Characterization of the cardio-craniofacial mesoderm during vertebrate embryogenesis

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For the past few years, our lab has been focusing on the identification of candidate signaling molecules and tissue-specific transcription factors that regulate cardiac and skeletal muscle formation during early vertebrate embryogenesis. During that time, our studies have yielded important new insights into the processes underlying head muscle development.[1-5] In particular, our findings provided valuable support for the current theory that the development of the head musculature differs profoundly from that of trunk myogenesis.[2,4] We identified extrinsic signaling pathways that regulate, both positively and negatively, the patterning and differentiation of cranial paraxial mesoderm.[1-4] We also revealed that head muscles are developmentally linked to cardiac formation.[1, 3, 5]

Lineage plasticity of the cranial paraxial mesoderm

The developing heart is a specialized muscular vessel that serves as a pump for both the systemic and pulmonary circuitry (Figure 1, Panel A). This extremely complicated organ is highly sensitive to genetic perturbations, which are reflected in the numerous congenital heart defects that affect ~1% of all live births. The multiplicity of cardiac progenitor populations in various vertebrate species is an emerging area of intense focus in many laboratories, due to the enormous therapeutic potential of these avenues for treating heart disease. During early embryogenesis, heart and skeletal muscle progenitor cells are thought to derive from distinct regions of the mesoderm (i.e., lateral plate mesoderm and paraxial mesoderm, respectively). We have been employing both *in vitro* and *in vivo* experimental systems in the avian embryo to explore how mesoderm progenitors in the head differentiate into both heart and skeletal muscles. Utilizing fate mapping studies, gene expression analyses, and manipulations of signaling pathways in the chick embryo, we demonstrated that cells from the cranial paraxial mesoderm contribute to both myocardial and endocardial cell populations within the cardiac outflow tract. We further showed that bone morphogenic protein (BMP) signaling affects the specification of mesoderm cells in the head: application of BMP4 to chick embryos, both *in vitro* and *in vivo*, induces cardiac differentiation in the cranial paraxial mesoderm, and blocks the differentiation of skeletal muscle precursors in these cells. Our results demonstrate that cells within the cranial paraxial mesoderm play a vital role in cardiogenesis, as a new source of cardiac progenitors that populate the cardiac outflow tract *in vivo* (Figure 1B and Figure 3).[3]

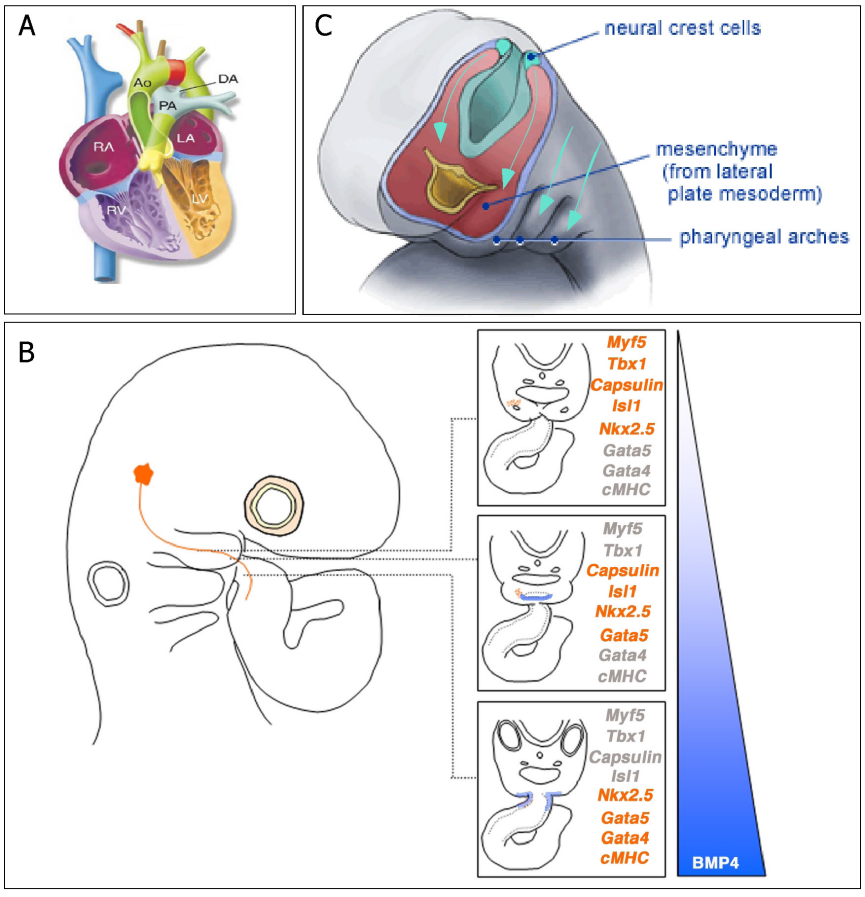

Fig. 1 *Cardio-craniofacial mesoderm specification*
A. An image of the adult four-chambered mammalian heart. B. Cranial paraxial mesoderm cells migrate through the branchial arches (pharyngeal arches), where they differentiate into the skeletal muscle lineage. We demonstrated that some of these mesodermal cells can migrate further, toward the aortic sac, which connects the branchial arches to the outflow tract. These cells eventually contribute to the myocardium and endocardium of the outflow tract. The gradual shift from a skeletal muscle to a cardiac cell fate is correlated with the spatiotemporal expression of BMP4. Ectopic application of BMP4 both in vitro and in vivo promoted cardiogenesis in the cranial paraxial mesoderm, and blocked the skeletal muscle differentiation program. C. The vertebrate head is an excellent developmental system for the study of both patterning and differentiation programs. During craniofacial development, progenitor cells derived from the cranial paraxial mesoderm fuse together to form a myofiber, which is attached to a specific skeletal element derived from the cranial neural crest in a highly coordinated manner.

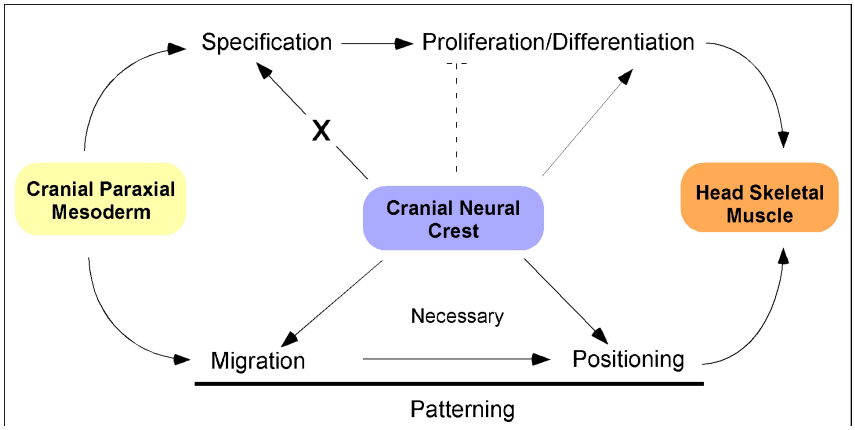

Fig. 2 *A model for the regulation of skeletal muscle formation by cranial neural crest cells Our studies of craniofacial muscle development in mouse and chick embryonic models have clarified the extent to which the myogenic program is intrinsic, or controlled by extrinsic environmental signals. We provide direct evidence that cranial neural crest cells play diverse and critical roles during skeletal muscle formation in vertebrates.*

Craniofacial muscle patterning

Craniofacial development requires the orchestrated integration of multiple interactions among progenitor cells derived from both the cranial paraxial mesoderm and the cranial neural crest (CNC; Figure 1C). Although it has long been suggested that the CNC plays an indirect role in the formation of the head musculature, the precise molecular underpinnings of this exquisitely tuned process, and the significance of the CNC's contribution to it, are far less clear. In a recent study, we analyzed head skeletal muscle patterning and differentiation *in vivo*, in three mouse models involving genetic perturbations of CNC development, as well as in CNC-ablated chick embryos. Our results demonstrated that although early specification of the skeletal muscle lineage is CNC-independent, CNC cells play an important role at later developmental stages, regulating the expression patterns of myogenic genes, the migration and axial registration of the mesoderm cells, and the subsequent differentiation of myoblasts in the branchial arches. Our findings support a model in which CNC cells control craniofacial development and patterning by regulating positional interactions with mesoderm-derived muscle progenitors that together shape the cranial musculoskeletal architecture during vertebrate embryogenesis (Figure 2).[4]

The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development

Heart development takes place in close apposition to the developing head. The term "cardio-craniofacial morphogenetic field" reflects the intimate developmental relationship between the head, face, and heart, which is also reflected in numerous cardiac and craniofacial birth defects (Hutson and Kirby, 2003). Nathan et al[5] have characterized the nature of the cardio-craniofacial mesoderm in both chick and mouse embryos, using several lineage tracing and gene expression techniques. We first performed cell lineage and molecular analyses of the splanchnic mesoderm (SpM), which contributes to the anterior or secondary heart field (AHF/SHF) and lies adjacent to the cranial paraxial mesoderm (CPM), in order to delineate the boundaries between the CPM, undifferentiated SpM progenitors of the AHF/SHF, and differentiating cardiac cells (Figure 3). We next revealed the regionalization of branchial arch mesoderm: CPM cells contribute to the proximal region of the myogenic core, and the adjacent SpM cells contribute to the distal region of the core (Figure 3).

We further demonstrated in the chick that proximal CPM cells (Myf5^{+}) contribute to the mandibular adductor complex, while Isl1-expressing myoblasts located distally in the first branchial arch, contribute to the

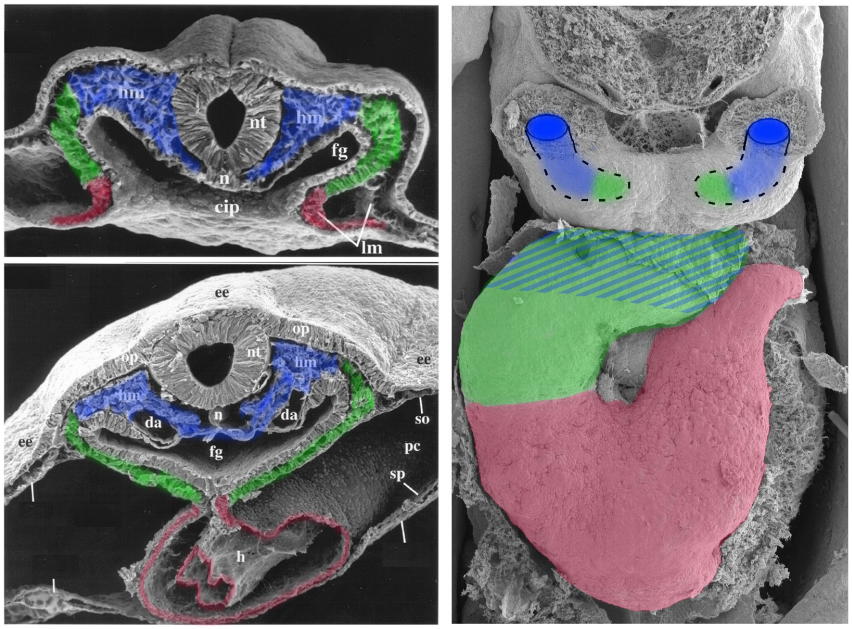

Fig. 3 *A model for the contribution of the cranial paraxial mesoderm and splanchnic mesoderm to both heart and skeletal muscle progenitors in the branchial arches. Scanning electron micrograph images of 2-3 day-old chick embryos are shown. Colors represent the distinct regionalization of the cardio-craniofacial mesoderm: CPM (blue); undifferentiated SpM (green); and differentiated SpM (pink).*

intermandibular muscle. Using *Isl1-Cre* mice, we revealed that, in a similar manner, Isl1^{+} cells contribute to the pharyngeal mesoderm (E10.5) and to a subset of branchiomeric muscles, but not to the extraocular or tongue muscles. In addition, we manipulated the Wnt/β-catenin signaling pathway in the chick, and provided evidence that it is capable of regulating the specification, differentiation and morphogenesis of cells derived from the Isl1/Nkx2.5/SpM field.[5]

Deciphering the embryonic origin(s) of satellite cells in the head musculature

Head muscles are generally classified anatomically; for example, the six extraocular muscles (EOM) move and rotate the eye in a highly coordinated manner; branchiomeric muscles control jaw movement, 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. Our recent lineage studies in both chick and mouse models provide clear evidence of the significant heterogeneity in this unique group of muscles (illustrated in Figure 4).

Numerous studies have provided a conceptual framework for understanding the relative contributions of cells of distinct mesodermal origins to cardiac and skeletal muscle tissues. None, however, have attempted to determine the nature of these putative progenitor cells by successively isolating them and characterizing their lineage relationships. Several recent studies have established that satellite cells attached to trunk muscles derive developmentally from the somites. At present, however, molecular and cellular investigations of skeletal muscle progenitors in the head are lacking. In particular, little is known about the embryonic origin(s) of satellite cells within the head musculature. In order to identify the origin(s) of craniofacial satellite cells in discrete head muscle subgroups, we applied various lineage-tracing strategies in both avian and mouse models. In the avian system, CPM cells were labeled using replication-defective viruses or quail-chick transplantations, and the contribution of these cells to the satellite cell population in the head was then assessed. In the mouse, the Cre-Lox genetic system was employed to perform long-term lineage tracing using Pax3-Cre (trunk skeletal muscle and neural crest), MesP1-Cre (cardio-craniofacial mesoderm) and Isl1-Cre (anterior/secondary heart field) mouse lines crossed with Z/EG or R26R reporters.

Taken together, our studies provide original insights relevant to an understanding of head muscle developmental programs, and may enhance our ability to effectively work toward the cure of cranial-specific muscle myopathies. Knowledge of the processes underlying the development of the cranial mesoderm, and the lineage relationships linking cardiovascular and skeletal muscle progenitor populations, will provide valuable insights that will bring us closer to our goal of programming stem cells towards a myogenic fate, to treat muscle disorders.

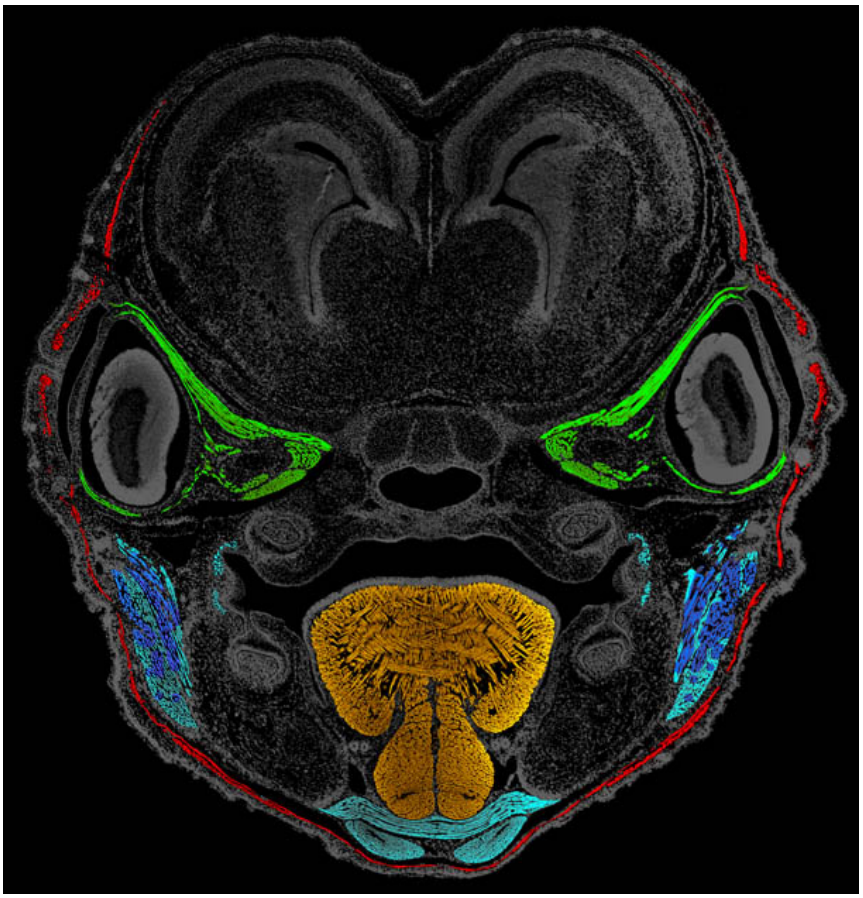

Fig. 4 *A model for the heterogeneity of the head musculature. Muscle staining of the frontal section of an E16.5 mouse embryo is shown. Based on our studies and those of others, we divided the cranial muscles into four subgroups: EOM, green; Tongue, orange; Isl1+ SpM-derived lower jaw, turquoise; Mastication, blue. We showed that Isl1+ cells partially contribute to the mastication muscles.*

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Nathan E, Monovich A, Tirosh-Finkel L, Harrelson Z, Rousso T, Rinon A, Evans S, Tzahor E (2008) The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development. *Development* 135: 647-657

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