Landmarks and Lineages in the Developing Heart

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One of the overarching concepts underpinning mammalian embryonic development is that of “regulation.” This implies that the lineage potential of a particular cell is broader than its actual fate. Although the influential experiments in this area performed by Hans Driesch in the late 1800s concerned the fate of individual embryonic blastomeres, the concept of regulative development has pervaded all aspects of embryology, overlapping with the concept of regenerative fields. In the context of the heart, a good example is that of frog embryo cells fated to form the dorsal mesocardium and dorsal pericardial (splanchnic) mesoderm but which can “regulate” and form myocardial tissue if the normal heart is injured or extirpated.1 As we delve into the molecular mechanisms of developmental biologists, no doubt influenced by the homeotic (HOMC) genes (Figure, A). This notion has been perpetuated as fact in embryology and medical textbooks with very little experimental support, indeed, in the fruit fly, the dorsal vessel, equivalent to the embryonic left (systemic) ventricle, with contributions also to other regions. Cells that arise from a distinct “second” lineage are added progressively from a dorsal proliferative center (the second heart field) to the poles of this early heart tube which serves as a scaffold. Within the second lineage, different cellular and oriented proliferative behaviors, and gene expression patterns, arise, depending on the stage of differentiation and site of deployment at the inflow or outflow poles. This lineage contributes to most regions of the heart but not to the embryonic left ventricle. Thus, although lineage restriction does play a role in heart development, myocyte clonal growth patterns in the heart are largely inconsistent with a lineage-based segmental prepattern for heart chamber formation.3 Recent evidence also suggests that some myocardial cells of the embryonic heart derive from the proepicardium, a distinct progenitor organ that separates early from the heart fields and gives rise to the outer epicardial layer of the heart and, subsequently, the coronary blood vessels.4 However, such a myocardial contribution is still controversial.5

In this issue of Circulation Research, Aanhaanen et al highlight an interesting complexity in the way myocardial cells are patterned in the forming heart tube.6 At the heart of the issue is how cardiac chamber and nonchamber myocardium come into being? There has been great progress in this area over the last decade and a half, to the extent that we now have a fairly clear notion of the anatomic building blocks of the heart and the first insights into the conserved transcription factor pathways that guide heart specification and morphogenesis. Growth of the early heart is extremely dynamic, with a proliferating undifferentiated precursor pool contributing cells to both inflow and outflow poles of the initial heart tube over a period of ~2 days (in the mouse). Fate mapping studies using Cre recombinase technology and a novel retrospective lineage technique show that this dynamic behavior has, at its base, an early lineage segregation (Figure, B), dating to a time at or likely before the specification of heart cell types.1 These experiments show that the initial heart tube forms from a precursor population that is quite distinct from the cells subsequently added. This “first” lineage begins to differentiate early while still within its crescent-shaped progenitor pool. Subsequently, this epithelial structure fuses to form the initial myogenic heart tube, which adopts a lower proliferative index and contributes cells mainly to the anatomic left (systemic) ventricle, with contributions also to other regions. Cells that arise from a distinct “second” lineage are added progressively from a dorsal proliferative center (the second heart field) to the poles of this early heart tube which serves as a scaffold. Within the second lineage, different cellular and oriented proliferative behaviors, and gene expression patterns, arise, depending on the stage of differentiation and site of deployment at the inflow or outflow poles. This lineage contributes to most regions of the heart but not to the embryonic left ventricle. Thus, although lineage restriction does play a role in heart development, myocyte clonal growth patterns in the heart are largely inconsistent with a lineage-based segmental prepattern for heart chamber formation.3 Recent evidence also suggests that some myocardial cells of the embryonic heart derive from the proepicardium, a distinct progenitor organ that separates early from the heart fields and gives rise to the outer epicardial layer of the heart and, subsequently, the coronary blood vessels.4 However, such a myocardial contribution is still controversial.5

In this issue of Circulation Research, Aanhaanen et al highlight an interesting complexity in the way myocardial cells are patterned in the forming heart tube.6 At the heart of the issue is how cardiac chamber and nonchamber myocardium come into being, if not by segmental or clonal restriction mechanisms. Existing genetic and gene expression evidence suggests that the specialized chamber myocardium of the atria and ventricles forms progressively from “primary” myocardium in regional domains along the growing heart tube.7 Such specialization involves activation of distinct genes, including Nppa/Anf, Smpx/Chisel, and Atp2a2/Serca2a, not expressed in cardiomyocytes of the early heart tube, sometimes referred to as “less differentiated” although...
clearly contractile. The heart tube is also less mature in that it has not developed trabeculae. Specified chambers become trabeculated and come to dominate the heart through selective growth only later. The remaining primary (nonchamber) myocardium adopts a distinct fate, becoming the less-specialized myocardium of the inner curvature, elements of the proximal conduction system (sinoatrial and AV node and His bundle), inflow and outflow vessel myocardium, and fibrotic tissue of the AV junction. Homozygous mutation of several mouse cardiac transcription factors, including the homeodomain factor Nkx2–5 and T-box factors Tbx5 and Tbx2, lead to formation of a rudimentary heart tube in which formation of chamber myocardium is blocked.

The family of T-box transcription factors play key roles in establishing the initial pattern of chamber and nonchamber myocardium, and different family members appear to work in an antagonistic fashion. A synergistic activity between Tbx5 with Nkx2–5 promotes expression of chamber genes, whereas the repressor Tbx2, expressed in nonchamber myocardium, competes with Tbx5 for interaction with Nkx2–5, forming a repressive complex that inhibits expression of chamber genes. The apparent restriction in the expression of Tbx2 and its upstream inducers Bmp2 and -4, to nonchamber myocardium, suggests that these factors play a key role in imposing nonchamber fate on the early heart tube.

In a careful study using Cre recombinase technology and mutant mice, Aanhaanen et al have now discovered that the initial Tbx2 expression domain encompassing nonchamber myocardium of the AVC can also give rise to a significant portion of the basal part of left ventricular chamber myocardium (Figure, C).6 De la Cruz had also reported additional later contributions to the inlet and outlet regions of the avian ventricles.8 Aanhaanen et al show in the mouse that this contribution had not occurred in the heart tube at embryonic day 9.25, but was evident at embryonic day 10.5 at the beginning of ventricular expansion. Cells that had expressed Tbx2 also extend further into the right ventricle. The findings in this article indicate that the primitive left ventricle mainly contributes to the apical part of the definitive left ventricle, proximal to, and including part of, the intraventricular septum. Given that the primitive left ventricle occupies a larger region than the primitive AVC, and has a higher proliferative index once chamber specification has occurred, it is certainly surprising that its contribution is ultimately smaller that that of AVC derived cells. To clearly assess the complementary contribution of the primitive ventricles and the degree of overlap with AVC derived cells, it would be interesting to systematically mark these cells in the early heart tube and follow their descendants. As far as clonal considerations are concerned, the AVC is constituted by cells of both first and second lineages, so that cells from this source that later invade the left ventricle may be of either origin. There is no suggestion that the AVC deployment represents a special progenitor pool. Rather, the work demonstrates that the initial Tbx2 expression domain does not faithfully define nonchamber myocardium and, as suggested by the lineage studies discussed above, that boundaries between chambers and their flanking regions are not clonally restricted, nor are they rigidly defined by signaling mechanisms at this early time. Formation of chamber/nonchamber boundaries in the heart is therefore regenerative and may occur in equal part by progressive restriction of cell dispersal and by changing signaling gradients. Indeed, transcription factors Hesr1 and Hesr2, which in some circumstances can act downstream of Notch signaling, have been implicated in the formation of the AVC boundary by repression of Tbx2 (Figure, C). We do not know exactly when the left ventricle/AVC boundary becomes fixed, but these studies suggest that the junctional region of the Tbx2-positive AVC at embryonic day 9.25 is coopted to chamber myocardium. This implies a positive pathway for chamber induction. The fact that the right ventricle was labeled also argues that specialization of chamber myocardium from...
primary nonchamber myocardium (in this case the outflow region) is a positive and progressive process and does not simply occur through imposition of a nonchamber state on a default chamber state by expression of Tbx2. Other observations, based on the use of Cre recombinase–expressing strains in which Cre is thought to mark only the second lineage, also showed more extensive marking of the heart at later stages, possibly also reflecting a similar expansion into chamber myocardium.10–12

Aanhaanen et al6 rightly suggest that errors in development of the early AVC may affect a larger region of the heart than anticipated, and the findings of the authors will be important for interpretation of congenital heart disease phenotypes and those in mouse genetic models. A final reflection, then, is that the early AVC is an ill-defined entity, in fact merely a constriction at best. It may be better to consider only that the definitive AVC is formed from cells that lie within the initial Tbx2 expression domain, which at the earliest time of heart tube formation covers, we now know from this study, a much broader domain than that of AVC precursors.

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**Disclosures**

None.

**References**


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