DEVELOPMENTAL DYNAMICS 238:3257–3265, 2009

Characterization of Molecular Markers to Assess Cardiac Cushions Formation in Xenopus

Young-Hoon Lee[1,2] and Jean-Pierre Saint-Jeannet[2]*

The valves and septa of the mature heart are derived from the cardiac cushions, which develop from discrete swellings in two regions of developing heart tube: the atrioventricular (AV) canal and the ventricular outflow tract (OFT). In higher vertebrates, three distinct lineages contribute to the heart valves and septa, the endocardium, the myocardium, and the cardiac neural crest that will populate the cardiac jelly of the OFT. Very little is known about cardiac cushions development in amphibians. Here, we describe the expression of eight genes during key stages of cardiac cushion development in Xenopus. Among these genes, the Wnt antagonist Frzb1 and the transcription factors Xl-Fli, Sox8, Sox9, and Sox10 are differentially expressed in the mesenchyme of the OFT and AV cushions. These genes can be used in combination with lineage-tracing experiments to determine the embryonic origin of the cardiac cushions mesenchyme in Xenopus. *Developmental Dynamics 238:3257–3265, 2009*. © **2009 Wiley-Liss, Inc.**

Key words: cardiac cushion; outflow tract; atrioventricular canal; mesenchyme; neural crest; SoxE; Fzb1; Xl-Fli; Xenopus

Accepted 1 October 2009

INTRODUCTION

The septa and valves of the mature heart are essential to maintain the separation between the systemic and pulmonary circulation and to preserve the directionality of the blood flow. Abnormal septation of the heart and valve anomalies are the most frequent forms of congenital heart defects (reviewed in Joziasse et al., 2008).

The heart starts as a simple tubular structure consisting of an outer myocardial cell layer separated from an inner endocardial tube by a sheet of extracellular matrix called the cardiac jelly. As the heart is going through the looping stages, this extracellular matrix starts to thicken in two regions, the atrioventricular (AV) canal and the ventricular outflow tract (OFT). These discrete swellings of cardiac jelly, known as cardiac cushions, will subsequently be colonized by different subpopulations of cardiac mesenchyme. During further maturation of the heart, the AV and the OFT cushions will undergo extensive remodeling to give rise to the valve leaflets and the septum of the OFT, respectively.

The AV and the OFT cushion mesenchyme are generated by epithelial-to-mesenchymal transformation of the endocardial cells lining the cushions (reviewed in Snarr et al., 2008). However, tissues ablation and transplantation experiments in avian embryos have shown that the OFT cushion also receives an important contribution from a population of neural crest cells arising from the posterior hindbrain region, known as the cardiac neural crest (reviewed in Hutson and Kirby, 2007). In mouse, fate-mapping studies using the Cre-Lox technology also strongly support a critical role of the cardiac neural crest in mediating OFT septation (reviewed in Stoller and Epstein, 2005). Interestingly, the neural crest does not contribute cells to the AV cushion.

The situation appears to be somewhat different in lower vertebrates. For example, the zebrafish does not have a separated systemic and pulmonary circulation; therefore, cardiac neural crest cells are not needed for septation of the OFT. Instead, lineage-tracing experiments indicate that the

[1]Department of Oral Anatomy, School of Dentistry, Chonbuk National University, Jeonju, South Korea
[2]Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania
Grant sponsor: University of Pennsylvania and the School of Veterinary Medicine.
***Correspondence to: Jean-Pierre Saint-Jeannet, Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104. E-mail: saintj@vet.upenn.edu**

DOI 10.1002/dvdy.22148
Published online 6 November 2009 in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Summary of Genes Expression in Xenopus Heart as Revealed by Whole Mount In Situ Hybridization

Genes	OFT region	Ventricle	Atrium	AVC region
Ap2	–	–	–	–
Bmp4	+	–	–	+
Fgf8	–	–	–	–
FrzA	+	+	+	+
Frzb1	+	–	–	+
Frz2	+	+	+	+
Frz3	–	–	–	–
Frz7	+	+	+	+
Frz9	–	–	–	–
Id2	+	+	+	+
Islet1	–	–	–	–
Msx1	–	–	–	+
Nkx2.5	+	+	+	+
Pax3	–	–	–	–
Shh	+	+	+	+
Snail1	–	–	–	–
Snail2	–	–	–	–
Sox8	+	–	–	+
Sox9	+	–	–	+
Sox10	+	–	–	–
Tbx5	–	+	+	+
Tbx20	+	+	+	+
Wnt1	–	–	–	–
Wnt2b	–	–	–	–
Wnt3a	–	–	–	–
Xl-Fli	+	+	+	+

"+" indicates positive expression, while "–" indicates that the gene was not detected. AVC, atrioventricular canal; OFT, outflow tract.

neural crest cells migrating into the zebrafish heart make a substantial contribution to the myocardial cell lineage in all regions of the developing heart (Sato and Yost, 2003; Li et al., 2003). In the frog *Xenopus laevis*, the formation of the OFT is incomplete, with a spiral OFT that directs blood flow to the pulmocutaneous or systemic arteries (Kolker et al., 2000; Mohun et al., 2000; Lohr and Yost, 2000). This structure is remarkably similar to the aorticopulmonary septum of the embryonic mammalian OFT. Transplantation experiments using grafts of *Xenopus borealis* into *Xenopus laevis* indicate that cells from the rhombencephalic neural crest cells can populate the wall of the OFT (Sadaghiani and Thiébaud, 1987). In another set of experiments, early ablation of neural crest progenitors resulted in a broad range of cardiac defects suggesting that neural crest cells also contribute to the cardiovascular system in Xenopus (Martinsen et al., 2004). However, in both studies heart formation was assessed at stage 39, before the mesenchymal colonization of the OFT cushion is initiated (stage 41; Kolker et al., 2000; Mohun et al., 2000; Lohr and Yost, 2000). Therefore, it is still unclear whether neural crest cells make a specific contribution to the OFT cushion in Xenopus, as seen in higher vertebrates.

To start to address this important question, we analyzed the expression of eight genes during key stages of cardiac cushion development. More specifically, we describe genes differentially expressed in the mesenchyme of the OFT and AV cushions that can be used in combination with lineage experiments to determine the embryonic origin of the cardiac cushions mesenchyme in Xenopus.

RESULTS AND DISCUSSION

Analysis of Genes Expressed in the Developing Cardiac Cushions

We examined the spatial and temporal expression pattern by whole mount in situ hybridization of 26 genes (Table 1). These genes were selected based on their expression and function during cardiac cushion formation described in other species. Among these, we selected 8 genes that are expressed in the heart at stages relevant to cardiac cushion formation (stages 39–45; Nieuwkoop and Faber, 1967; Kolker et al., 2000; Mohun et al., 2000). These genes include Bmp4 (Fig. 1A,I,Q,Y; Jones et al., 1992), Msx1 (Fig. 1B,J,R,Z; Suzuki et al., 1997), Nkx2.5 (Fig. 1C,K,S,A'; Cleaver et al., 1996), Frzb1 (Fig. 1D,L,T,B'; Wang et al., 1997), Sox8 (Fig. 1E,M,U,C'; O'Donnell et al., 2006), Sox9 (Fig. 1F,N,V,D'; Spokony et al., 2002), Sox10 (Fig. 1G,O,W,E'; Aoki et al., 2003), and Xl-Fli (Fig. 1H,P,X,F'; Meyer et al., 1995). Bmp4, Nkx2.5, Sox9, and Xl-Fli expression domains overlap with the prospective region of both the OFT and AV cushions as early as stage 39 (Fig. 1A,C,F,H), and will persist in these regions at least until stage 45 (Fig. 1I,K,N,P,Q,S,V,X,Y, A',D',F'). Frzb1 follows a fairly similar expression profile starting at stage 40 (Fig. 1D,L,T,B'). Among these genes. Nkx2.5 and Xl-Fli are in fact expressed throughout the entire developing heart tube (Fig. 1C,H,K, P,S,X,A',F'). Msx1 is restricted to the AV cushions throughout the process of valve formation (Fig. 1B,J,R,Z). Sox8 is first detected in the OFT and AV region around stage 40 (Fig. 1M) and stage 41 (Fig. 1U), respectively. Sox8 expression is maintained in both domains up to stage 45 (Fig. 1C'). Sox10 expression is initiated around stage 41 in a region that corresponds to the future OFT cushions, and persists in this domain at least until stage 45 (Fig. 1W,E').

Cardiac Cushions Development in Xenopus

To evaluate more precisely the cell type–specific expression of these genes, we performed in situ hybridization on section at stages 39, 41, and 45. Sections were performed along two different axes to assess OFT (transverse section) and AV (sagittal section) cushion development (Fig. 2). The development of the cardiac tissue in Xenopus has been

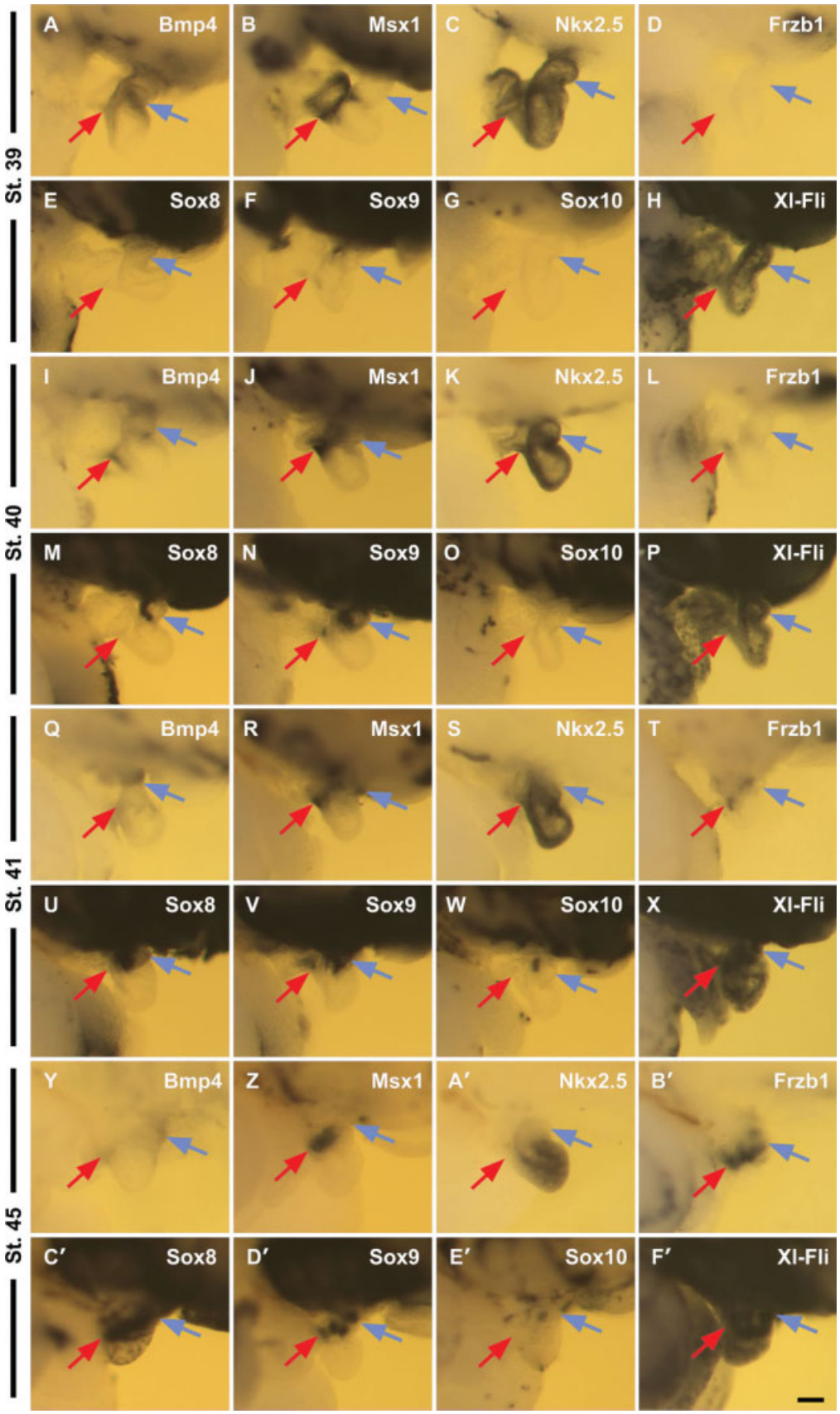

Fig. 1. Cardiac genes expression at stages 39, 40, 41, and 45 by whole-mount in situ hybridization. In all panels, a lateral view of the cardiac region is shown, anterior to right, dorsal to top. Blue and red arrows indicate the position of the OFT and AV valves, respectively. Scale bar = 100 μm.

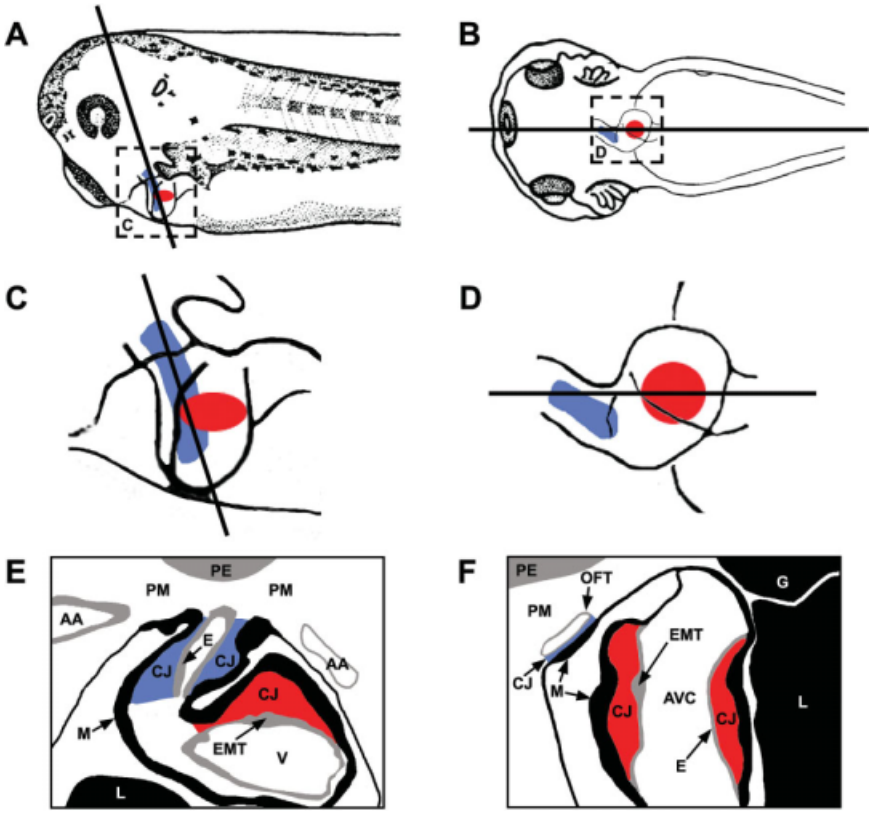

Fig. 2.

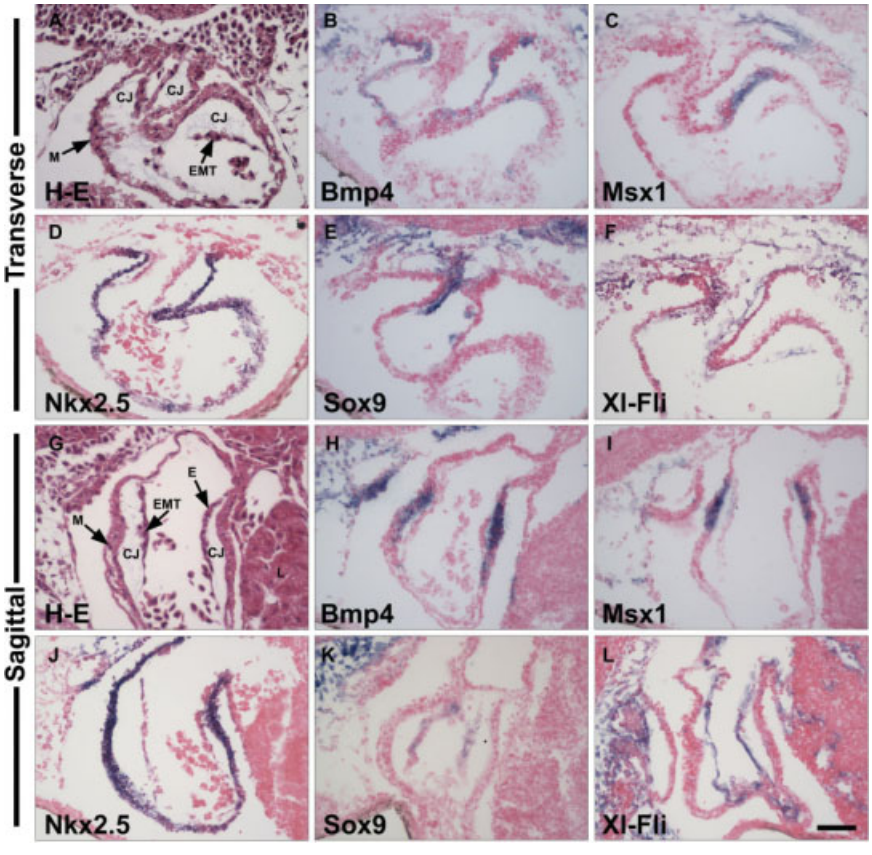

Fig. 3.

described in detail and valve formation is believed to occur between stage 41 and stage 44 (Nieuwkoop and Faber, 1967; Kolker et al., 2000; Mohun et al., 2000; Lohr and Yost, 2000). At stage 39 (the first time-point of this study), the OFT and AV cushions are already clearly visible. They are formed of endothelial cells separated from the myocardium by a layer of acellular cardiac jelly (Figs. 2E,F, 3A,G). At stage 39, a small number of mesenchymal cells have started to invade the cardiac jelly of the AV cushion by epithelial-to-mesenchymal transformation of the endocardium (Figs. 2E,F, 3A,G), while the cardiac jelly of the OFT cushion remains acellular until stage 40 (not shown). It is at stage 41 that the cardiac jelly of the OFT cushion starts to be populated by mesenchymal cells (Fig. 4A). As development proceeds, the mesenchymal cells start to condensate in the OFT and AV cushions. During further maturation of the heart, the AV and the OFT cushions will undergo extensive remodeling to give rise to

Fig. 2. Diagram illustrating the orientation of the transverse and sagittal sections in subsequent figures. Lateral view (**A**) and ventral view (**B**) of a stage-39 embryo. The black lines indicate the level of the transverse (A) and sagittal (B) sections. The diagrams are modified from Nieuwkoop and Faber (1967). **C, D:** Higher magnification of the two regions boxed in A and B. The position of the developing OFT and AV canal is indicated in blue and red, respectively. **E, F:** Schematic representation of the H-E stained sections as shown in Figure 3A and G, respectively, illustrating the topography of the tissues as they relate to cardiac cushion formation. The positions of future OFT (blue) and AV (red) cushions are highlighted. AA, aortic artery; AVC, atrioventricular canal; CJ, cardiac jelly; E, endocardium; EMT, site of epithelial-to-mesenchymal transformation; G, gut; L, liver; M, myocardium; OFT, outflow tract; PE, pharyngeal endoderm; PM, pharyngeal mesoderm; V, ventricle.

Fig. 3. Cardiac gene expression at stage 39 by in situ hybridization on section. Transverse and sagittal sections are at the level of the OFT and AV canal, respectively. **A, G:** Hematoxylin/eosin (H-E) stained sections. Bmp4 (**B, H**), Msx1 (**C, I**), and Nkx2.5 (**D, J**) are expressed in the myocardium while Sox9 (**E, K**) and Xl-Fli (**F, L**) are detected in the endocardium. CJ, cardiac jelly; E, endocardium; EMT, site of epithelial-to-mesenchymal transformation; L, liver; M, myocardium. Scale bar = 50 μm.

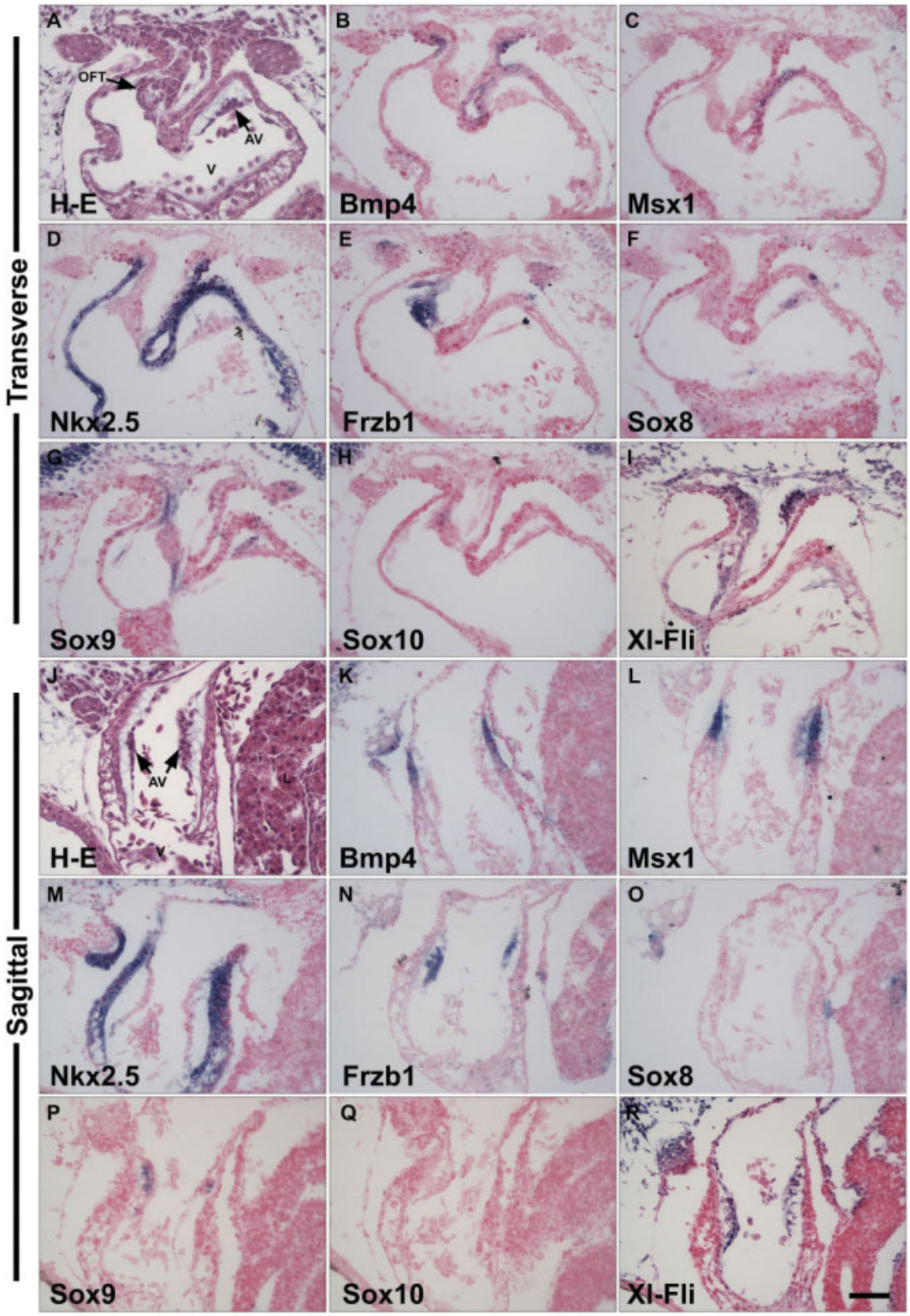

Fig. 4. Cardiac gene expression at stage 41 by in situ hybridization on section. Transverse and sagittal sections are at the level of the OFT and AV canal, respectively. **A, J:** Hematoxylin/eosin (H-E) stained sections. Bmp4 (**B, K**), Msx1 (**C, L**), and Nkx2.5 (**D, M**) are expressed in the myocardium. Frzb1 (**E, N**), Sox8 (**F, O**), Sox9 (**G, P**), and Xl-Fli (**I, R**) are expressed in the mesenchyme of the OFT and AV cushions. Sox10 (**H, Q**) is weakly expressed in the OFT mesenchyme. AV, atrioventricular cushion; L, liver; OFT, outflow tract cushion; V, ventricle. Scale bar = 50 μm.

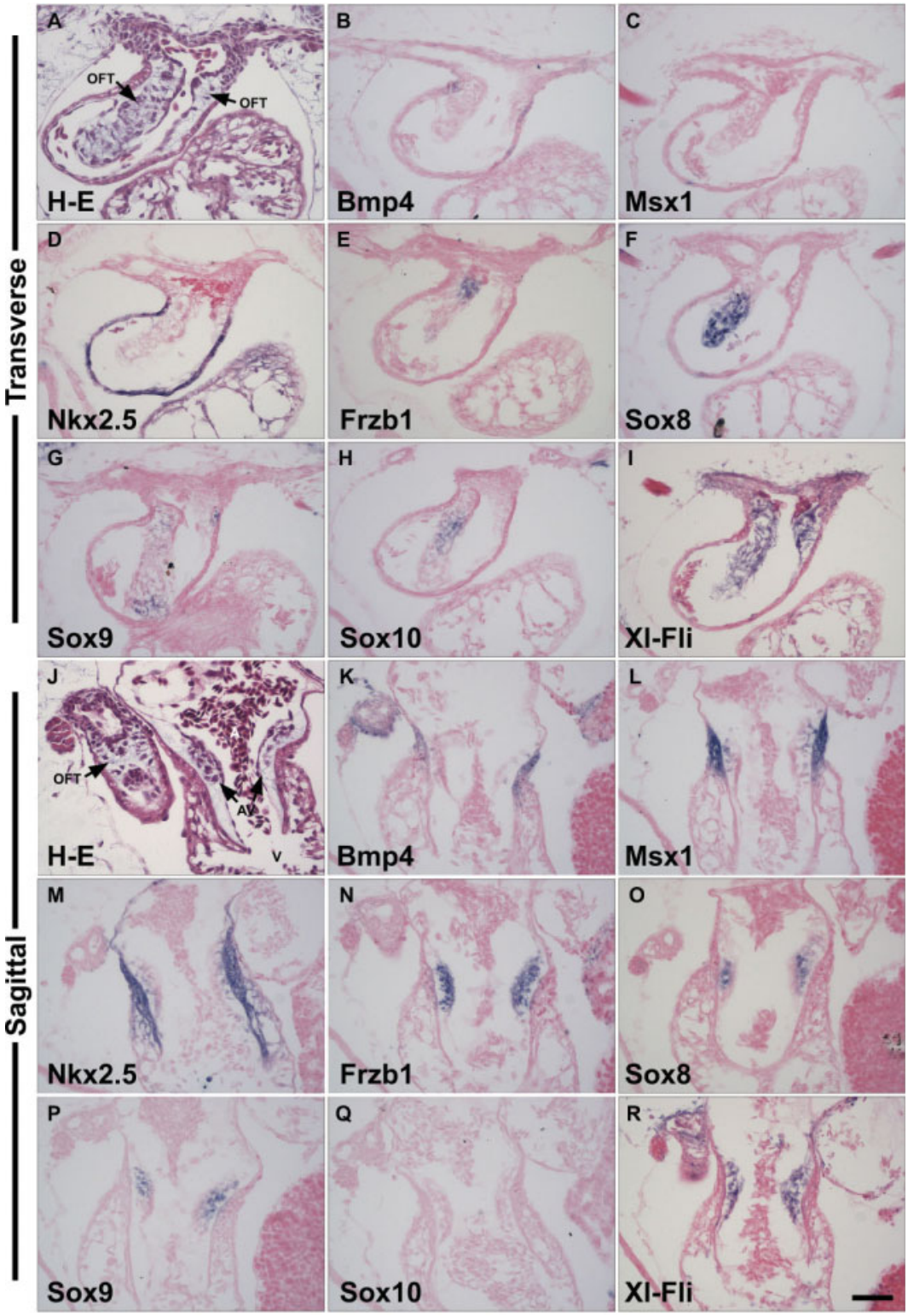

Fig. 5. Cardiac genes expression at stage 45 by in situ hybridization on section. Transverse and sagittal sections are at the level of the OFT and AV canal, respectively. **A,J:** Hematoxylin/eosin (H-E) stained sections. Bmp4 (**B, K**), Msx1 (**C, L**), and Nkx2.5 (**D, M**) are expressed in the myocardium. Frzb1 (**E, N**), Sox8 (**F, O**), Sox9 (**G, P**), and Xl-Fli (**I, R**) are differentially expressed in the mesenchyme of the OFT and AV cushions. Sox10 (**H, Q**) is detected in the middle portion of the OFT cushion. A, atrium; AV, atrioventricular valve; OFT, outflow tract valve; V, ventricle. Scale bar = 50 μm.

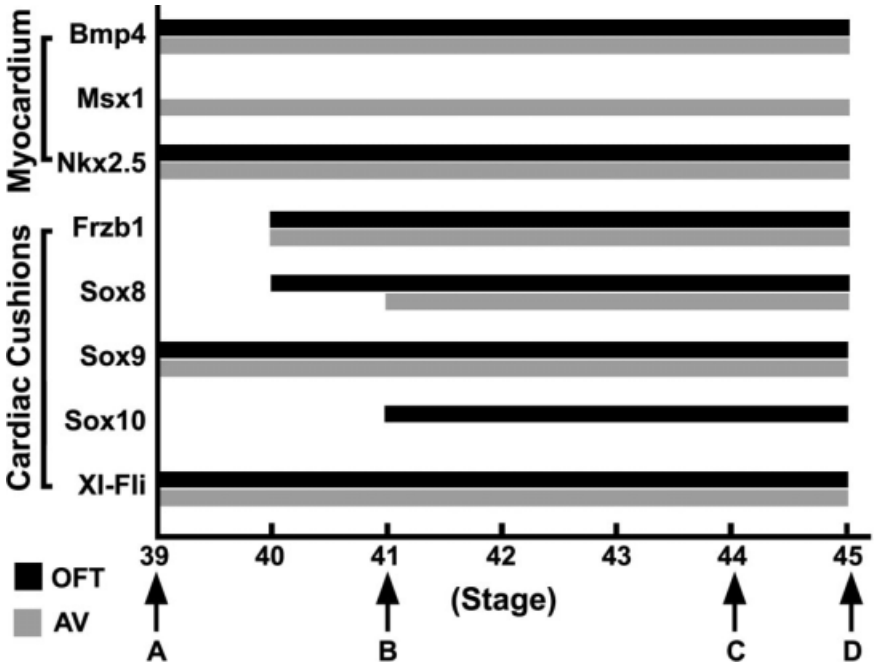

Fig. 6. Summary of the timeline of cardiac genes expression in Xenopus as they relate to cardiac cushions formation. The letters indicate key stages of heart development based on this study and previously published work (Nieuwkoop and Faber, 1967; Kolker et al., 2000; Mohun et al., 2000; Lohr and Yost, 2000). **A:** Initiation of epithelial-to-mesenchymal transformation of the endocardium lining the AV cushion. **B:** Initiation of epithelial-to-mesenchymal transformation of the endocardium lining the OFT cushion. **C:** OFT spiral septum and AV valves are formed. **D:** Atrial septation is completed.

the AV valve leaflets and the spiral septum of the OFT, respectively (Fig. 5A,J). Atrial septation is completed around stage 45 (not shown; Nieuwkoop and Faber, 1967).

Bmp4, Msx1, and Nkx2.5 Expression During OFT and AV Cushions Formation

The signaling molecule Bmp4 is asymmetrically expressed in the heart anlage in Xenopus, where it has been implicated in heart looping (Breckenridge et al., 2001). During endocardial cushion development, Bmp4 is strongly expressed in the myocardial cells surrounding the OFT and the AV canal as early as stage 39 (Fig. 3B,H). Bmp4 expression persists in this tissue at stage 41 (Fig. 4B,K) and stage 45 (Fig. 5B,K); however, the expression level declines as the cushions mature into a valve. In chick and mouse embryos, Bmp4 is expressed in the muscle layer of the OFT and AV cushions but not in the cardiac cushion mesenchyme (Keyes et al., 2003; Jiao et al., 2003). In mice, Bmp4 is critically required for AV septation after cushions have formed (Jiao et al., 2003).

The homeodomain transcription factor Msx1, a downstream effector of Bmp signaling, has been implicated in the regulation of AV and OFT cushions formation in chick and mouse embryos (Chan-Thomas et al., 1993; Chen et al., 2007, 2008). In Xenopus, Msx1 expression is restricted to the myocardium immediately adjacent to the AV canal at stage 39 (Fig. 3C,I). Overtime Msx1 expression level increases in this region of the myocardium. It is also detected at low levels in the mesenchyme of the AV cushion, as the epithelial-to-mesenchymal transformation takes place (Figs. 4L, 5L). Unlike its chick and mouse counterparts, Xenopus Msx1 does not appear to be expressed during OFT cushion formation (Figs. 3C, 4C, 5C).

Nkx2.5, the vertebrate homolog of the Drosophila tinman gene, is expressed in the cardiac lineage of fish, chick, and mouse (Chen and Fishman, 1996; Buchberger et al., 1996; Lints et al., 1993). It is essential for myogenic and morphogenetic differentiation in the mammalian heart (Lyons et al., 1995). In Xenopus, Nkx2.5 is expressed in early heart progenitors and is maintained in the cardiac tissue throughout development (Cleaver et al., 1996; Newman and Krieg, 1998). During endocardial cushion development, Nkx2.5 is detected throughout the myocardium at all stages examined (Figs. 3D,I, 4D,M, 5D,M).

Differential Gene Expression in the OFT and AV Cushion Mesenchyme

Among the 8 genes showing expression domains in regions of the developing heart that will form the cardiac cushions (Fig. 1), 5 were more specifically expressed in the mesenchyme of the OFT and/or AV cushions. These genes include the Wnt antagonist Frzb1, a member of the Ets family of transcription factors, Xl-Fli, and the three members of the SoxE class of transcriptional regulators, Sox8, Sox9, and Sox10.

Frzb1 is expressed in the endocardial and mesenchymal cells of the OFT and the AV cushions at stage 41 (Fig. 4E,N). This expression persists in the mesenchymal cells that populate the OFT and AV cushions at least until stage 45 (Fig. 5E,N). Frzb1 appears to be restricted to the proximal portion of the OFT cushion (Fig. 5E). A similar expression pattern in both the OFT and AV cushions has also been reported in birds (Ladher et al., 2000; Person et al, 2005).

All three SoxE genes are expressed in the OFT mesenchyme. Sox8 is first expressed in the region of the OFT at stage 40 (Fig. 1M). By Stage 41, Sox8 is also detected in the mesenchyme of the AV cushion (Fig. 4F). Sox8 expression levels increase over time in both the OFT and AV cushion (Figs. 4F, 5F). Interestingly, Sox8 is excluded from the most proximal portion of the OFT mesenchyme (Fig. 4F). Sox9 is initially expressed in the endocardial cells lining the OFT and the AV canal at stage 39 (Fig. 3E,K). As development proceeds, Sox9 expression is lost in the endocardial lineage, and

appears to be now restricted to the proximal and distal portions of the OFT cushion at stage 41 (Fig. 4G) and stage 45 (Fig. 5G). It is also detected throughout the AV cushion (Figs. 4P, 5P). Sox10 is first detected in the OFT cushion around stage 41 (Figs. 1W, 4H). By stage 45, Sox10 is clearly restricted to the middle portion of the OFT cushion (Fig. 5H). In contrast to the other two SoxE genes, Sox10 is not detected in the AV cushion (Figs. 4Q, 5Q). In chick embryos, Sox8 and Sox9 are also expressed in both the OFT and AV cushions. However, Sox10 is strictly restricted to the AV cushion in birds (Montero et al., 2002). In mouse, Sox9 is expressed in all mesenchymal cells of the endocardial cushions, and conditional inactivation of Sox9 results in hypoplastic cardiac cushions (Akiyama et al., 2004). It is believed that Sox9 regulates epithelial-to-mesenchymal transformation during endocardial cushions formation (Akiyama et al., 2004).

Finally, Xl-Fli is first detected in the endocardial cells lining both cushions at stage 39 (Fig. 3F,L) and will persist in this lineage throughout valve formation. At stage 41, Xl-Fli expression domain expands to the mesenchyme of the developing OFT and AV cushions (Fig. 4I,R), and these cells will continue to express Xl-Fli at least until stage 45 (Fig. 5I,R). Unlike SoxE genes, which are restricted to distinct but partially overlapping domains of the OFT cushion, Xl-Fli is expressed throughout the entire OFT mesenchyme (Fig. 5I).

In summary, we report the expression of a number of genes expressed during cardiac cushions formation in Xenopus (Fig. 6). We identified genes that are expressed throughout the entire myocardium (NKx2.5) or restricted to the muscle lineage surrounding the developing cushions (Bmp4 and Msx1). We also describe genes that are detected in the endocardium lining the OFT and AV cushions (Sox9 and Xl-Fli), as well as genes that are differentially activated in the mesenchyme of the OFT (Frzb1, Sox8, Sox9, and Sox10) or the AV (Sox9 and Xl-Fli) cushion. With these tools in hand, we are now in an excellent position to perform lineage-tracing experiments to determine the embryonic origin of the cardiac cushions mesenchyme in Xenopus, a question that has never been fully addressed in this organism.

EXPERIMENTAL PROCEDURES

Histology

Embryos were staged according to Nieuwkoop and Faber (1967) and fixed in half-strength Karnovsky's solution (1.5% glutaraldehyde, 1.5% paraformaldehyde, 0.1 M phosphate buffered solution, pH 7.4) for 1 hr. After dehydration through a graded series of ethyl-alcohol, embryos were embedded in Paraplast+. Transverse or sagittal serial sections (6 μm) were performed on an Olympus rotary microtome and stained with Hematoxylin and Eosin.

In Situ Hybridization

For whole-mount in situ hybridization, the epidermis was manually removed in the cardiac area to allow for a better penetration of the probes. Embryos were then fixed in MEMFA and processed for in situ hybridization as previously described (Harland, 1991). For in situ hybridization on sections, after fixation embryos were embedded in Paraplast+, and 12-μm serial sections hybridized according to the procedure described by Lemaire and Gurdon (1994). Sections were then briefly counterstained with Eosin. Antisense DIG-labeled probes (Genius Kit, Roche) were synthesized using template cDNA encoding Ap2 (Luo et al., 2003), Bmp4 (Jones et al., 1992), Fgf8 (Christen and Slack, 1997), FrzA (Xu et al., 1998), Frzb1 (Wang et al., 1997), Frz2 (Deardorff and Klein, 1999), Frz3 and Frz7 (Deardorff et al., 2001), Frz9 (Wheeler and Hoopler, 1999), Id2 (Martinsen et al., 2004), Islet1 (Brade et al., 2007), Msx1 (Suzuki et al., 1997), Nkx2.5 (Cleaver et al., 1996), Pax3 (Bang et al., 1997), Shh (Ekker et al., 1995), Snail1 (Essex et al., 1993), Snail2 (Mayor et al., 1995), Sox8 (O'Donnell et al., 2006), Sox9 (Spokony et al., 2002), Sox10 (Aoki et al., 2003), Tbx5 and Tbx20 (Brown et al., 2005), Wnt1 and Wnt3a (Wolda et al., 1993), Wnt2b (Landesman and Sokol, 1997), and Xl-Fli (Meyer et al., 1995).

ACKNOWLEDGMENTS

We thank Drs. Frank Colon, Jamie Lohr, Peter Klein, Paul Krieg, Roberto Mayor, Malcolm Moss, Petra Pandur, Pierre Remy, Thomas Sargent, Sergei Sokol, and Masanori Taira for plasmids. This work was supported by Bridge Funds from the University of Pennsylvania and the School of Veterinary Medicine.

REFERENCES

Akiyama H, Chaboissier MC, Behringer RR, Rowitch DH, Schedl A, Epstein JA, de Crombrugghe B. 2004. Essential role of Sox9 in the pathway that controls formation of cardiac valves and septa. Proc Natl Acad Sci USA 101:6502–507.

Aoki Y, Saint-Germain N, Gyda M, Magner-Fink E, Lee YH, Credidio C, Saint-Jeannet JP. 2003. Sox10 regulates the development of neural crest-derived melanocytes in Xenopus. Dev Biol 259: 19–33.

Bang AG, Papalopulu N, Goulding MD, Kintner C. 1999. Expression of Pax-3 in the lateral neural plate is dependent on a Wnt-mediated signal from the posterior non-axial mesoderm. Dev Biol 212: 366–380.

Brade T, Gessert S, Kühl M, Pandur P. 2007. The amphibian second heart field: Xenopus islet-1 is required for cardiovascular development. Dev Biol 311: 297–310.

Breckenridge RA, Mohun TJ, Amaya E. 2001. A role for BMP signalling in heart looping morphogenesis in Xenopus. Dev Biol 232:191–203.

Brown DD, Martz SN, Binder O, Goetz SC, Price BM, Smith JC, Conlon FL. 2005. Tbx5 and Tbx20 act synergistically to control vertebrate heart morphogenesis Development132:553–563.

Buchberger A, Pabst O, Brand T, Seidl K, Arnold HH. 1996. Chick NKx-2.3 represents a novel family member of vertebrate homologues to the Drosophila homeobox gene tinman: differential expression of cNKx-2.3 and cNKx-2.5 during heart and gut development. Mech Dev 56:151–163.

Chan-Thomas PS, Thompson RP, Robert B, Yacoub MH, Barton PJ. 1993. Expression of homeobox genes Msx-1 (Hox-7) and Msx-2 (Hox-8) during cardiac development in the chick. Dev Dyn 197:203–216.

Chen JN, Fishman MC. 1996. Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. Development 122:3809–3816.

Chen YH, Ishii M, Sun J, Sucov HM, Maxson RE Jr. 2007. Msx1 and Msx2 regulate survival of secondary heart field precursors and post-migratory proliferation of cardiac neural crest in the outflow tract. Dev Biol 308:421–437.

Chen YH, Ishii M, Sucov HM, Maxson RE Jr. 2008. Msx1 and Msx2 are required

for endothelial-mesenchymal transformation of the atrioventricular cushions and patterning of the atrioventricular myocardium. BMC Dev Biol 8:75.
Christen B, Slack JMW. 1997. FGF-8 is associated with anteroposterior patterning and limb regeneration in Xenopus. Dev Biol 192:455–466.
Cleaver OB, Patterson KD, Krieg PA. 1996. Overexpression of the tinman-related genes XNkx-2.5 and XNkx-2.3 in Xenopus embryos results in myocardial hyperplasia. Development 122: 3549–3556.
Deardorff MA, Klein PS. 1999. Xenopus frizzled-2 is expressed highly in the developing eye, otic vesicle and somites. Mech Dev 87:229–233.
Deardorff MA, Tan C, Saint-Jeannet J-P, Klein PS. 2001. A role for frizzled-3 in neural crest development. Development 128:3655–3663.
Ekker SC, McGrew LL, Lai CJ, Lee JJ, von Kessler DP, Moon RT, Beachy PA. 1995. Distinct expression and shared activities of members of the hedgehog gene family of *Xenopus laevis*. Development 121:2337–2347.
Essex LJ, Mayor R, Sargent MG. 1993. Expression of Xenopus Snail in mesoderm and prospective neural fold ectoderm. Dev Dyn 198:108–122.
Harland RM. 1991. In situ hybridization: an improved whole-mount method for Xenopus embryos. Methods Cell Biol 36:685–695.
Hutson MR, Kirby ML. 2007. Model systems for the study of heart development and disease. Cardiac neural crest and conotruncal malformations. Semin Cell Dev Biol 18:101–110.
Jiao K, Kulessa H, Tompkins K, Zhou Y, Batts L, Baldwin HS, Hogan BL. 2003. An essential role of Bmp4 in the atrioventricular septation of the mouse heart. Genes Dev 17:2362–2367.
Jones CM, Lyons KM, Lapan PM, Wright CV, Hogan BL. 1992. DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in Xenopus mesoderm induction. Development 115: 639–647.
Joziasse IC, van de Smagt JJ, Smith K, Bakkers J, Sieswerda GJ, Mulder BJ, Doevendans PA. 2008. Genes in congenital heart disease: atrioventricular valve formation. Basic Res Cardiol 103: 216–227.
Keyes WM, Logan C, Parker E, Sanders EJ. 2003. Expression and function of bone morphogenetic proteins in the development of the embryonic endocardial cushions. Anat Embryol (Berl) 207:135–147
Kolker SJ, Tajchman U, Weeks DL. 2000. Confocal imaging of early heart development in *Xenopus laevis*. Dev Biol 218:64–73.
Ladher RK, Church VL, Allen S, Robson L, Abdelfattah A, Brown NA, Hattersley G, Rosen V, Luyten FP, Dale L, Francis-West PH. 2000. Cloning and expression of the Wnt antagonists Sfrp-2 and Frzb during chick development. Dev Biol 218:183–198.
Landesman Y, Sokol SY. 1997. Xwnt-2b is a novel axis-inducing Xenopus Wnt, which is expressed in embryonic brain. Mech Dev 63:199–209.
Lemaire P, Gurdon JB. 1994. A role for cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of the goosecoid and Xwnt-8 genes along the dorsoventral axis of early Xenopus embryos. Development 120:1191–1199.
Li YX, Zdanowicz M, Young L, Kumiski D, Leatherbury L, Kirby ML. 2003. Cardiac neural crest in zebrafish embryos contributes to myocardial cell lineage and early heart function. Dev Dyn 226:540–550.
Lints TJ, Parsons LM, Hartley L, Lyons I, Harvey RP. 1993. Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development 119: 419–431.
Lohr JL, Yost HJ. 2000. Vertebrate model systems in the study of early heart development: Xenopus and zebrafish. Am J Med Genet 97:248–257.
Luo T, Lee Y-H, Saint-Jeannet J-P, Sargent TD2003. Induction of neural crest in Xenopus by transcription factor AP2α. Proc Natl Acad Sci USA 100:532–537.
Lyons I, Parsons LM, Hartley L, Li R, Andrews JE, Robb L, Harvey RP. 1995. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2–5. Genes Dev 9:1654–1666.
Martinsen BJ, Frasier AJ, Baker CV, Lohr JL. 2004. Cardiac neural crest ablation alters Id2 gene expression in the developing heart. Dev Biol 272:176–190.
Mayor R, Morgan R, Sargent MG. 1995. Induction of the prospective neural crest of Xenopus. Development 121: 767–777.
Meyer D, Stiegler P, Hindelang C, Mager AM, Remy P. 1995. Whole-mount in situ hybridization reveals the expression of the Xl-Fli gene in several lineages of migrating cells in Xenopus embryos. Int J Dev Biol 39:909–919.
Mohun TJ, Leong LM, Weninger WJ, Sparrow DB. 2000. The morphology of heart development in *Xenopus laevis*. Dev Biol 218:74–88.
Montero JA, Giron B, Arrechedera H, Cheng YC, Scotting P, Chimal-Monroy J, Garcia-Porrero JA, Hurle JM. 2002. Expression of Sox8, Sox9 and Sox10 in the developing valves and autonomic nerves of the embryonic heart. Mech Dev 118:199–202.
Newman CS, Krieg PA. 1998. tinman-related genes expressed during heart development in Xenopus. Dev Genet 22: 230–238.
Nieuwkoop PD, Faber J. 1967. Normal table of Xenopus laevis (Daudin). Amersterdam: North Holland Publishing Company.
O'Donnell M, Hong CS, Huang X, Delnicki RJ, Saint-Jeannet,J.P.,2006. Functional analysis of Sox8 during neural crest development in Xenopus. Development 133:3817–3826.
Person AD, Garriock RJ, Krieg PA, Runyan RB, Klewer SE. 2005. Frzb modulates Wnt-9a-mediated beta-catenin signaling during avian atrioventricular cardiac cushion development. Dev Biol 278:35–48.
Sadaghiani B, Thiebaud CH. 1987. Neural crest development in the *Xenopus laevis* embryo, studied by interspecific transplantation and scanning electron microscopy. Dev Biol 124:91–110.
Sato M, Yost HJ. 2003. Cardiac neural crest contributes to cardiomyogenesis in zebrafish. Dev Biol 257:127–139.
Snarr BS, Kern CB, Wessels A. 2008. Origin and fate of cardiac mesenchyme. Dev Dyn 237:2804–2819.
Spokony RF, Aoki Y, Saint-Germain N, Magner-Fink EK, Saint-Jeannet JP. 2002. The transcription factor Sox9 is required for cranial neural crest development in Xenopus. Development 129:421–432.
Stoller JZ, Epstein JA. 2005. Cardiac neural crest. Semin Cell Dev Biol 16: 704–715.
Suzuki A, Ueno N, Hemmati-Brivanlou A. 1997. Xenopus msx1 mediates epidermal induction and neural inhibition by BMP4. Development 124:3037–3044.
Wang S, Krinks M, Moos M Jr. 1997. Frzb-1, an antagonist of Wnt-1 and Wnt-8, does not block signaling by Wnts -3A, -5A, or -11. Biochem Biophys Res Commun 236:502–504.
Wheeler GN, Hoopler S. 1999. Two novel Xenopus frizzled genes expressed in developing heart and brain. Mech Dev 86:203–207.
Wolda SL, Moody CJ, Moon RT. 1993. Overlapping expression of Xwnt-3a and Xwnt-1 in neural tissue of *Xenopus laevis* embryos. Dev Biol 155:46–57.
Xu Q, D'Amore PA, Sokol SY. 1998. Functional and biochemical interactions of Wnts with FrzA, a secreted Wnt antagonist. Development 125:4767– 4776.

Developmental Dynamics