Heart Fields: Spatial Polarity and Temporal Dynamics

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ABSTRACT

In chick and mouse, heart fields undergo dynamic morphological spatiotemporal changes during heart tube formation. Here, the dynamic change in spatial polarity of such fields is discussed and a new perspective on the heart fields is proposed. The heart progenitor cells delaminate through the primitive streak and migrate in a semicircular trajectory craniolaterally forming the bilateral heart fields as part of the splanchnic mesoderm. They switch their polarity from anteroposterior to mediolateral. The anterior intestinal portal posterior descent inverts the newly formed heart field mediolateral polarity into lateromedial by 125° bending. The heart fields revert back to their original anteroposterior polarity and fuse at the midline forming a semi heart tube by completing their half circle movement. Several names and roles were assigned to different portions of the heart fields: posterior versus anterior, first versus second, and primary versus secondary heart field. The posterior and anterior heart fields define basically physical fields that form the inflow–outflow axis of the heart tube. The first and second heart fields are, in contrast, temporal fields of differentiating cardiomyocytes expressing myosin light chain 2a and undifferentiated and proliferating precardiac mesoderm expressing Isl1 gene, respectively. The two markers present a complementary pattern and are expressed transiently in all myocardial lineages. Thus, Isl1 is not restricted to a portion of the heart field or one of the two heart lineages as has been often assumed. Anat Rec, 297:175–182, 2014.

Key words: splanchnic mesoderm; morphogenesis; heart tube; heart fields

The development of the heart in chick and mouse, two amniotes, a bird and a mammal, is a highly dynamic process in terms of space (3D) and time (4D). It starts from the delamination and migration of precardiac mesodermal cells through the primitive streak (PS) during the Hamburger–Hamilton stage 3 (HH3) of embryonic development in the chick or embryonic day 6 (E6) in mice, and proceeds to the completion of aortic/pulmonary septation at stages HH34 or E14.5, respectively (Schoenwolf and Garcia-Martinez, 1995; Kinder et al., 2001). Heart progenitor cells which contribute to the three heart layers (endocardium, myocardium, and epicardium) and the several heart segments [conotruncus or outflow tract (OFT), right (RV) and left ventricle (LV), atrioventricular canal (AVC), right (RA) and left atrium (LA), and sinus venosus (SV)] all derive from a small pool of cells located in the epiblast on either side of the PS (Garcia-Martinez and Schoenwolf, 1993; Tam et al., 1997). These cells go through an epithelial to mesenchymal transition and delaminate through the PS, migrating in a semicircular trajectory and proliferating simultaneously. They initially move craniolaterally before converging medially while forming the lateral plate mesoderm (LPM) and other mesodermal derivatives with little or no

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cellular mixing (Yang et al., 2002; Yue et al., 2008; Cui et al., 2009). The LPM splits into the dorsal somatic and ventral splanchnic mesoderm. The dorsal somatic mesoderm gives rise to the body wall and other derivatives, while the ventral splanchnic mesoderm gives rise to all heart components mentioned earlier and other derivatives. The only nonmesodermal contribution to the heart is from neural crest cells that are of ectodermal origin and contribute to the aortic/pulmonary septum, smooth muscles tunics of the great arteries, and possibly to some smooth muscle of the coronary vessels as well (Kirby and Hutson, 2010; Arima et al., 2012). The first cells to separate and commit to a specific fate are those of the endocardium; they delaminate and segregate from the splanchnic mesoderm to form the bilateral endocardial tubes (reviewed in Harris and Black, 2010). The remaining mesodermal cells continue their migration in the same trajectory as a sheet until the completion of addition of all heart components at stage HH22 in chick and E12.5 in mouse, respectively. During this process, the mesodermal cells reorganize themselves first into LPM and then into splanchnic mesoderm (Waldo et al., 2005; Ilagan et al., 2006). The cranial portions of the bilateral heart fields fuse at the midline, and bulge ventrally, forming a semi (myocardial) heart tube or trough that soon begins its peristaltic contraction (HH10-E8.5) (Stalsberg and DeHaan, 1969; DeRuiter et al., 1992). Most of the remaining future myocardium remains as extended splanchnic mesodermal layer that continues to converge into the heart tube for the next few days of development. Addition of the myocardial progenitors takes place simultaneously with the folding of the endoderm and the formation of the foregut pocket. In fact, it is the anterior intestinal portal (AIP) that enables the forming myocardial tube to move ventrally and caudally. Meanwhile, the bilateral endocardial tubes are also brought together by the formation of the foregut where they fuse ventrally and medially, giving rise to a single closed endocardial tube, which is separated from the myocardial wall by cardiac jelly. The endocardial tube allows blood circulation in a closed system. While simple circulation provides the essential needs of the embryo, the myocardial heart is just starting to form, and the epicardium is not yet specified. The proepicardium forms later (HH14, E9.5), also from the splanchnic mesoderm, to give rise to the epicardium, some of the coronary vessel components and other derivatives (van Wijk et al., 2009; Del Monte and Harvey, 2012).

In this article, the focus will be on two major topics, the first deals with the spatial polarities of the myocardial precursor cells during their journey from the PS to the heart tube by comparing and contrasting the classic and revised heart tube formation models based mainly on data from the chick, the second topic deals with the different names and roles of the heart fields by proposing a temporal role for the first and second heart fields based mainly on data from the mouse.

HEART TUBE FORMATION MODELS

The anteroposterior polarity of heart progenitors from their entry into the PS to the formation of the heart tube is preserved (Schoenwolf and Garcia-Martinez, 1995; Kinder et al., 2001). How this polarity is maintained is not as obvious as it may appear (Fishman and Chien 1997). If the migrating cells follow simply a semicircular pattern without any folding, then the polarity would invert, or in other words what was anterior would become posterior and vice versa (Fig. 1A). In the classic chick model of heart tube formation, the mesodermal cells of the anterior portion of the PS inexplicably have to migrate to the anterior of the LPM/splanchnic mesoderm and the posterior have to migrate to the posterior portion in order to preserve polarity while forming the

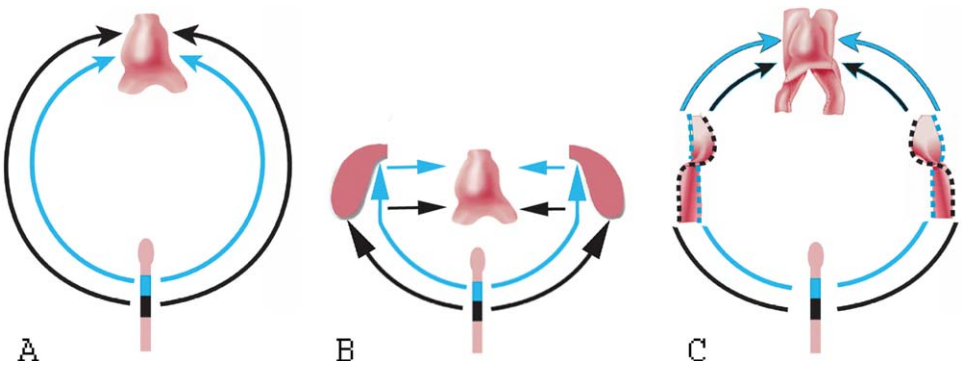

Fig. 1. Chick Heart Fields craniocaudal and mediolateral polarity changes during representative embryonic stages. (**A**) Shows how polarity would be inverted if mesodermal cells migrate from the primitive streak (PS) in a semicircular fashion without any major folding between stages HH3 and HH10. (**B**) Shows how the classic model accounts for maintaining polarity. It shows cranial (blue) and caudal (black arrow) cell migration from the primitive streak at stage HH3 and formation of the bilateral heart fields (crescent like) at stage HH6-7. The fields move medially and fuse to form the heart tube (HT) at stage HH10, keeping the same polarity all the way from the PS to HT. (**C**) shows the semicircular migration of mesodermal cells from the PS (HH3) to the lateral plate mesoderm (LPM) (HH8) to semi heart tube (HH10) and surrounding precardiac splanchnic mesoderm. First, they move craniolaterally to position themselves in mediolateral polarity in the LPM. Secondly, the anterior intestinal portal (AIP) elongation and 125° heart field bending inverts their polarity into lateromedial. Finally, they revert back again into craniocaudal polarity while converging into the heart tube allowing the embryo to maintain the same polarity between the PS and HT. The most anterior cells (blue line) in the PS migrate to a medial position then become lateral in the heart field (dashed blue line), coinciding with the AIP and end up anterior in the semi heart tube (blue arrow). However, the posterior cells (black line) in the PS migrate to a lateral position then become medial in the heart field (dashed black line), coinciding again with the AIP and end up posterior in the semi heart tube (black arrow). All panels are viewed ventrally.

presumed cardiac crescent (DeHaan, 1965; Fishman and Chien 1997; Srivastava and Olson, 2000). The bilateral cardiogenic plates as a "crescent" of the splanchnic mesoderm converge directly in a straight line to the midline, fuse cranially, and then the fusion moves caudally in a zipper-like fashion. Concomitantly, the bilateral plates curve ventrally so the medial edges fuse first ventrally forming the heart outer curvature, and then the lateral edges fuse dorsally forming the heart inner curvature resulting in a closed heart tube. The forming heart tube, in the classic model, not only represents the entire heart (it needs only to grow and remodel), but also maintains exactly the same spatial polarity before and after fusion as well (DeHaan, 1965; Fishman and Chien 1997; Srivastava and Olson, 2000) (Fig. 1B). In the revised heart tube formation model, heart progenitor cells delaminate through the PS and migrate in a semicircular trajectory craniolaterally forming the bilateral heart fields as separate entities of the splanchnic mesoderm sheets. The anteroposterior polarity of the heart progenitors in the PS switches to mediolateral in the splanchnic mesoderm. The AIP posterior descent inverts the newly formed heart fields into lateromedial with 125° bending. The heart fields revert to anteroposterior and fuse at the midline as a semi heart tube completing their half circle movement (Fig. 1C) (Cui et al., 2009; Camp et al., 2012).

The bilateral splanchnic mesoderm layers are patterned along the mediolateral axis as stripes; each stripe contributes to a segment of the myocardium (Fig. 2). The most lateral stripe becomes medial after AIP elongation and contributes to the most caudal segment of the forming heart tube (SV) that is recruited into the heart fields only at later stage (Fig. 2C). The most medial stripe becomes lateral after AIP elongation and contributes to the most cranial segment of the forming heart tube, namely the OFT (Stalsberg and DeHaan, 1969; Abu-Issa and Kirby, 2007, 2008) (Fig. 2A–C). Concomitantly, the heart is displaced caudally along the AIP caudal elongation (Waldo et al., 2001). The stripes in between will contribute sequentially to the rest of the myocardial segments (RV and LV, AVC, and RA and LA). The lateral stripes that form the inflow travel a shorter distance and thus contribute to the heart earlier while the medial stripes travel a longer distance and thus get incorporated later into the heart proper (Abu-Issa and Kirby, 2007, 2008). The early semi myocardial tube represents several segments, but is largely composed of ventricular myocardium (Fig. 2B, B′) (Stalsberg and DeHaan, 1969; de la Cruz et al., 1998). The first portion to fuse medially is composed mainly of left ventricular cardiomyocytes (Fig. 2A, A′) which is followed sequentially and bidirectionally (Moreno-Rodriguez et al., 2006) by adjacent

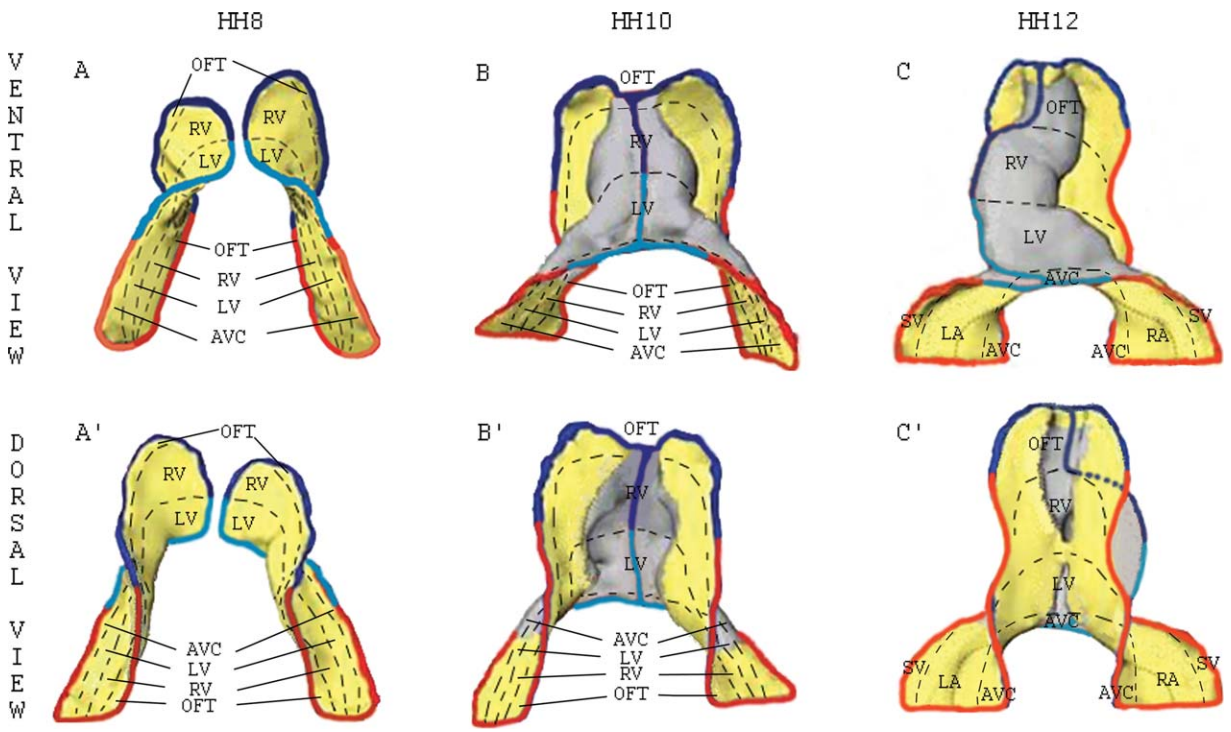

Fig. 2. Patterning and polarities of 3D heart field reconstructions at few relevant chick embryonic stages. (**A, A′**) show dorsal and ventral view of the distinct bilateral heart fields at stage HH8 with different stripes (black dashed lines) that represent the outflow (OFT), right ventricle (RV), left ventricle (LV) and atrioventricular canal (AVC) with ~125° bending that coincide with the anterior intestinal portal (AIP). The edges of the heart fields represent the future heart outer curvature (light and dark blue) and the inner curvature (red). (**B, B′**) show dorsal and ventral view of the cardiac mesoderm as a semi heart tube (gray) and precardiac mesoderm (yellow) at stage HH10. It shows different stripes and outer/inner curvatures as in (A, A′). (**C, C′**) show dorsal and ventral view of the looping heart tube (gray) and precardiac mesoderm (yellow) at stage HH12. It shows strips of the right atrium (RA), left atrium (LA) and sinus venosus (SV) in addition to the stripes and outer/inner curvatures shown in (A, A′). The dorsal view (C′) shows the dorsal mesocardium initial fusion at the midregion. 3D reconstructions are adapted from Moorman et al., 2010 with permission.

segments on both sides giving rise to the outer heart curvature (Fig. 2, blue line). The RV follows cranially and the AVC and the two atria follow caudally resulting in the SV and conotruncus to be added last (Fig. 2B, C). Additionally, the initial heart tube at HH10 (Fig. 2A′) is indeed a semi heart tube where the dorsal roof (dorsal mesocardium) closure initiates later at stage HH12 in the midregion and extends bidirectionally as seen for the ventral mesocardium and gives rise to the inner curvature (Fig. 2C′, red line) (Patten, 1922; Moreno-Rodriguez et al., 2006). The semi heart tube represents only a small portion of the bilateral heart fields that continue to be recruited into the heart proper for several days (HH10-HH22) (Waldo et al., 2001; Mjaatvedt et al., 2001; Waldo et al., 2005). The size and proportion of each segment may differ greatly from one stage to another; particularly for those segments that undergo major morphological remodeling as seen in the myocardial outflow which shrinks over four times during the remodeling process. The OFT elongates from HH12 up to HH24 and shortens afterward until stage HH31 (Rana et al., 2007). Similar examples are seen in the AV canal and sinus venous, which are relatively prominent at early stages and become compact and smaller after remodeling (van den Berg and Moorman, 2011). Similarly, in mice, the bilateral splanchnic mesoderm sheet is also patterned along the mediolateral axis as stripes inferred by the dynamic expression of Fgf10 and Fgf8. (Kelly et al., 2001; Ilagan et al., 2006).

Heart tube formation takes place during major embryonic changes that affect all three germ layers; the embryo is transformed simultaneously from a flat trilaminar (endo-, meso-, and ectoderm) disc in chick, and a trilaminar cup in mouse, into three tubes within a larger fourth tube structure. The three tubes are the neural, gut and heart tubes within the larger body wall tube. The major morphogenetic movements begin with the folding of the ectoderm dorsally, which results in the formation of the neural tube (Smith and Schoenwolf, 1997; Massarwa et al., 2013). The folding of the endoderm ventrally results in the formation first of the foregut, followed by the hindgut. Lastly, the mesoderm continues its semicircular movement that started at the PS using the endoderm as its substrate. The mesoderm is in fact in adherence with the endoderm, thus is passively subject to the folding of the endoderm, as well as to its own active movement. The coordination of the two movements leads to heart tube formation (Varner and Taber, 2012; Gavrilov and Lacy, 2013). The combination of ecto-meso- and endoderm movements leads to the formation of the body wall. Even though each of the three morphogenetic movements of the three germ layers is likely to have its own autonomous mechanical force, the disruption of one would constrain the proper formation of the others either passively when they are in direct contact such the foregut endoderm and cranial splanchnic mesoderm and/or by simple physical hindrance.

HEART FIELDS

Heart fields have been the subject of numerous reviews recently, but there are still unsolved issues and ambiguities concerning the differences and similarities among the several names and roles of the heart fields (Kelly, 2012; Xavier-Neto et al., 2012). The bilateral cardiogenic mesoderm layers that contribute to the heart are specified by multiple positive inductive signals such as members of TGFβs, BMPs, noncanonical WNTs, FGFs, HHs signaling, and by negative signals such as members of the canonical WNTs and retinoic acid signaling from subjacent endoderm, adjacent notochord and overlaying ectoderm (Vincent and Buckingham, 2010). These signals lead to the determination of the cardiogenic region of the splanchnic mesoderm sheet and the expression of a battery of transcription factors including MESPs (Mesp1), NKs (Nkx2.5), GATAs (Gata4 and 6), FOXs (Foxh1, Foxc1 and 2), ISLs (Isl1), HANDs (Hand1 and 2), MEFs (Mef2c), and TBXs (Tbx20) (Lopez-Sanchez and Garcıa-Martınez, 2011; Vincent and Buckingham, 2010). The expression of several of these transcription factors presents a crescent pattern at the head fold stage embryo, which often becomes synonymous with cardiac marker. The crescent pattern is not as simple as it may seem: first, genes are often not expressed in the same pattern but rather in different or partially overlapping crescents. It is difficult to make an accurate judgment due to the lack of fixed morphological landmarks unless two probes are used. An example of this is the nonoverlapping crescent pattern for Isl1 and MLC2a (Cai et al., 2003) and the partial overlapping of Gata4 and Nkx2.5 (Nathan et al., 2008). Second, the crescent may occur exclusively in the splanchnic mesoderm or in combination with other tissues such as endoderm or ectoderm. To determine the exact tissues that express a gene, histological sections are needed. An example, Nkx2.5, which in addition to the LPM, is expressed in the adjacent endo- and ectoderm as well (Abu-Issa and Kirby, 2008; Nathan et al., 2008). Finally, the initial crescent expression of these genes may diverge at later stages, possibly because of the dynamic temporal expression pattern of different cell populations at different times as seen again for Isl1 and MLC2a (Cai et al., 2003). A comprehensive cardiac marker or real pan-heart marker is difficult to find. For instance, Nkx2.5 expression includes most, but not all, heart progenitors at one time/stage, but eventually all heart cells seem to express it (Ma et al., 2008). A more promising pan-heart marker is Baf60c/Smarcd3, a subunit of the BAF chromatin-remodeling complex. Smarcd3 is expressed before the formation of the cardiac crescent in the region predicted to contain cardiac progenitors of the midgastrula mouse embryo and continues to later stages. Lineage tracing of this population specifically labels the entire heart layers and segments, including the endo-, myo-, and epicardium (Lickert et al., 2004; Devine et al., 2012).

Posterior versus Anterior Heart Field

The expression of Fgf10 using a mouse transgenic line carrying LacZ inserted into the Fgf10 locus was quite revealing. Fgf10-LacZ is expressed in a crescent-like pattern at the head fold stage (Fig. 3A). At the heart looping stage, it is expressed specifically in the outflow and RV of the heart proper and in the pharyngeal mesoderm surrounding the outflow, some of which is recruited into the outflow at later stages (Fig. 3B, C) (Kelly et al., 2001). This same expression pattern was also seen for Fgf8, a critical player in outflow and RV formation using a lacZ knock-in line (Abu-Issa et al., 2002; Ilagan et al., 2006). This Fgf10 domain is called the anterior heart field because it labels the anterior or cranial portion of

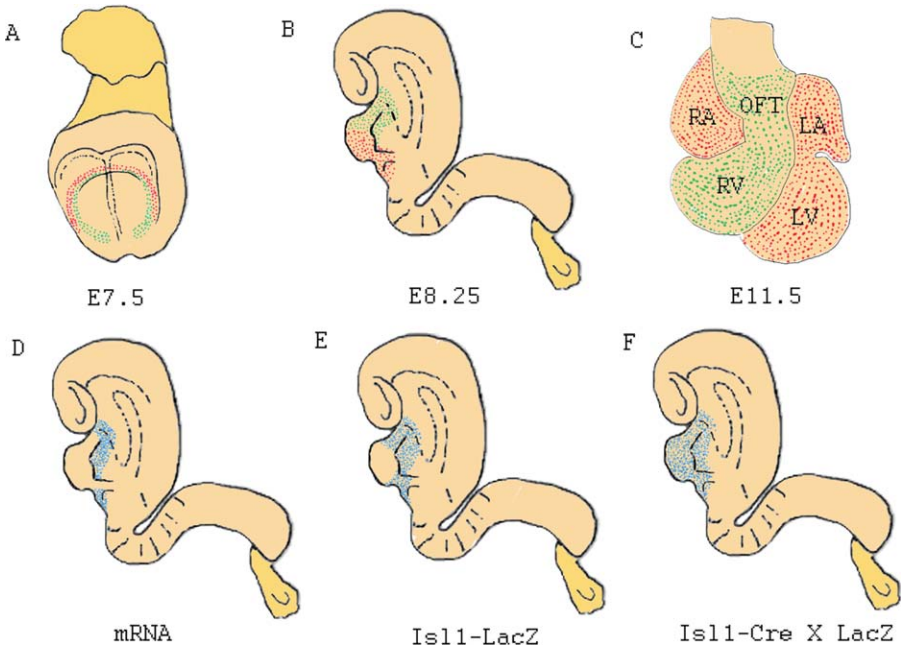

Fig. 3. Schematic representation of gene expression patterns and heart fields during mouse embryonic development. (**A, B, C**) Shows the expression of anterior heart field marker Fgf10 revealed by LacZ insertion depicted as green dots and posterior heart field marker Tbx5 depicted as red dots at three different representative stages. (A) Shows an E7.5 embryo with expression of Fgf10 and Tbx5 as two distinct but complementary crescents. (B) Shows an E8.25 embryo with simple heart tube expressing Fgf10 and Tbx5 in the anterior and posterior portion of heart tube, respectively. (C) Shows an E11.5 embryonic heart where Fgf10 is expressed in outflow tract (OFT) and right ventricle (RV) and Tbx5 in the left ventricle (LV), left atrium (LA) and right atrium (RA). (**D, E, F**) shows the expression pattern of Isl1 depicted as blue dots in an E8.25 embryo. (D) Shows the Isl1 expression in the heart surrounding mesoderm revealed by mRNA *in situ* hybridization. (E) Shows Isl1 expression in the heart surrounding mesoderm and outflow tract revealed by Isl1-LacZ knock-in. (F) Shows Isl1 expression in both heart proper and surrounding mesoderm revealed by lineage tracing approach using Isl1-Cre line crossed with a LacZ reporter line.

the heart tube (outflow + RV) but not the posterior or caudal portion (LV +two atria + SV) (Kelly et al., 2001). The expression of Tbx5 was found to be complementary to Fgf10. Tbx5 is expressed first in a crescent which is nonoverlapping with that of Fgf10 at head fold stages (Fig. 3A), then in the caudal portion of the heart proper and in the surrounding mesoderm (Fig. 3B, C); Tbx5 is required for inflow formation (Bruneau et al., 2001). These two heart portions, the anterior (cranial) and the posterior (caudal), have clear physical borders and genetic programs that define each following specification and determination of precardiac mesoderm. The anterior and posterior fields of the heart are also affected by the more general craniocaudal body axis program (Hochgreb et al., 2003; Bertrand et al., 2011). It is important not to confuse the posterior heart field with the "primary" heart field. The primary heart field is defined as the first cells to differentiate in the cardiac crescent, which will give rise to the early heart tube as seen with sarcomeric myosin (MF20) expression pattern (Han et al., 1992). In this manner, it is similar to the first heart field as explained presently in the next paragraph.

First versus Second Heart Field

Heart lineage analysis in mice produced intriguing results. During or before gastrulation, heart lineages split into two that possibly belong to a common lineage originally; the first lineage gives rise to the major portion of the LV and some cells of the two atria, whereas the second lineage gives rise to the outflow, RV, both atria, and some cells of the LV. The two lineages intermix partially leaving no clear border between the two; the former moves into the heart relatively earlier than the latter and the two lineages were called first and second heart fields, respectively (Meilhac et al., 2004). The significance of this lineage split remains enigmatic, but expression of Isl1 offered hope to solve this mystery because the Isl1 lacZ knock-in is expressed in the heart outflow, RV and partially in the two atria at the heart tube looping stage (Fig. 3E) (Cai et al., 2003) overlapping reasonably well with the second heart lineage. However, at the same stage, Isl1 mRNA is expressed, instead, in precardiac mesoderm and not in any segment of the heart proper using *in situ* hybridization (ISH) (Fig. 3D). The mRNA expression pattern of Isl1 (but not the lacZ insertion) is complementary to the expression of differentiating marker atrial myosin light chain 2 (MLC2a) from the early head fold stage embryo with two clear complementary crescents and continues to be complementary until all precardiac cells differentiate into cardiomyocytes. Isl1 is expressed in the precardiac mesoderm and is required to keep them in an undifferentiated and proliferative state (Cai et al., 2003). Cre and reporter lineage tracing studies showed conclusively that Isl1 is expressed in all the heart cells (Fig. 3F) (Sun et al., 2007; Ma et al., 2008). The Cre lines trace not only all the cells that expressed the gene at

any time during their lifespan but also their descendants as well. The expression pattern of Isl1 lacZ insertion is intermediate between the pattern of Isl1 mRNA and the pattern using Cre line; this may reflect the longer half-life of lacZ protein relative to the Isl1 mRNA molecule and/or ISH lower sensitivity, a similar scenario has been observed for both Fgf10 and 8 (Kelly et al., 2001; Ilagan et al., 2006). The discrepancy between the expression patterns for Isl1 generated using ISH and LacZ staining on one hand, and that of Cre lineage tracing on the other, argues strongly for the transient expression of Isl1 in all precardiac mesodermal cells before they differentiate into cardiomyocytes. A similar scenario was found for Fgf10, Fgf10 is expressed in the heart progenitors and repressed when these cells enter the heart outflow as differentiating cardiomyocytes (Watanabe et al., 2012). Although there is no exact spatial overlap between Isl1 mRNA expression domain and second heart field as defined originally by lineage, the fact that the Isl1 expressing cells are recruited into the heart in sequential temporal waves implies temporal correlation between the two. MLC2a and Isl1 expressing fields are complementary to each other and define a temporal aspect of heart development. Their expression domains have been called first and second heart fields, respectively. In this definition, the primary heart field overlaps with the first heart field (Buckingham et al., 2005). In the chick, lacking similar transgenic lines, it is not possible to make such accurate predictions, but the expression pattern of Isl1, Tbx5 and differentiation markers are consistent with what is observed in mice (Yamada et al., 2000; Yuan and Schoenwolf, 2000; Nathan et al., 2008).

The dynamic temporal aspect of heart formation is of paramount importance for understanding the underlying processes involved, as well as, the phenotypic outcomes of mutations disrupting these processes. Heart progenitor cells proliferate and differentiate in time and space in a highly coordinated fashion. These two processes are somehow mutually exclusive; each limits the other. The proliferation versus differentiation dichotomy is manifested in low proliferation and high differentiation of cells in the early heart tube proper and in high proliferation and low differentiation in those cells that are awaiting outside the heart proper (Cai et al., 2003; van den Berg et al., 2009; de Voer et al., 2012). The proliferation increases dramatically later in the heart chambers as they are ballooning out (Moorman et al., 2010).

Disruption of such delicate balance seems to underlie several mutations with heart phenotypes. In addition to Isl1, Nkx2.5 controls the spatiotemporal switch between proliferation and differentiation states of heart progenitor cells (Prall et al., 2007), and Hand1 is also involved in the balance between proliferation and differentiation in the developing heart (Riserbro et al., 2006). In Nkx2.5 and other cardiac mutants, cardiogenesis seems to initiate but is somehow blocked too early during development, leading to the formation of a reduced pool of cardiogenic precursors and a truncated heart. In these mutants, cardiac genes are often only downregulated or upregulated and/or expressed in a smaller domain but not completely abolished (Lyons et al., 1995). It is possible that within these mutants, the program for cardiogenesis begins but then fails too early somewhere down the line. One may ask how to explain the initiation of cardiogenesis? It is possible that the proteins encoded by cardiac genes act cooperatively and have overlapping functions; the knockout of multiple genes may be required to completely abolish cardiogenesis. Double knockouts support such possibility because they show enhanced mutant heart phenotypes such as Nkx2.5; Mef2c double KO (Vincentz et al., 2008) and close interaction between them (Clark et al., 2013). An alternative possibility is that other genes may be required at earlier stages for cardiogenesis.

Secondary and Other Heart Fields

Another heart field is the secondary heart field, which is defined by a small mesodermal population that is added to the truncus cardiomyocyte and to the smooth muscle cells of the base of the great arteries or aortic sac (Waldo et al., 2001; Dyer and Kirby, 2009). Using Cre-based lineage tracing, Tbx1 labels the pharyngeal mesoderm and heart outflow. It plays a role in both growth and differentiation of cells of the outflow (Xu et al., 2004) and the heart expression domain overlaps closely with the secondary heart field described earlier. Other expression patterns are also restricted to a portion or segment of the heart and play a specific role in the development of that segment. Similarly, some of the same genes seen earlier that play a wide role initially, have a more restricted role later on. Examples of such subregions are seen with Tbx2 and Tbx3 expression domains. Tbx2 is expressed in both the OFT and AVC, and Tbx3 is expressed only in the OFT. These two genes play critical roles in the formation of the subregions where they are expressed (reviewed by Greulich et al., 2011). Further examples of subregions are the two atria, with each atrium deriving from splanchnic mesoderm of the same side (ipsilaterally); RA derives from splanchnic mesoderm of the right heart field, and the LA derives from splanchnic mesoderm of the left heart field (Abu-Issa and Kirby, 2008; Galli et al., 2008). Recently, a "Third" or "Tertiary Heart Field" that represents the precursor of chick pacemaker cells is found to derive from a posterior region of the heart fields mentioned previously and seem not to expresses the same set of genes (Bressan et al., 2013).

CONCLUSIONS

Our understanding of early heart development made strides in the last decade, but there is still a long way to a comprehensive understanding of this complex process. The heart tube is formed in a dynamic fashion, both in physical and temporal dimensions along with other structures such as the neural, gut, and body wall tube. The importance of the temporal aspect of the embryonic movements cannot be overemphasized; every cellular process and movement must happen at a precise location and time in order for the embryo to form properly. The heart fields constitute initially one field (Abu-Issa et al., 2004), which is then subdivided into smaller and smaller domains or subregions, both spatially and temporally. The most prominent subdivision is the division of the heart tube into the anterior and posterior portions. An unusual subdivision is a temporal one; the MLC2a and Isl1 transiently expressing domains designated as the first and second heart fields, respectively. The

understanding of the first and second fields adopted by this article addresses the confusion encountered in the literature dealing with different heart field names and roles especially regarding the relationship between second and anterior heart fields. As established in this article, the former is a temporal whereas the latter is a physical field, but as the second heart field has a physical aspect to it, the precardiac cell population that express Isl1 transiently, the anterior heart field has a temporal aspect to it as it travels a longer distance and thus is added later into the heart proper relative to the posterior heart field. The novel understanding of the first and second fields also suggest that the lineage split in early precardiac mesoderm plays a role in temporal regulation of heart development. Finally, smaller subdivisions are observed in which smaller portions of the heart express and are controlled by specific genes. These examples show that the heart formation undergoes a long chain of events; each built on the previous one and advances another process that is smaller in extent. Heart formation goes through a hierarchical cascade that is tightly connected and coordinated which starts with the specification of the whole cardiogenic mesoderm during gastrulation and extends to the most specialized process such as the aortic/pulmonary septation at much later stages.

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