



Published in final edited form as:

Science. 2010 July 30; 329(5991): 565–568. doi:10.1126/science.1190181.

Early Chordate Origins of the Vertebrate Second Heart Field

Alberto Stolfi¹, T. Blair Gainous¹, John J. Young¹, Alessandro Mori^{1,*}, Michael Levine^{1,†}, and Lionel Christiaen^{1,2,†}

¹Center for Integrative Genomics, Division of Genetics, Genomics and Development, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

²Center for Developmental Genetics, Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003, USA

Abstract

The vertebrate heart is formed from diverse embryonic territories, including the first and second heart fields. The second heart field (SHF) gives rise to the right ventricle and outflow tract, yet its evolutionary origins are unclear. We found that heart progenitor cells of the simple chordate *Ciona intestinalis* also generate precursors of the atrial siphon muscles (ASMs). These precursors express *Islet* and *Tbx1/10*, evocative of the splanchnic mesoderm that produces the lower jaw muscles and SHF of vertebrates. Evidence is presented that the transcription factor COE is a critical determinant of ASM fate. We propose that the last common ancestor of tunicates and vertebrates possessed multipotent cardiopharyngeal muscle precursors, and that their reallocation might have contributed to the emergence of the SHF.

The vertebrate heart initially forms as a tube from a population of precursor cells termed the first heart field (FHF). Cells from the adjacent second heart field (SHF) are then progressively added to the developing heart (1, 2). In avian and mammalian hearts, the FHF contributes mainly to the left ventricle, whereas the SHF gives rise to the outflow tract and large portions of the right ventricle and atria. Both fields arise from common mesodermal progenitors, although the detailed lineage relationships between FHF and SHF remain uncertain (1, 3). SHF-like territories have been identified in frog (4, 5), zebrafish (6), and lamprey (7), yet evidence for a deeper evolutionary origin remains obscured by the absence of a clear SHF in invertebrates (8).

Tunicates are the sister group to the vertebrates (9). Studies on a model tunicate, the ascidian *Ciona intestinalis*, have revealed conserved regulatory mechanisms underlying chordate heart development (10). The *Ciona* heart arises from a pair of blastomeres (named the B7.5 cells, by Conklin's nomenclature) in gastrulating embryos (11). Localized expression of

[†]To whom correspondence should be addressed. mlevine@berkeley.edu (M.L.); lc121@nyu.edu (L.C.).

*Present address: Forest Laboratories Inc., 909 Third Avenue, New York, NY 10022, USA.

Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5991/565/DC1

Materials and Methods

Figs. S1 to S11

Movies S1 to S4

MesP in B7.5 cells determines their competence to form the heart (Fig. 1A) (12, 13). Subsequently, fibroblast growth factor signaling induces expression of *FoxF* and the heart determinants *NK4* (*tinman/Nkx2.5*), *GATAa*, and *Hand-like/NoTrlc* in the anterior B7.5 granddaughter cells [the trunk ventral cells (TVCs)] (Fig. 1B) (13, 14). *FoxF* activates downstream target genes that control the migration of the TVCs to the ventral trunk region (Fig. 1C) (14, 15).

After metamorphosis, descendants of the B7.5 lineage give rise to the heart (Fig. 1D). B7.5 descendants also generate the atrial siphon muscles (ASMs) that surround the excurrent openings of the peribranchial atrium (Fig. 1D), as well as longitudinal muscles (LoMs) arising from the ASMs during metamorphosis (fig. S7). The contribution of the B7.5 lineage to ASMs is consistent with conventional lineage tracing performed in the distantly related ascidian *Halocynthia roretzi* (16). Cardiomyocytes and ASMs are distinguished by expression of the myosin heavy chain (MHC) genes *MHC2* and *MHC3* (17), respectively (fig. S3). Thus, they constitute distinct muscle types arising from common progenitor cells.

Live imaging of the B7.5 lineage cells allowed the characterization of events leading to the separation of heart and ASMs (Fig. 1E). After their migration to the ventral trunk region, each TVC undergoes two successive asymmetric divisions along the mediolateral axis to produce six cells on either side of the ventral midline (Fig. 1, E and F, time points 0 to 2; movie S1). The larger daughter cells (lateral TVCs) are positioned lateral to the smaller medial TVCs. Subsequent symmetric cell divisions result in an array of ~24 cells: eight lateral TVCs (four on either side) bracketing 16 medial TVCs (Fig. 1, E and F, time points 3 to 5; movie S2) (18).

A second migration occurs several hours after hatching. Each group of lateral TVCs detaches from the medial TVCs and migrates dorsally as a polarized cluster of cells on either side of the trunk. They eventually form a ring of cells underneath the atrial siphon primordia (Fig. 1, G and H, and movie S3). Targeted inhibition of TVC specification blocked the formation of ASMs (fig. S4). These observations demonstrate that the lateral TVCs correspond to the precursors of the ASMs.

The ASMs are evocative of vertebrate jaw muscles arising from lateral/splanchnic mesoderm (SpM): *Ciona* TVCs and vertebrate anterior SpM both express orthologs of *Nkx2.5* and *FoxF* and derive from progenitors that expressed *MesP* during gastrulation (5, 19, 20). In chick and mouse embryos, much of the anterior SpM gives rise to the SHF, but some precursors migrate into the first branchial arch and form intermandibular muscles (21, 22). A key marker of the anterior SpM and SHF is the LIM-homeodomain transcription factor *Islet1* (*Isl1*) (4–6, 21). The single *Ciona Islet* (23) gene is expressed in several tissues including the ASM precursors, which maintain *Islet* expression during their migration away from the medial TVCs (fig. S1). The latter possibly show weak and transient *Islet* expression, which is reminiscent of that reported in the FHF of vertebrates (19).

Islet expression was further characterized using defined enhancers (fig. S2). Reporter transgenes containing ~3.2 kb of the *Islet 5'* flanking region exhibited localized expression in the lateral TVCs, and in ASMs in juveniles (Fig. 2, A to C). In contrast, *MesP* reporter

transgenes labeled the entire B7.5 lineage, including both ASMs and heart (e.g., Fig. 1, C and D, and fig. S5). The heart primordium is situated ventrally and medially to the *Islet*⁺ ASM progenitors (Fig. 2A). This is reminiscent of the positioning of the FHF relative to *Isl1*⁺ SHF/pharyngeal mesoderm in basal vertebrates (4, 5, 7). Furthermore, LoM precursors segregating from the ASMs express the *Ciona* ortholog of *Tbx1* (24), an important regulator of SHF and pharyngeal mesoderm development in vertebrates (25) (Fig. 2, D to F, and fig. S7). Taken together, these results suggest homology between the ASM/LoM precursors of tunicates and the progenitors of lower jaw muscles and SHF of vertebrates.

Preliminary functional assays suggest that *Islet* is not instructive for the specification of ASMs (fig. S10). In the course of these studies, we found that the transcription factor *Collier/Olf1/EBF* (*COE*) is expressed early in the ASM precursors (Fig. 2G and fig. S11). To determine whether this localized expression is instructive for ASM specification, we used the *FoxFTVC* enhancer to misexpress *COE* in all TVCs (13). In 96% of transfected embryos, all TVC descendants migrated toward the atrial siphon placodes and expressed *Islet>GFP* (Fig. 3, A and B, fig. S6, and movie S4). More than half (56%) of the transfected embryos grew into juveniles that lacked a heart but still had ASMs (Fig. 3, D, E, and G to J, and fig. S6), which suggests that *COE* is sufficient to specify a lateral TVC identity and subsequent ASM fate.

Similar misexpression of a repressor form of *COE* (*COE: :WRPW*) resulted in the reciprocal phenotype: All TVC descendants remained in the ventral trunk, and *Islet>GFP* expression was abolished (Fig. 3, A and C, and fig. S6). Upon metamorphosis, TVC descendants differentiated into enlarged hearts (Fig. 3, D, F to H, K, and L, and fig. S8). Inhibition of *COE* function thus transforms the entire TVC lineage into heart tissue, indicating that *COE* activity is required for ASM specification.

The *COE* homolog *Collier/Knot* is involved in muscle type specification in *Drosophila* (26), but a role for *COE* in vertebrate SHF or jaw muscle development has not been reported. As a first step toward determining whether *COE* factors might play a conserved role in vertebrates, we performed in situ hybridization of the *COE* orthologs *Xebf2* and *Xebf3* in *Xenopus tropicalis* embryos (Fig. 4, A to C, and fig. S9). *Xebf2* expression was seen in *Nkx2.5*⁺/*Isl1*⁺ anterior lateral mesoderm, where *Tbx1* is also expressed (5).

The preceding results suggest that the last common ancestor of tunicates and vertebrates had a population of cardiopharyngeal mesoderm, which (i) arose from *MesP*-expressing early mesoderm, (ii) expressed orthologs of *FoxF* and *Nkx2.5*, and (iii) had the potential to give rise to both heart tissue and pharyngeal muscles, which (iv) correlated with differential maintenance of *Islet* expression. Moreover, *COE* might be a conserved determinant of chordate pharyngeal mesoderm development.

We propose that the reallocation of *Islet*⁺ cardiopharyngeal progenitors among the heart and cranial myogenic fields supported the emergence of the SHF (Fig. 4, D and E). In *Ciona*, *Islet*⁺ cells do not contribute to the heart, but targeted expression of *COE: :WRPW* was sufficient to convert them into cardiomyocytes. It is conceivable that the reallocation of

Islet⁺ pharyngeal muscle progenitors toward SHF depended on the intercalation of cardiac regulatory network components (e.g., *GATA4*, *Mef2c*) downstream of *Islet* (27).

An ancient connection between heart and craniofacial muscles has been proposed on the basis of the role of an *Nkx2.5* ortholog in the pharynx of the nematode *Caenorhabditis elegans*, which lacks a heart (28). *Haikouella lanceolata*, a fossil chordate from the Lower Cambrian, shows a putative heart adjacent to a muscularized pharyngeal atrium (29). The shared lineage of cardiomyocytes and pharyngeal muscles in tunicates hints that a pool of common precursors could have formed both heart and pharyngeal atrium muscles in a *Haikouella*-like ancestor. Northcutt and Gans (30) proposed that muscular ventilation of the pharyngeal arches was a key transition in the evolution of the vertebrates. Therefore, the cardiopharyngeal mesoderm could have been instrumental in the coevolution of circulatory, respiratory, and feeding functions in tunicates and vertebrates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank D. Hendrix for computational analysis, A. Cooc for technical assistance, and R. Harland, I. Philipp, and T. J. Park for advice. Supported by NSF grant IOB 0445470 (M.L.), NIH training grant 0745322 (T.B.G.), and a New York University Faculty of Arts and Science start-up package (L.C.).

References and Notes

1. Buckingham M, Meilhac S, Zaffran S. Nat Rev Genet. 2005; 6:826. [PubMed: 16304598]
2. Abu-Issa R, Kirby ML. Annu Rev Cell Dev Biol. 2007; 23:45. [PubMed: 17456019]
3. Meilhac SM, Esner M, Kelly RG, Nicolas JF, Buckingham ME. Dev Cell. 2004; 6:685. [PubMed: 15130493]
4. Brade T, Gessert S, Kühl M, Pandur P. Dev Biol. 2007; 311:297. [PubMed: 17900553]
5. Gessert S, Kuhl M. Dev Biol. 2009; 334:395. [PubMed: 19660447]
6. de Pater E, et al. Development. 2009; 136:1633. [PubMed: 19395641]
7. Kokubo N, et al. Evol Dev. 2010; 12:34. [PubMed: 20156281]
8. Perez-Pomares JM, Gonzalez-Rosa JM, Munoz-Chapuli R. Int J Dev Biol. 2009; 53:1427. [PubMed: 19247975]
9. Delsuc F, Brinkmann H, Chourrout D, Philippe H. Nature. 2006; 439:965. [PubMed: 16495997]
10. Davidson B. Semin Cell Dev Biol. 2007; 18:16. [PubMed: 17223594]
11. Satou Y, Imai KS, Satoh N. Development. 2004; 131:2533. [PubMed: 15115756]
12. Christiaen L, Stolfi A, Davidson B, Levine M. Dev Biol. 2009; 328:552. [PubMed: 19389354]
13. Davidson B, Shi W, Beh J, Christiaen L, Levine M. Genes Dev. 2006; 20:2728. [PubMed: 17015434]
14. Beh J, Shi W, Levine M, Davidson B, Christiaen L. Development. 2007; 134:3297. [PubMed: 17720694]
15. Christiaen L, et al. Science. 2008; 320:1349. [PubMed: 18535245]
16. Hirano T, Nishida H. Dev Biol. 1997; 192:199. [PubMed: 9441662]
17. Ogasawara M, et al. Dev Genes Evol. 2002; 212:173. [PubMed: 12012232]
18. Davidson B, Shi W, Levine M. Development. 2005; 132:4811. [PubMed: 16207759]
19. Prall OW, et al. Cell. 2007; 128:947. [PubMed: 17350578]
20. Kang J, Nathan E, Xu SM, Tzahor E, Black BL. Dev Biol. 2009; 334:513. [PubMed: 19580802]

21. Nathan E, et al. *Development*. 2008; 135:647. [PubMed: 18184728]
22. Tzahor E. *Dev Biol*. 2009; 327:273. [PubMed: 19162003]
23. Giuliano P, Marino R, Pinto MR, De Santis R. *Mech Dev*. 1998; 78:199. [PubMed: 9858732]
24. Takatori N, et al. *Dev Dyn*. 2004; 230:743. [PubMed: 15254908]
25. Zhang Z, Huynh T, Baldini A. *Development*. 2006; 133:3587. [PubMed: 16914493]
26. Crozatier M, Vincent A. *Development*. 1999; 126:1495. [PubMed: 10068642]
27. Olson EN. *Science*. 2006; 313:1922. [PubMed: 17008524]
28. Okkema PG, Ha E, Haun C, Chen W, Fire A. *Development*. 1997; 124:3965. [PubMed: 9374394]
29. Chen JY, Huang DY, Li CW. *Nature*. 1999; 402:518.
30. Gans C, Northcutt RG. *Science*. 1983; 220:268. [PubMed: 17732898]

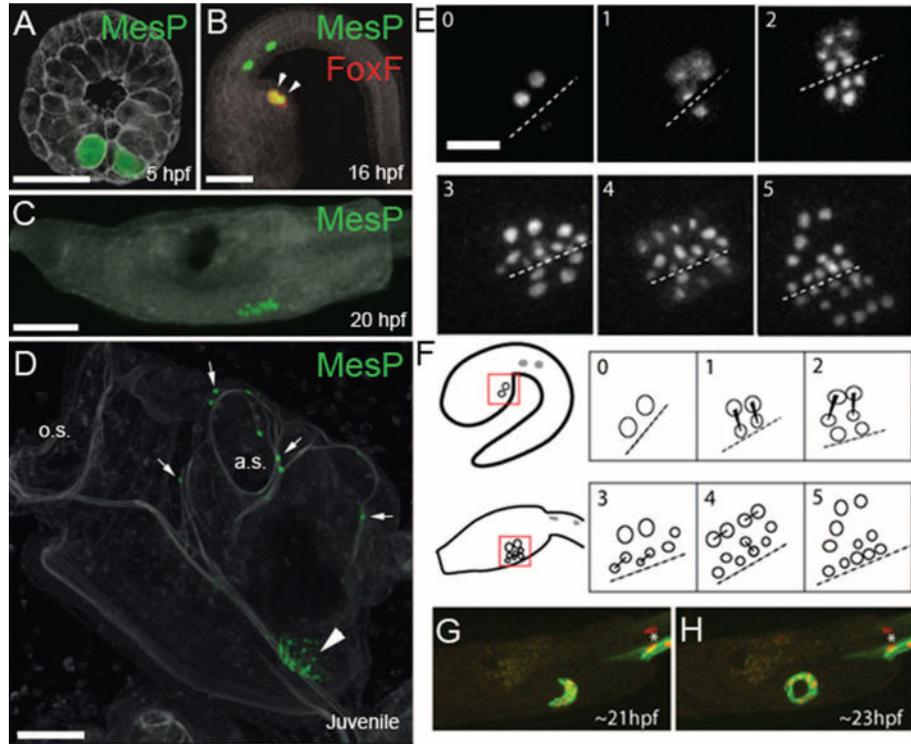


Fig. 1. Contribution of trunk ventral cells (TVCs) to heart and atrial siphon muscles (ASMs). (A) Immunodetection of β -gal expression (green) in B7.5 cells in a gastrulating embryo transfected with *MesP>lacZ* transgene. (B) Visualization of *MesP>lacZ* (green) and *FoxF[TVCh enhancer]>mCherry* (red) expression in B7.5 descendants in a tailbud-stage embryo. (C) Expression of a *MesP>Histone2B(H2B):GFP* fusion protein (green) in a tadpole. (D) Visualization of *MesP>H2B:CFP* (green) in a stage 38 juvenile [\sim 100 hours post-fertilization (hpf)]. Expression is visible in the heart (arrowhead), ASMs, and longitudinal muscles (LoMs) (arrows). *MesP* is activated only in the B7.5 pair of cells at the gastrula stage. a.s., atrial siphon; o.s., oral siphon. (E) Frames from movie S1 (time points 0 to 2) and S2 (time points 3 to 5). Dashed line indicates ventral midline. Right-side cells are partially visible at time points 1 and 2. Stereotyped cell divisions (see text) result in four lateral TVCs on either side of the embryo flanking \sim 16 medial TVCs. The four lateral TVCs on either side detach and migrate to form ASMs. Medial cells form the heart. Cells were visualized as two independent time-lapse sequences of embryos transfected with *MesP>H2B:GFP/CFP*. (F) Cartoon representing the events in (E). (G and H) Frames from movie S3 showing left-side ASM precursors expressing *MesP>H2B:mCherry* (red) and *MesP>PH:GFP* (green). ASM precursors encircle the siphon primordium, between 21 hpf (G) and 23 hpf (H). Scale bars, 50 μ m [(A) to (D)], 20 μ m (E). Asterisks identify anterior tail muscles (ATMs).

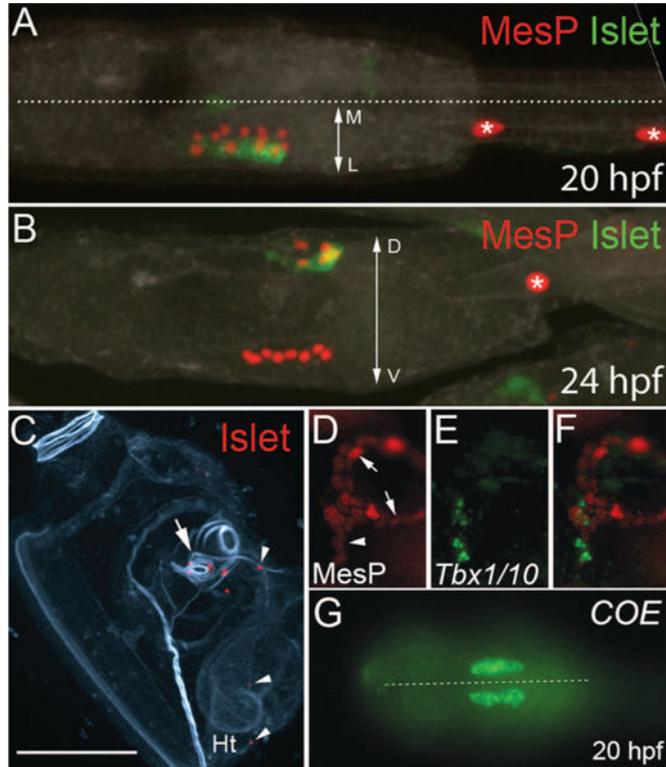


Fig. 2. ASM-specific gene expression. **(A)** Dorsal view of electroporated larva exhibiting mosaic incorporation (left side) of *Islet>GFP* (green) and *MesP>H2B::mCherry* (red) transgenes at 20 hpf, before the migration of the lateral TVCs. *Islet>GFP* expression is restricted to lateral TVCs. Dotted line indicates midline; M and L indicate medial and lateral, respectively. **(B)** Lateral view of larva expressing same transgenes as in **(A)** at 24 hours, after migration of *Islet>GFP*-positive lateral TVC descendants around the atrial siphon primordium. D, dorsal; V, ventral. **(C)** Juvenile (~100 hpf) raised from embryo transfected with *Islet>H2B::mCherry* (red), with transgene expression visible around atrial siphons (arrow) and longitudinal muscles (arrowheads), but not heart (Ht). F-actin is stained by phalloidin (blue-green). Scale bar, 100 μ m. **(D)** Magnified view (see fig. S7) of LoMs (arrowhead) segregating from ASMs (arrows) during metamorphosis, visualized by *MesP>lacZ* expression (red). Panel width is ~100 μ m. **(E)** In situ hybridization of *Tbx1/10* (green). **(F)** Merged view of **(D)** and **(E)**, showing activation of *Tbx1/10* in LoMs. **(G)** COE expression in lateral TVCs at 20 hpf revealed by in situ hybridization. Dotted line indicates ventral midline.

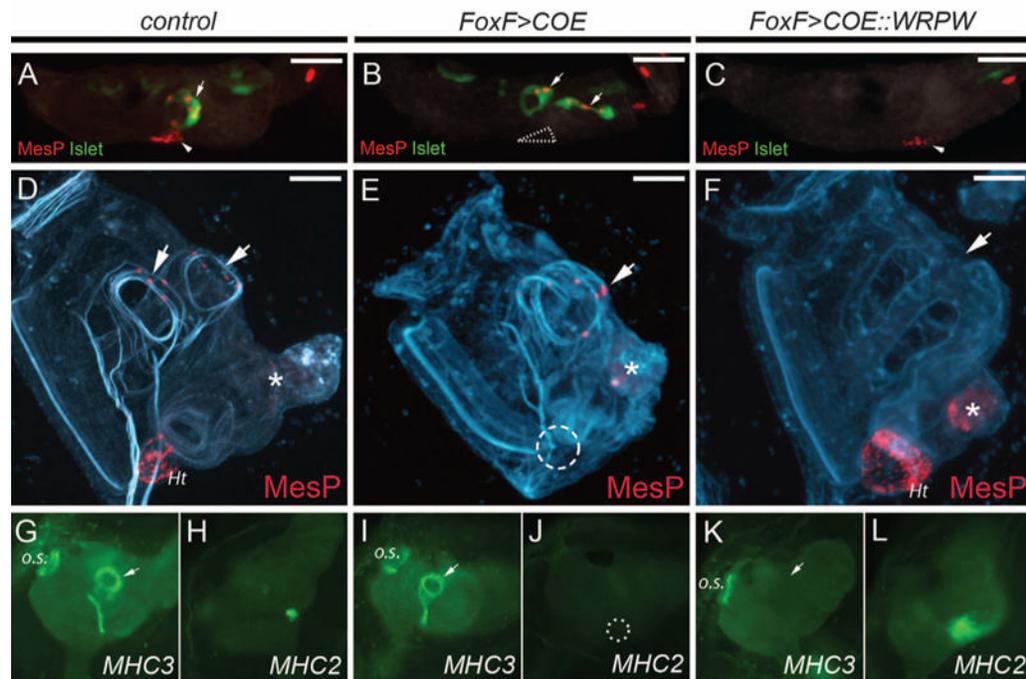


Fig. 3.

COE controls ASM specification. (A to C) Larvae cotransfected with *MesP>H2B::mCherry* (red), *Islet>GFP* (green), and (A) *FoxF>lacZ*, (B) *FoxF>COE*, or (C) *FoxF>COE::WRPW*. (B) All TVCs are transformed into ASM precursors (arrows). No heart primordium is formed (dashed triangle). (C) All TVC descendants form a heart (arrowhead) with no *Islet>GFP* expression. (D) Juveniles raised from embryos transfected with *MesP>H2B::mCherry* (red), counterstained with phalloidin (blue-green). *H2B::mCherry*⁺ B7.5 descendants populate the heart (Ht) and ASMs (arrows) (residual larval muscle staining indicated by asterisk). (E) Cotransfection with the *FoxF>COE* transgene, resulting in no heart (usual location indicated by dashed circle) but normal ASMs (arrow). (F) Coexpression of the *FoxF>COE::WRPW* transgene. TVCs form an expanded heart and there are no ASMs (arrow). (G and H) In situ hybridization on wild-type juveniles, showing *MHC3* and *MHC2* expression in ASMs (arrow) and heart, respectively. (I and J) *FoxF>COE* results in loss of *MHC2* expression (dashed circle), but not *MHC3* expression (arrow). (K and L) *FoxF>COE::WRPW* abolishes *MHC3* expression (arrow) and leads to expanded *MHC2* expression. Scale bars, 50 μ m; o.s., oral siphons.

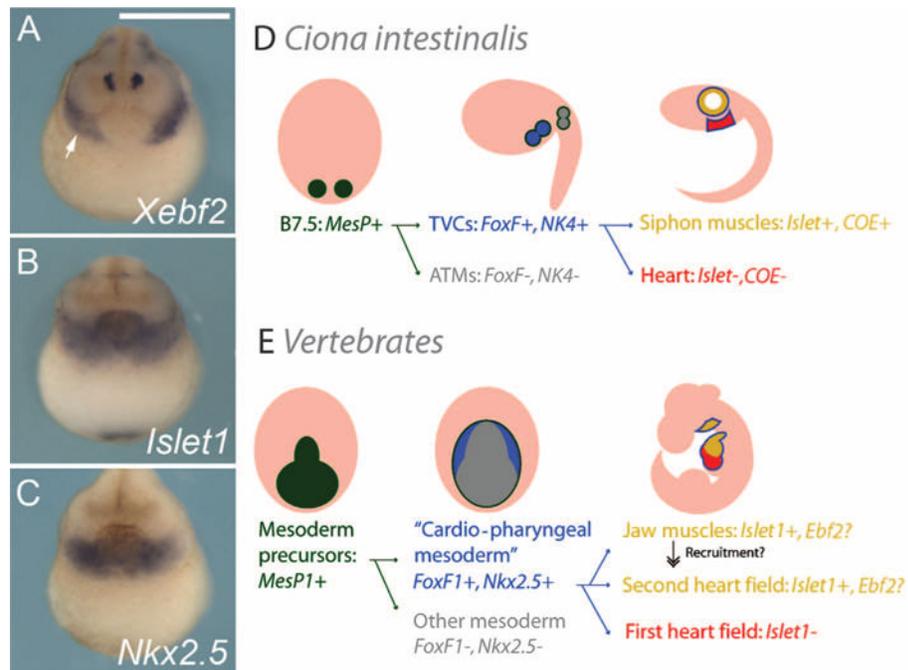


Fig. 4. Comparison to vertebrate pharyngeal mesoderm. (A to C) Expression of the *COE* ortholog *Xebf2* and anterior lateral mesoderm/SHF markers *Nkx2.5* and *Islet1* in *X. tropicalis* embryos at NF stage 20. (A) Expression of *Xebf2* (white arrow) partially overlaps that of *Islet1* (B) and *Nkx2.5* (C) in pharyngeal mesoderm lateral to the heart primordium. Scale bar, 500 μ m. (D and E) The cardiopharyngeal lineages of *Ciona* (D) and vertebrates (E). (D) Summary of differential expression of selected regulatory genes in the B7.5 lineage. (E) Expression of orthologous genes in mouse development. Evolutionary reallocation of *Islet1*⁺ cardiopharyngeal precursors toward the heart might have given rise to the SHF.