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Review

Heart fields: one, two or more?

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In the mouse, the earliest heart precursors can be traced to approximately 50 founder cells located on both sides of the midline in the epiblast of early gastrula stage embryos (E6.5) (Tam et al., 1997). During gastrulation, the heart precursor cells are confined to the anterior and middle primitive streak, and migrate with other cells that will form the definitive endoderm and the mesodermal germ layers (Garcia-Martinez and Schoenwolf, 1993). After their ingression, they rapidly move laterally and cranially until they assume residence in the lateral plate mesoderm (Yang et al., 2002). The heart founders located here, bilaterally in the anterior–lateral plate mesoderm have been designated the primary heart fields.

The primary heart fields were characterized in chick embryos by Rawles (1943) who first determined which cells in the mesoderm had the potential to form myocardium (Rawles, 1943). Lineage tracing studies have been carried out by several groups including Garcia-Martinez and Schoenwolf (1993), Lopez-Sanchez et al. (2001), Rosenquist and DeHaan (1966), and Stalsberg and DeHaan (1969). The primary heart fields were also defined molecularly using cardiac expression markers such as Nkx2.5 and Gata4. Recently, a study using dye marking and molecular expression in the same embryos demonstrated that there is no exact correspondence of the two types of labeling, adding more complexity to the definition of the primary heart fields in the chick (Redkar et al., 2001). In addition, while the earlier studies of Rosenquist and DeHaan (1966) found a spatiotemporal correspondence between the anterior–posterior polarity of the heart field and the heart tube as early as stage 5 in chick, Redkar et al. (2001) did not find such polarity until stage 8, which is just before fusion of the bilateral heart primordia. Thus, although the existing data are not necessarily incompatible much remains to be known about the primary heart fields.

After the cardiac precursor cells take up residence in the lateral plate mesoderm as the primary heart fields, they are segregated into the splanchnic layer of lateral plate mesoderm (Linask, 1992; Linask et al., 1997). The splanchnic layer lies adjacent to the endoderm which is thought to provide inductive signals to begin myocardial differentiation (Lough and Sugi, 2000). The cranial most parts of the heart fields are then drawn to the midline by complex morphogenetic movements at the anterior intestinal portal creating the cardiac crescent, a horseshoe-shaped ‘‘field’’ composed of the bilateral heart fields joined in the midline (Fig. 1). In the chick, a considerable period elapses between formation of the heart fields [Hamburger-Hamilton stage (HHS) 5] and appearance of the cardiac crescent (HHS 8); however, in the mouse, the time between formation of the heart fields and cardiac crescent is short and therefore the bilateral unconnected heart fields are difficult to see (Abu-Issa, unpublished observation). As the anterior intestinal portal continues to move caudally forming the foregut pocket, the heart fields are brought together in the ventral midline forming the cardiac tube that begins to beat and loop almost immediately. Several recent reviews have been valuable in focusing on different aspects of early heart development (Brand, 2003; Kelly and Buckingham, 2002).

Early studies of heart development using iron oxide particles to mark different locations of the forming heart in chicks have shown that elongation of the heart tube results not only from expansion of the tissue already in the tube but also from progressive addition of cells to both the arterial (outflow) pole and to a lesser extent the venosus (inflow) pole (Stalsberg and DeHaan, 1969). A more focused study of the arterial (outflow) pole using a similar marking technique also showed that the majority of the truncus (cranial part of the outflow) is added during looping, which occurs between stages 13 and 22 in chick embryos (De la Cruz et al., 1977).

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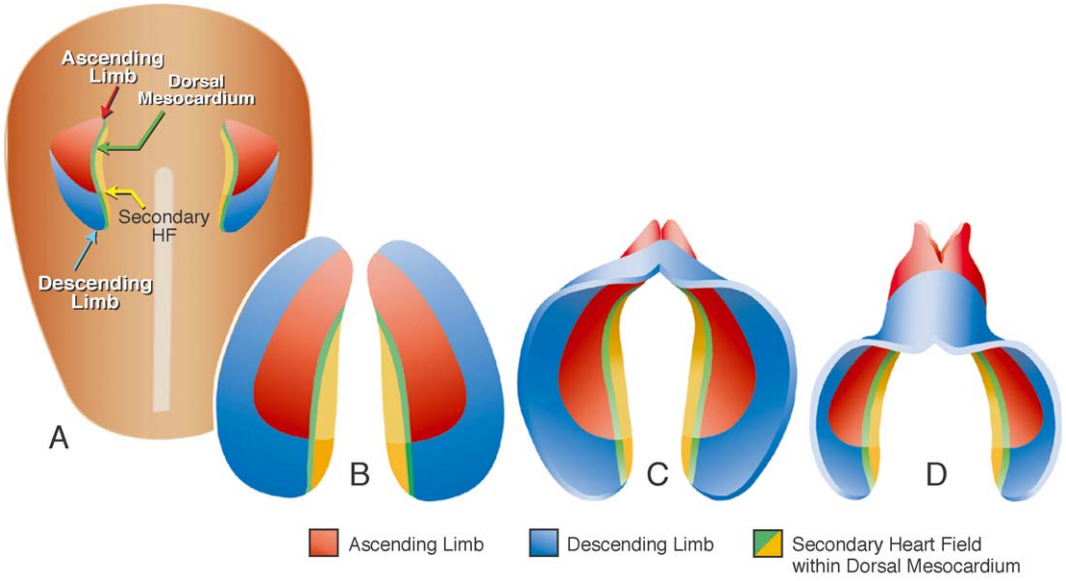

Fig. 1. Schematic representation, using both chick and mouse data, of heart development from the early gastrula stage to early heart tube stage with emphasis on the embryonic components of the heart (color-coded) derived from the "heart field". (A) Gastrula stage. The heart field is formed by continuous migration of the mesodermal cells through the primitive streak into bilateral positions. (B) Headfold stage. The heart field is still a flat layer of splanchnic mesoderm where cells move medially and the components of the heart change positions relative to each other. (C) Cardiac crescent stage. As the heart fields converge medially, the descending limb fuses first. The cells that will form the anterior limb become cranial as the descending limb folds 180° caudally. (D) Early heart tube stage. The folding continues caudally giving rise to the heart tube with the ascending and descending limbs in a cranial/caudal position.

In all of the marking studies to identify the primary heart field done to date, the embryos have been explanted from the yolk in a technique known as New culture. Although the technique provides clearer visibility both in placing the labels and in following the labeled cells, embryos are not viable past 48 h in these cultures. Indeed, most studies have not been carried out past stage 12. (Chapman et al., 2001; New, 1955). The significance of terminating the experiments at stage 12 has become apparent since the discovery of the addition of the outflow myocardium between stages 13 and 22 by De la Cruz et al. (1977) and confirmed by Waldo et al. (2001). We therefore do not know the relationship of the cell source for the outflow myocardium to the primary heart fields.

An early study in mice showed that the cells added to the outflow myocardium originate in the splanchnic mesoderm where mesenchymal cells differentiate into cardiomyocytes between E8 and E11 (Viragh and Challice, 1973). A second study also based on the morphological analysis of heart development in humans and rats showed that the arterial (outflow) pole myocardium is extended by prepharyngeal mesoderm (de Vries, 1981).

Twenty years later, in three papers using both old and new techniques, it was rediscovered that the elongation of the heart takes place because of the addition of newly formed cells from the surrounding mesoderm (Kelly et al., 2001; Mjaatvedt et al., 2001; Waldo et al., 2001).

Waldo et al. (2001) showed, using marking experiments and quail-chick chimeras, that cells in the pharyngeal mesenchyme caudal to the outflow tract are added to the elongating outflow tract (Fig. 2). This is the same region termed by de Vries (1981) as "prepharyngeal" mesoderm. Gene expression revealed that these progenitor cells of the outflow myocardium recapitulate the expression profile of Nkx2.5 and Gata4 seen in the primary heart field. Using different marking techniques in chick, Mjaatvedt et al. (2001) showed that the definitive outflow tract (truncus + conus) is derived from a rapidly elongating band of cephalic undifferentiated mesoderm, in pharyngeal mesenchyme immediately adjacent and surrounding the distal end of the heart tube (Fig. 2). These investigators also showed that the truncal myocardium still developed after ablation of the primary heart fields, suggesting that this myocardium originates from a distinct heart field. Although this is the strongest evidence to this date that the outflow myocardium originates from cells outside of the primary heart fields, one is left wondering if these ablations could have missed part of the field since the study by Redkar et al. (2001) raises questions about the precise location of the primary heart fields in the chick.

A field of the same name was identified in mice by Kelly et al. (2001), who reported a lacZ transgenic insertion into the mouse Fgf10 locus in which lacZ activity was found in the entire ascending limb of the looped heart, that is, outflow and right ventricle (Fig. 2). The lacZ-positive outflow myocardium was continuous with the splanchnic mesoderm and the mesodermal core of the pharyngeal arches, a similar location to that proposed by Mjaatvedt et al. (2001). Using DiI labeling, these investigators showed that the pharyngeal mesoderm progressively moved into the lengthening heart

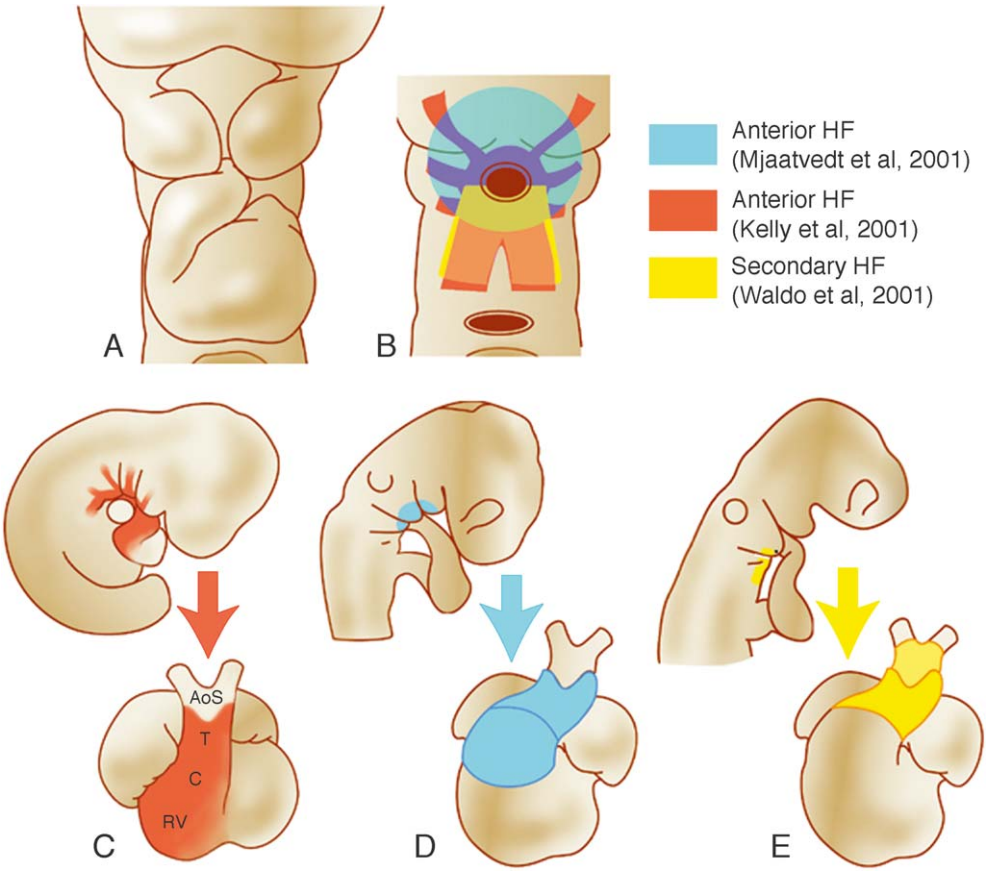

Fig. 2. Schematic representation of the location, extent, and contribution of cells that are added into the heart after the formation of the heart tube. (A) Ventral view of the looped heart of an E9.5 mouse embryo (comparable to chicken: stage 12). (B) Heart removed. The two anterior and secondary heart fields (color coded) are superimposed on each other in a ventral view. Note the color change when two or three colors overlap. (C) Lateral view of a looped heart in a mouse embryo (E9.5) and a ventral view of a four-chambered heart (E11.5). The red area as proposed by Kelly et al. (2001) represents the anterior pole before and after its addition to the heart. (D) Lateral view of a looped heart in a chicken embryo (stage 16) and a ventral view of a four-chambered heart (stage 22). The blue area: as proposed by Mjaatvedt et al. (2001) represents the anterior heart field before and after its addition to the heart. (E) Lateral view of a looped heart in a chicken embryo (stage 14) and a ventral view of a four-chambered heart (stage 22). The yellow area as proposed by Waldo et al. (2001) represents the secondary heart field before and after its addition to the heart. Note that the outflow is divided from anterior to posterior into aortic sac (AoS), truncus (T), conus (C), and right ventricle (RV).

tube. This movement of the pharyngeal mesoderm and the difference seen in the location of Fgf10 mRNA versus beta galactosidase activity suggest that the areas of lacZ expression translocate into and become components of the heart. These data also suggest that a larger population of cells contribute to the outflow tract and right ventricle in mouse as compared to chick via ingression through the outflow connection with the pharynx. Based only on the lacZ expression pattern and without external marking, Kelly et al. (2001) proposed that this field represents a separate population of cells located medial to, but separate from, the primary heart fields. However, since the ‘‘molecular’’ location of the primary heart fields in the chick does not map to the position of cells located by dye tracing experiments (Redkar et al., 2001), combined studies are needed to make this assertion.

In the chick, the cranial part of the right ventricle and proximal outflow (conus) are part of the initial tubular heart, although they appear to be divided from the ventral pharynx somewhat later than the left ventricle and caudal right ventricle (Manner, 2000). The differences in chick and mouse concerning the addition of pharyngeal mesoderm through the outflow may have to do with the more rapid closure of the foregut seen in mouse.

Two different names were given to this newly identified field that constitutes the cells added to the developing outflow limb of the heart tube: the secondary heart field or anterior heart field. These names define somewhat different, but perhaps overlapping areas of embryonic mesoderm (Fig. 2). The most restricted is the secondary heart field which refers to the prepharyngeal mesoderm caudal to the outflow tract (Waldo et al., 2001). More recent studies suggest that this field contributes only the region of transition from the heart to the base of great arterial vessels, that is, the distal myocardium of the outflow tract (probably the truncus in the embryonic heart) and smooth muscle in the tunica media at the base of the mature aorta and pulmonary trunk (Waldo et al., in preparation). The anterior heart field described by Mjaatvedt et al. (2001) is in the same regions

as that described by Kelly et al. (2001) but contributes to the distal conus and truncus similar to that described by Waldo et al. (2001). However, the anterior heart field as described by Kelly et al. (2001) contributes the myocardium of the right ventricle, conus and truncus, that is, the entire ascending limb of the looped heart. The field is much broader than that described as secondary heart field and encompasses not only the "prepharyngeal mesenchyme" but also the lateral and more anterior splanchnic mesoderm that extends into middle of the cranial pharyngeal arches. The secondary heart field defined by Waldo et al. (2001) contributes to a very limited region where the distal myocardium meets the smooth muscle of the base of the aorta and pulmonary trunk while the anterior heart field defined by Kelly et al. (2001) forms the right ventricle and conus.

Interestingly, it has recently been shown that a mutation in mouse Isl-1 results in embryonic hearts lacking the outflow limb (conus + right ventricle) and also partially the atria (Cai et al., 2003). It is not known if the truncus is also missing in these mice. Expression and lineage tracing data show that Isl-1 is expressed in a more medial/posterior field relatively to cells expressing myosin light chain 2a (MLC2a) at E7.0, and that these cells move cranially and are added mainly to the outflow limb of the looped heart although some also end up in the inflow limb (Cai et al., 2003). This pattern mimics that seen in the Fgf10-lacZ knock-in and supports the idea that there may be differential mediolateral specification of cells within the primary heart field.

The differences between secondary and anterior heart fields may reflect simply the differences of the stages analyzed, and the tissues and the techniques for cell marking. Regardless, the results converge on a unifying principle, namely that the tubular or preseptation heart is composed of two different segments which form an inflow and an outflow limb. The outflow limb is further augmented by the cells that form the junction of the arterial pole of the heart with the arterial vascular system. If all the cells originate in the primary heart fields, these differences reflect an unsuspected and complex patterning of these fields such that cells in different locations are set aside early in development to contribute to the formation of the different parts and segments of the heart. A great deal of molecular data has accrued in the last few years suggesting that regional differences in myocardial cells are established early (Christoffels et al., 2000; Franco et al., 2000). The current data argue that the regional differences are established even earlier than originally suspected. However, the data from the Isl-1 null mouse also argue that the story may be more complex than a simple model of the field being divided into a prospective inflow and outflow limb but instead that the heart is constructed from several populations in the primary heart field that converge in a coordinated fashion from different locations and at different times with different programs and give rise to this amazing organ (Fig. 1). In any case, until tracing studies show that there are more than a single "heart field", the current evidence points to complex patterning in the primary heart field rather than the existence of multiple fields. If this is true and there is only a single primordial heart field with various subdivisions, then the task at hand is to find a common language to characterize these regions.

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