates the epicardial vs. myocardial lineage switch at the inflow pole of the heart.[57]

What lies downstream of BMP signalling in the transition from cardiac progenitors to cardiomyocytes? In the chick, the transcription factor Msx1, expressed primarily by neural crest cells, has been shown to function between BMP and FGF pathways to promote cardiomyocyte differentiation.[51] Likewise, double knockout of Msx1/2 in the mouse resulted in increased proliferation of second heart field cells.[58] How Msx genes expressed in neural crest cells promote myocardial differentiation of PM cells is currently not clear. A recent study revealed another means by which BMP signalling can promote PM differentiation: the Martin laboratory found that the PM progenitor

markers Isl1 and Tbx1 are repressed by BMP signalling, via the induction of microRNAs (miRNA 17–92 cluster).[59]

6. Regulation of pharyngeal mesoderm by other tissues

PM progenitors are exposed to signals from pharyngeal endoderm, ectoderm, and neural crest cells that, together, create a complex regulatory system (reviewed in references 8,49). Perturbation of the balance of signals within this system can lead to abnormal heart development. Neural crest ablation in the chick, for example, results in increased FGF signalling and elevated proliferation in PM.[60–62] These studies suggest that both cardiac neural crest (affecting caudal PM progenitors) and cranial neural crest cells (affecting cranial PM) (shown in *Figure 1D*), buffer proliferative signals (presumably FGFs) secreted from the endoderm and ectoderm to promote PM migration and differentiation.

Other examples for the roles of cardiac/cranial neural crest in the regulation of PM cells may be found in mice lacking either *Smad4,* a member of the Smad family of signal transduction proteins involved in BMP signalling[63] or the BMP receptor *ALK2*[64] in neural crest cells. Both of these mouse models resulted in abnormal differentiation of PM progenitors that led to severe cardiac outflow defects. Furthermore, these studies are consistent with a BMP-dependent signalling mechanism involving neural crest cells that regulate these progenitors. Similarly, interference with Notch signalling in neural crest, PM or endothelial cells, all result in arterial pole and arch artery defects (reviewed in references 8,65). Taken together, these studies demonstrate that multiple signals from various tissues are intertwined in an as-yet unclear manner, to control the development of PM cells.

It has long been suggested that neural crest—PM crosstalk is involved in patterning of the head musculature (reviewed in reference 66). Several recent studies suggest that pharyngeal muscle patterning and differentiation are tightly regulated by the interaction of PM-derived muscle progenitors with adjacent cranial neural crest cells that give rise to most of the vertebrate skeletal system, including bones, cartilage, and connective tissues in the face.[62,67–70] It is therefore believed that CNC-derived connective tissue progressively imposes the characteristic anatomical musculoskeletal architecture upon PM muscle progenitors.

7. Pharyngeal mesoderm cells are multipotent cardiac and skeletal muscle progenitors

While it is now well-established that PM progenitors contribute to both pharyngeal muscles and heart progenitors within the arterial pole of the heart,[3,4,12] the question remained whether these head and heart cells originated from single PM cells or from a predetermined, mixed population of PM cells. Using a retrospective clonal analysis (that they developed) in the mouse, the Buckingham laboratory demonstrated that head muscles and second heart field derivatives originate from multipotent PM progenitors.[16] Two myogenic lineages linking groups of head muscles to different parts of the heart were identified (*Figure 4A–C*): The first muscle lineage (blue) gives rise to the temporalis and masseter, two first pharyngeal arch muscles, as well as to the EOMs. Strikingly, this single-cell lineage also contributes to myocardial cells in the right ventricle (*Figure 4C*). The second lineage gives rise to a broad range of muscles controlling facial expression, which derive from mesoderm of the second pharyngeal arch, and also contributes myocardial cells to the arterial pole of the heart (*Figure 4B* and *C*). These findings highlight the dynamic posterior shift in the alignment of the cardiac outflow tract with the pharyngeal arches, and the spatiotemporal deployment of PM into heart and head muscles. Interestingly, *Mef2c-AHF-Cre*, activated in PM progenitors,[22,23] can distinguish between first- and second-pharyngeal arch myogenic lineages. Further sublineages distinguish myocardium at the base of the aorta or pulmonary trunk, with a clonal relationship to right or left head muscles, respectively.[16]

In conclusion, we have attempted to summarize novel insights into how pharyngeal muscles and certain parts of the heart arise from PM. In doing so, we emphasized key findings concerning the anatomical, cellular, and molecular characteristics of PM progenitors. We also discussed signalling mechanisms that regulate PM proliferation and differentiation, and the evolution of PM-derived cardiac and skeletal muscle progenitors. The developmental paths that lead to the formation of skeletal muscles in the head appear to be distinct from those operating in the trunk. Considerable cellular and genetic variations among the different craniofacial muscle groups are also seen. A deeper understanding of PM development is instrumental in deciphering the aetiology of heart and craniofacial birth defects.

Acknowledgements

We deeply thank Miriam Brodt-Ivenshitz and Itamar Harel from the Tzahor laboratory for the artwork. We thank Brad Davidson and Mike Levine for insightful discussions. E.T. is the incumbent of the Gertrude and Philip Nollman Career Development Chair.

Conflict of interest: none declared.

Funding

The Tzahor laboratory is supported by research grants from the Association Française Contre les Myopathies; the Israel Science Foundation; the United States-Israel Binational Science Foundation (BSF, with S.M.E); the Helen and Martin Kimmel Institute for Stem Cell Research; the German Israeli Foundation (GIF); and the Minerva Foundation. S.M.E. would like to acknowledge funding from NIH and BSF.

References

1. Psychoyos D, Stern CD. Fates and migratory routes of primitive streak cells in the chick embryo. *Development* 1996;**122**:1523–1534.
2. Kinder SJ, Tsang TE, Quinlan GA, Hadjantonakis AK, Nagy A, Tam PP. The orderly allocation of mesodermal cells to the extraembryonic structures and the anteroposterior axis during gastrulation of the mouse embryo. *Development* 1999;**126**:4691–4701.
3. Nathan E, Monovich A, Tirosh-Finkel L, Harrelson Z, Rousso T, Rinon A *et al*. The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development. *Development* 2008;**135**:647–657.
4. Kelly RG, Brown NA, Buckingham ME. The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. *Dev Cell* 2001;**1**:435–440.
5. Mjaatvedt CH, Nakaoka T, Moreno-Rodriguez R, Norris RA, Kern MJ, Eisenberg CA *et al*. The outflow tract of the heart is recruited from a novel heart-forming field. *Dev Biol* 2001;**238**:97–109.
6. Waldo KL, Kumiski DH, Wallis KT, Stadt HA, Hutson MR, Platt DH *et al*. Conotruncal myocardium arises from a secondary heart field. *Development* 2001;**128**:3179–3188.
7. Buckingham M, Meilhac S, Zaffran S. Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet* 2005;**6**:826–835.
8. Vincent SD, Buckingham ME. How to make a heart: the origin and regulation of cardiac progenitor cells. *Curr Top Dev Biol* 2010;**90**:1–41.
9. Dyer LA, Kirby ML. The role of secondary heart field in cardiac development. *Dev Biol* 2009;**336**:137–144.

10. Evans SM, Yelon D, Conlon FL, Kirby ML. Myocardial lineage development. *Circ Res* 2010;**107**:1428–1444.
11. Moorman AF, Christoffels VM, Anderson RH, van den Hoff MJ. The heart-forming fields: one or multiple? *Philos Trans R Soc Lond B Biol Sci* 2007;**362**:1257–1265.
12. Tirosh-Finkel L, Elhanany H, Rinon A, Tzahor E. Mesoderm progenitor cells of common origin contribute to the head musculature and the cardiac outflow tract. *Development* 2006;**133**:1943–1953.
13. Tzahor E. Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. *Dev Biol* 2009;**327**:273–279.
14. Grifone R, Kelly RG. Heartening news for head muscle development. *Trends Genet* 2007;**23**:365–369.
15. Kelly RG. Core issues in craniofacial myogenesis. *Exp Cell Res* 2010;**316**:3034–3041.
16. Lescroart F, Kelly RG, Le Garrec JF, Nicolas JF, Meilhac SM, Buckingham M. Clonal analysis reveals common lineage relationships between head muscles and second heart field derivatives in the mouse embryo. *Development* 2010;**137**:3269–3279.
17. Wachtler F, Jacob M. Origin and development of the cranial skeletal muscles. *Bibl Anat* 1986;**29**:24–46.
18. Black BL. Transcriptional pathways in second heart field development. *Semin Cell Dev Biol* 2007;**18**:67–76.
19. Tzahor E, Lassar AB. Wnt signals from the neural tube block ectopic cardiogenesis. *Genes Dev* 2001;**15**:255–260.
20. Rana MS, Horsten NC, Tesink-Taekema S, Lamers WH, Moorman AF, van den Hoff MJ. Trabeculated right ventricular free wall in the chicken heart forms by ventricularization of the myocardium initially forming the outflow tract. *Circ Res* 2007;**100**:1000–1007.
21. Bothe I, Dietrich S. The molecular setup of the avian head mesoderm and its implications for craniofacial myogenesis. *Dev Dyn* 2006;**235**:2845–2860.
22. Dong F, Sun X, Liu W, Ai D, Klysik E, Lu MF *et al*. Pitx2 promotes development of splanchnic mesoderm-derived branchiomeric muscle. *Development* 2006;**133**:4891–4899.
23. Verzi MP, McCulley DJ, De Val S, Dodou E, Black BL. The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain within the secondary/anterior heart field. *Dev Biol* 2005;**287**:134–145.
24. Harel I, Nathan E, Tirosh-Finkel L, Zigdon H, Guimaraes-Camboa N, Evans SM *et al*. Distinct origins and genetic programs of head muscle satellite cells. *Dev Cell* 2009;**16**:822–832.
25. Sambasivan R, Tajbakhsh S. Skeletal muscle stem cell birth and properties. *Semin Cell Dev Biol* 2007;**18**:870–882.
26. Noden DM, Francis-West P. The differentiation and morphogenesis of craniofacial muscles. *Dev Dyn* 2006;**235**:1194–1218.
27. Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly R *et al*. Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. *Dev Cell* 2009;**16**:810–821.
28. Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS. Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. *Dev Biol* 2010;**337**:29–41.
29. Porter JD, Israel S, Gong B, Merriam AP, Feuerman J, Khanna S *et al*. Distinctive morphological and gene/protein expression signatures during myogenesis in novel cell lines from extraocular and hindlimb muscle. *Physiol Genomics* 2006;**24**:264–275.
30. Emery AE. The muscular dystrophies. *Lancet* 2002;**359**:687–695.
31. Lu JR, Bassel-Duby R, Hawkins A, Chang P, Valdez R, Wu H *et al*. Control of facial muscle development by MyoR and capsulin. *Science* 2002;**298**:2378–2381.
32. Kelly RG, Jerome-Majewska LA, Papaioannou VE. The del22q11.2 candidate gene Tbx1 regulates branchiomeric myogenesis. *Hum Mol Genet* 2004;**13**:2829–2840.
33. Shih HP, Gross MK, Kioussi C. Cranial muscle defects of Pitx2 mutants result from specification defects in the first branchial arch. *Proc Natl Acad Sci USA* 2007;**104**:5907–5912.
34. Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J *et al*. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell* 2003;**5**:877–889.
35. Laugwitz KL, Moretti A, Caron L, Nakano A, Chien KR. Islet1 cardiovascular progenitors: a single source for heart lineages? *Development* 2008;**135**:193–205.
36. Lin L, Cui L, Zhou W, Dufort D, Zhang X, Cai CL *et al*. Beta-catenin directly regulates Islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. *Proc Natl Acad Sci U S A* 2007;**104**:9313–9318.
37. Sun Y, Liang X, Najafi N, Cass M, Lin L, Cai CL *et al*. Islet 1 is expressed in distinct cardiovascular lineages, including pacemaker and coronary vascular cells. *Dev Biol* 2007;**304**:286–296.
38. Moretti A, Caron L, Nakano A, Lam JT, Bernshausen A, Chen Y *et al*. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell* 2006;**127**:1151–1165.
39. Fukushige T, Brodigan TM, Schriefer LA, Waterston RH, Krause M. Defining the transcriptional redundancy of early bodywall muscle development in C. elegans: evidence for a unified theory of animal muscle development. *Genes Dev* 2006;**20**:3395–3406.
40. Baugh LR, Hunter CP. MyoD, modularity, and myogenesis: conservation of regulators and redundancy in *C. elegans*. *Genes Dev* 2006;**20**:3342–3346.
41. Harfe BD, Fire A. Muscle and nerve-specific regulation of a novel NK-2 class homeodomain factor in *Caenorhabditis elegans*. *Development* 1998;**125**:421–429.
42. Haun C, Alexander J, Stainier DY, Okkema PG. Rescue of *Caenorhabditis elegans* pharyngeal development by a vertebrate heart specification gene. *Proc Natl Acad Sci U S A* 1998;**95**:5072–5075.
43. Olson EN. Gene regulatory networks in the evolution and development of the heart. *Science* 2006;**313**:1922–1927.
44. Davidson B. *Ciona intestinalis* as a model for cardiac development. *Semin Cell Dev Biol* 2007;**18**:16–26.
45. Satou Y, Imai KS, Satoh N. The ascidian Mesp gene specifies heart precursor cells. *Development* 2004;**131**:2533–2541.
46. Davidson B, Shi W, Beh J, Christiaen L, Levine M. FGF signaling delineates the cardiac progenitor field in the simple chordate, *Ciona intestinalis*. *Genes Dev* 2006;**20**:2728–2738.
47. Ogasawara M, Sasaki A, Metoki H, Shin-i T, Kohara Y, Satoh N *et al*. Gene expression profiles in young adult *Ciona intestinalis*. *Dev Genes Evol* 2002;**212**:173–185.
48. Stolfi A, Gainous TB, Young JJ, Mori A, Levine M, Christiaen L. Early chordate origins of the vertebrate second heart field. *Science* 2010;**329**:565–568.
49. Rochais F, Mesbah K, Kelly RG. Signaling pathways controlling second heart field development. *Circ Res* 2009;**104**:933–942.
50. Tzahor E. Wnt/beta-catenin signaling and cardiogenesis: timing does matter. *Dev Cell* 2007;**13**:10–13.
51. Tirosh-Finkel L, Zeisel A, Brodt-Ivenshitz M, Shamai A, Yao Z, Seger R *et al*. BMP-mediated inhibition of FGF signaling promotes cardiomyocyte differentiation of anterior heart field progenitors. *Development* 2010;**137**:2989–3000.
52. Park EJ, Watanabe Y, Smyth G, Miyagawa-Tomita S, Meyers E, Klingensmith J *et al*. An FGF autocrine loop initiated in second heart field mesoderm regulates morphogenesis at the arterial pole of the heart. *Development* 2008;**135**:3599–3610.
53. Zhang J, Lin Y, Zhang Y, Lan Y, Lin C, Moon AM *et al*. Frs2alpha-deficiency in cardiac progenitors disrupts a subset of FGF signals required for outflow tract morphogenesis. *Development* 2008;**135**:3611–3622.
54. Ilagan R, Abu-Issa R, Brown D, Yang YP, Jiao K, Schwartz RJ *et al*. Fgf8 is required for anterior heart field development. *Development* 2006;**133**:2435–2445.
55. Watanabe Y, Miyagawa-Tomita S, Vincent SD, Kelly RG, Moon AM, Buckingham ME. Role of mesodermal FGF8 and FGF10 overlaps in the development of the arterial pole of the heart and pharyngeal arch arteries. *Circ Res* 2010;**106**:495–503.
56. Hutson MR, Zeng XL, Kim AJ, Antoon E, Harward S, Kirby ML. Arterial pole progenitors interpret opposing FGF/BMP signals to proliferate or differentiate. *Development* 2010;**137**:3001–3011.
57. van Wijk B, van den Berg G, Abu-Issa R, Barnett P, van der Velden S, Schmidt M *et al*. Epicardium and myocardium separate from a common precursor pool by crosstalk between bone morphogenetic protein- and fibroblast growth factor-signaling pathways. *Circ Res* 2009;**105**:431–441.
58. Chen YH, Ishii M, Sun J, Sucov HM, Maxson RE Jr. Msx1 and Msx2 regulate survival of secondary heart field precursors and post-migratory proliferation of cardiac neural crest in the outflow tract. *Dev Biol* 2007;**308**:421–437.
59. Wang J, Greene SB, Bonilla-Claudio M, Tao Y, Zhang J, Bai Y *et al*. Bmp signaling regulates myocardial differentiation from cardiac progenitors through a MicroRNA-mediated mechanism. *Dev Cell* 2010;**19**:903–912.
60. Waldo KL, Hutson MR, Stadt HA, Zdanowicz M, Zdanowicz J, Kirby ML. Cardiac neural crest is necessary for normal addition of the myocardium to the arterial pole from the secondary heart field. *Dev Biol* 2005;**281**:66–77.
61. Hutson MR, Zhang P, Stadt HA, Sato AK, Li YX, Burch J *et al*. Cardiac arterial pole alignment is sensitive to FGF8 signaling in the pharynx. *Dev Biol* 2006;**295**:486–497.
62. Rinon A, Lazar S, Marshall H, Buchmann-Moller S, Neufeld A, Elhanany-Tamir H *et al*. Cranial neural crest cells regulate head muscle patterning and differentiation during vertebrate embryogenesis. *Development* 2007;**134**:3065–3075.
63. Jia Q, McDill BW, Li SZ, Deng C, Chang CP, Chen F. Smad signaling in the neural crest regulates cardiac outflow tract remodeling through cell autonomous and non-cell autonomous effects. *Dev Biol* 2007;**311**:172–184.
64. Kaartinen V, Dudas M, Nagy A, Sridurongrit S, Lu MM, Epstein JA. Cardiac outflow tract defects in mice lacking ALK2 in neural crest cells. *Development* 2004;**131**:3481–3490.
65. High FA, Epstein JA. The multifaceted role of Notch in cardiac development and disease. *Nat Rev Genet* 2008;**9**:49–61.
66. Noden DM, Trainor PA. Relations and interactions between cranial mesoderm and neural crest populations. *J Anat* 2005;**207**:575–601.
67. Tzahor E, Kempf H, Mootoosamy RC, Poon AC, Abzhanov A, Tabin CJ *et al*. Antagonists of Wnt and BMP signaling promote the formation of vertebrate head muscle. *Genes Dev* 2003;**17**:3087–3099.
68. Grenier J, Teillet MA, Grifone R, Kelly RG, Duprez D. Relationship between neural crest cells and cranial mesoderm during head muscle development. *PLoS ONE* 2009;**4**:e4381.
69. Tokita M, Schneider RA. Developmental origins of species-specific muscle pattern. *Dev Biol* 2009;**331**:311–325.
70. Heude E, Bouhali K, Kurihara Y, Kurihara H, Couly G, Janvier P *et al*. Jaw muscularization requires Dlx expression by cranial neural crest cells. *Proc Natl Acad Sci U S A* 2010;**107**:11441–11446.