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Dynamic positional fate map of the primary heart-forming region

Cheng Cui [a,1], Tracey J. Cheuvront [a], Rusty D. Lansford [b], Ricardo A. Moreno-Rodriguez [c], Thomas M. Schultheiss [d], Brenda J. Rongish [a,*]

[a] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160, USA
[b] Biology Division, California Institute of Technology, Pasadena, CA 91125, USA
[c] Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC 29425, USA
[d] Department of Anatomy and Cell Biology, Technion-Israel Institute of Technology, Haifa 31096, Israel

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ABSTRACT

Here we show the temporal–spatial orchestration of early heart morphogenesis at cellular level resolution, in vivo, and reconcile conflicting positional fate mapping data regarding the primary heart-forming field(s). We determined the positional fates of precardiac cells using a precision electroporation approach in combination with wide-field time-lapse microscopy in the quail embryo, a warm-blooded vertebrate (HH Stages 4 through 10). Contrary to previous studies, the results demonstrate the existence of a "continuous" circle-shaped heart field that spans the midline, appearing at HH Stage 4, which then expands to form a wide arc of progenitors at HH Stages 5–7. Our time-resolved image data show that a subset of these cardiac progenitor cells do not overlap with the expression of common cardiogenic factors, Nkx-2.5 and Bmp-2, until HH Stage 10, when a tubular heart has formed, calling into question when cardiac fate is specified and by which key factors. Sub-groups and anatomical bands (cohorts) of heart precursor cells dramatically change their relative positions in a process largely driven by endodermal folding and other large-scale tissue deformations. Thus, our novel dynamic positional fate maps resolve the origin of cardiac progenitor cells in amniotes. The data also establish the concept that tissue motion contributes significantly to cellular position fate — i.e., much of the cellular displacement that occurs during assembly of a midline heart tube (HH Stage 9) is NOT due to "migration" (autonomous motility), a commonly held belief. Computational analysis of our time-resolved data lays the foundation for more precise analyses of how cardiac gene regulatory networks correlate with early heart tissue morphogenesis in birds and mammals.

Introduction

Avian and human embryonic hearts share similar morphologies (De la Cruz et al., 1977; De la Cruz et al., 1983; De La Cruz et al., 1989; Abu-Issa and Kirby, 2008) and many cardiovascular malformations found in the chicken embryo are similar to those found in humans (Nishibatake et al., 1987). However, unlike mammals, the avian embryo is readily amenable to ex ovo culture and imaging. This optical accessibility allows direct observation of the cellular movements comprising heart formation in a warm-blooded experimental system.

During amniote cardiogenesis, epiblast cells ingress through the primitive streak and the newly formed mesodermal cells move cranio-laterally to segregate into two layers: the splanchnic mesoderm and the somatic mesoderm. At Hamburger and Hamilton (HH) Stage 6 (Hamburger and Hamilton, 1951), as the quail anterior endoderm folds, heart progenitor cells in the splanchnic mesoderm translocate to the midline and fuse to form a tubular heart. As the "foregut", or anterior intestinal portal (AIP), regresses caudally, a primitive heart "trough" is formed; open along its dorsal aspect (Moreno-Rodriguez et al., 2006; Stalsberg and DeHaan, 1969). The anterior and posterior portions of the dorsal roof of the trough continue to close, and the tube elongates bidirectionally (Moreno-Rodriguez et al., 2006) forming a "linear" heart tube between HH Stages 9 and 11. The linear heart tube consists of the apical portions of both ventricles. By HH Stage 11 the heart begins to bulge to the embryonic-right. At HH Stage 12 the beating heart has formed a loop, and is comprised of a proximal primitive outlet, which connects to the two ventral aortae cephalically, the apical portions of both ventricles, the primitive atria and a primitive inlet (De La Cruz et al., 1989).

Beginning with Rawles (1936, 1943), researchers have proposed positional fate maps of the heart-forming regions by observing the location of heart precursor cells at progressive developmental stages (DeHaan, 1963; Garcia-Martinez and Schoenwolf, 1993; Rosenquist, 1970; Stalsberg and DeHaan, 1969; and reviewed in Abu-Issa et al. (2004), Abu-Issa and Kirby (2007), Yutzey and Kirby (2002)). Presumptive molecular "markers" have also been used to identify precardiac cells in the primary heart-forming field (Ehrman and

* Corresponding author. Fax: +1 913 588 2710.
E-mail address: brongish@kumc.edu (B.J. Rongish).
[1] Current address: Laboratory of Developmental Biology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA.

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Yutzey, 1999; Schultheiss et al., 1995; Yuan and Schoenwolf, 2000). Among these, the homeobox gene, *Nkx-2.5* (Evans, 1999; Harvey, 1996; Schultheiss et al., 1995; reviewed in Olson (2006)), Bmp-2 (Schultheiss et al., 1997) and the transcription factor, GATA-4, along with other GATA family members (Jiang et al., 1998; Kostetskii et al., 1999), are commonly acknowledged as markers of cardiogenesis. GATA-4 and Nkx-2.5 are expressed in cardiogenic precursors in a pattern that largely overlaps with the Bmp-2 expression in the endoderm. Nkx-2.5 and Bmp-2 gene expression is observed in a crescent shape pattern at HH Stages 5–8 (Schultheiss et al., 1995), with Bmp-2 expressed more caudally than Nkx-2.5 (Schultheiss et al., 1997; Schultheiss et al., 1995). In contrast, GATA-4 is absent at the anterior midline prior to HH8 (Jiang et al., 1998; Kostetskii et al., 1999).

Numerous other studies have focused on if, and when, precardiac cells are pre-patterned to occupy a certain positional fate in the tubular heart. These studies generated a wide range of hypotheses regarding the spatial and temporal boundaries of the heart-forming region of amniotes. While valuable, this literature is difficult to reconcile into a unified perspective, and underscores the critical need for unbiased, dynamic cell and tissue positional fate maps.

The reliability and interpretation of previous heart fate mapping studies was limited by the existing technical methods of the time. For example, early workers such as DeHaan, Stalsberg and Rosenquist, could not exclusively label mesodermal cells for tracking, fluorescently or otherwise. The use of lipophilic fluorescent dyes, such as DiI, does not permit simultaneously tagging of large numbers of mesodermal cells at distinct locations in the same embryo — which, in turn, precludes computationally mapping their relative anterior–posterior and dorsal–ventral positions at high spatiotemporal resolution (Ehrman and Yutzey, 1999; Hochgreb et al., 2003; Redkar et al., 2001; Xavier-Neto et al., 2001). In short, cellular resolution, "real-time" precardiac cellular displacements were not recorded in previous studies.

Using a precision DNA plasmid electroporation protocol, and our well-established computational time-lapse microscopy, we show the trajectories of cardiac precursor cells as they contribute to forming the tubular heart. A major advantage of this approach is that the technology allows one to "run the movies backward". This important feature permits an observer to identify cells within the definitive linear heart (HH Stage 10) and to follow these cells, or their progenitors, frame-by-frame, backwards in time to their original positions in the mesoderm.

Here we show dynamic positional fate maps of precardiac cells. In doing so, we demonstrate that a bilateral, continuous primary heart-forming region exists at early stages in the avian embryo. More important, we document relative positional changes of precardiac cells as they exit the primitive streak and form the tubular heart (between HH Stage 3+ and HH10). When these empirical positional fate map data are compared with the expression patterns of Nkx-2.5 and Bmp-2 (HH Stages 5 and 10), the results identify a subset of cardiac progenitor cells that do not share overlapping expression patterns with these purported cardiogenic markers — until the tubular heart stage. Importantly, our time-lapse data reveal that the bulk of the precardiac cellular displacements during heart tube formation are driven by long-range tissue convection as opposed to cellular autonomous motility.

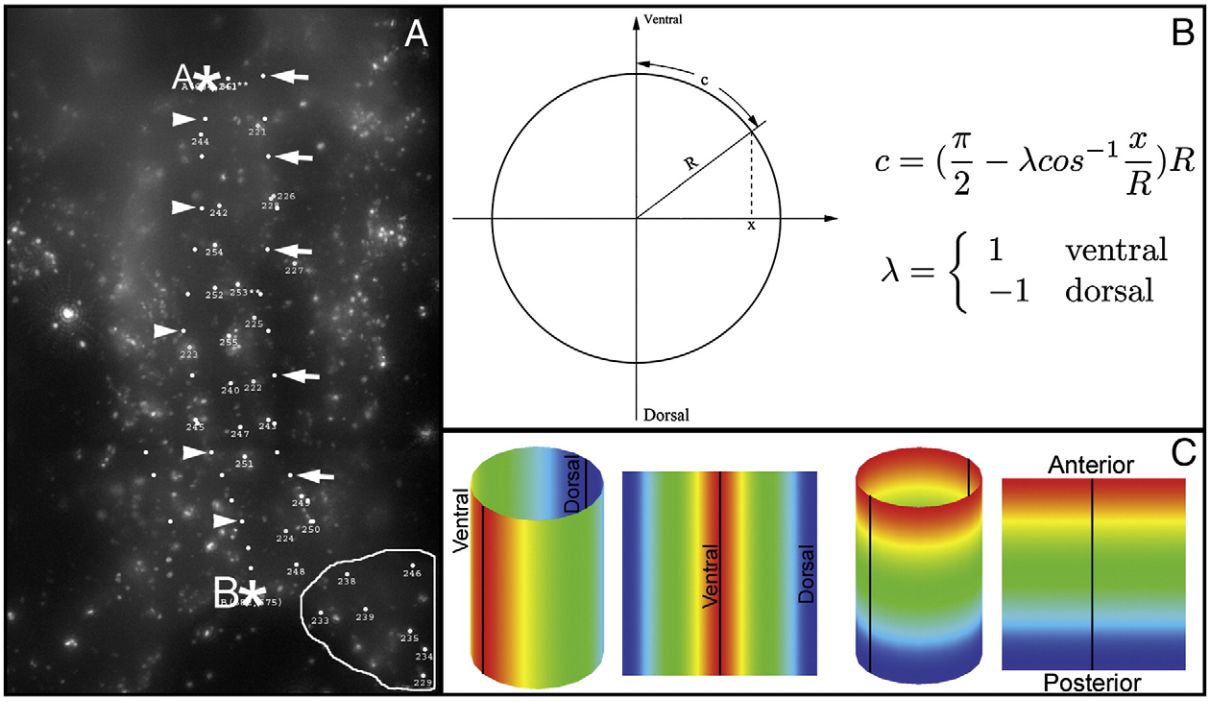

Fig. 1. Colors were assigned to tracked heart precursor cells in quail embryos to denote their positions at HH Stage 10 along both the antero-posterior (A-P) and the ventral–dorsal (V-D) axes. Fluorescent tracked precardiac cells were selected from a population of electroporated epiblastic cells that gastrulated, formed splanchnic mesodermal cells, and came to reside on the surface of the tubular heart. (A) A fluorescent image of an HH Stage 10 heart with tracked cells labeled with grayscale (intensity) values. To simplify, we assume that the heart tube is a cylinder with a radius *R*: defined as the distance from the midline reference points (arrowheads) to lateral reference points (arrows) at successive A-P levels; and that all tracked cells are on the cylinder's surface. The radius *R* represents the longest distance between a lateral point (white arrow) and the corresponding midline point (white arrowhead) at the same A-P level. The radius measurement can vary at different A-P levels. (B) The distance (*x*) between a tracked cell and the midline position of the heart tube at the same A-P level is used to calculate the tracked cell's position on the cylinder surface (*c*) using the equation shown. (C) The tracked cell is then assigned one color according to its position on the cylinder's surface to indicate its relative position along the V-D axis. The color red represents the ventral-most positions; the color blue represents the dorsal-most positions and the color green represents the lateral-most positions. Along the A-P axis, the tracked cells are assigned a separate color according to their positions relative to two reference points: A, the anterior-most point and B, the posterior-most point of the heart tube (see panel A asterisks). The color red represents the anterior-most positions; the color blue represents the posterior-most positions and the color green represents the middle positions. The cells that reside caudal to the heart (future omphalomesenteric veins) by HH Stage 10 (cells in the white circle in panel A) are assigned the color pink (see Fig. 2 and Movie 3).