Right Ventricular Myocardium Derives From the Anterior Heart Field

Stéphane Zaffran, Robert G. Kelly, Sigolène M. Meilhac, Margaret E. Buckingham, Nigel A. Brown

Abstract—The mammalian heart develops from a primary heart tube, which is formed by fusion of bilateral cardiac territories in which myocardial and endothelial cells have already begun to differentiate from splanchnic mesoderm. A population of myocardial precursors has been identified in pharyngeal mesoderm, anterior to the early heart tube. Cell labeling studies have indicated that this novel territory, called the anterior heart field (AHF), gives rise to the myocardial wall of the outflow tract. We now report that not only the myocardium of the outflow tract but also myocardial cells of the embryonic right ventricle are derived from this source. Explants of pharyngeal mesoderm or of the early heart tube were cultured from transgenic mice in which transgene expression marks different regions of the heart. Pharyngeal mesoderm from 5 to 7 somite embryos gives rise to cardiomyocytes with right ventricular and outflow tract identities, whereas the heart tube as this stage has an essentially left ventricular identity. Dil labeling confirms that the early heart tube is destined to contribute to the embryonic left ventricle and indicates that right ventricular myocardium is added from extracardiac mesoderm. Retrospective clonal analysis of the heart at embryonic day (E) 10.5 reveals the existence of a clonal boundary in the interventricular region, which appears during ventricular septation, underlining different origins of the two ventricular compartments. This study demonstrates the differences in the embryological origin of right and left ventricular myocardium, which has important implications for congenital heart disease. (Circ Res. 2004;95:000-000.)

Key Words: cardiac development • anterior heart field • outflow tract • right ventricle • explants

Normal formation of a 4-chambered heart is critically dependent on the early steps of cardiogenesis. These involve the specification and morphogenesis of functional embryonic cardiac chambers, which become remodeled during development of the definitive heart. The initial event in cardiac morphogenesis is the formation of a tubular heart from cardiomyocytes of the cardiac crescent, derived from anterior splanchnic mesoderm (see review1). The linear heart tube is initially orientated along a craniocaudal axis with an anterior outflow (or arterial) pole and a posterior inflow (or venous) pole. Repositioning of the outflow and inflow poles of the heart is brought about by rightward looping, with a dorsoanterior movement of the venous pole behind the developing ventricles (see review2). Cardiac looping in the mouse is initiated at embryonic day (E) 8.25 and is complete by E10.5; during this period, the heart tube grows rapidly in length through the addition of cells to the venous and arterial poles.3,4 At the arterial pole, precursor cells have been shown to originate from a distinct heart field situated in pharyngeal mesoderm, designated the anterior heart field (AHF).5-7

Identification of this novel source of myocardial cells raises a number of major questions concerning early events in heart morphogenesis. In particular, it is important to establish the extent of its contribution to the myocardium. In the chick, labeling experiments have suggested that the linear heart tube is composed of the primordia of the apical region of the right ventricle (cranially) and of the left ventricle (caudally).8 We have investigated this question in the developing mouse heart. Multiple lines of evidence suggest that future right ventricular myocardium is also derived from the AHF. Comparison of the early expression patterns of nlaZ transgenes, which provide unique markers of the future right or left ventricles of the midgestation heart, and explant experiments using these transgenic lines, suggest that the linear heart tube contributes predominantly to the embryonic left ventricle and that only the most anterior region contains future right ventricular cells. Explants of pharyngeal mesoderm give rise to myocardial cells with right ventricular as well as outflow tract identity. Dil labeling of cells, followed by embryo culture and analysis of their ventricular location, supports the conclusion that the AHF contributes to the right ventricle.

Materials and Methods

Transgenic and Targeted Mice

The transgenic lines Mlc3f-2, Mlc3f-9, and Mlc1v-24 have been described previously.9,10 α-cardiac actin nlacZ mice have been
Table 1. No. of Explants Analyzed for Transgene Expression

<table>
<thead>
<tr>
<th>Explant</th>
<th>No. of Somites</th>
<th>Mlc1v-24/TD</th>
<th>Mlc3f-9</th>
<th>Mlc3f-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharyngeal mesoderm</td>
<td>AHF</td>
<td>4/5</td>
<td>2 (+ + +)</td>
<td>3 (+ + +)</td>
</tr>
<tr>
<td></td>
<td>AHF</td>
<td>6/7</td>
<td>5 (+ + +)</td>
<td>3 (+)</td>
</tr>
<tr>
<td>Heart tube</td>
<td>Anterior</td>
<td>5</td>
<td>2 (+)</td>
<td>3 (+ + +)</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>7</td>
<td>2 (−)</td>
<td>3 (+)</td>
</tr>
<tr>
<td></td>
<td>Anterior</td>
<td>7</td>
<td>2 (+)</td>
<td>4 (+)</td>
</tr>
</tbody>
</table>

Results shown in Figures 2 and 5 were consistently obtained with the different transgenes in all experiments listed here. No. of explants and the extent of X-gal staining per beating explant (in parenthesis) are indicated. + + + indicates staining of the complete beating region; −, no X-gal labeling.

Embryonic Explant Cultures

The evening of the vaginal plug was taken as E0.5. After isolation of the embryos from the extraembryonic membranes, somites were counted before dissection. The heart tube alone or pharyngeal mesoderm (including pharyngeal endoderm) was dissected using microdissection scissors in PBS and transferred to culture medium in collagen (1%) coated multiwell dishes. Collagen coating promoted attachment of the explants to the culture well. Explants were incubated for 24 to 48 hours at 37°C with 5% CO2 in 250 μL of minimal Eagle’s medium (MEM) containing 5% horse serum, 100 mmol/L ascorbic acid, and 50 μg/mL kanamycin. After incubation, each sample was washed in PBS, followed by fixation in 4% paraformaldehyde in PBS, and three PBS rinses, before staining for β-galactosidase activity using whole-mount X-gal solution as described. Explant experiments were repeated several times for each transgenic line and tissue point (Table 1). Fluorescent immunohistochemistry was done with the following antibodies: polyclonal anti-β-galactosidase (β-gal) using whole-mount immunostaining. 

Dil Injection

Embryo culture and Dil labeling were performed as described; labeling at about E8.5 was performed lateroventrally, through the yolk sac, amnion, and pericardial wall. At E8 to E8.25 (4 to 7 somites), Dil was injected into the ventral myocardium of the cardiac crescent or of the heart tube, respectively.

Production and Description of nlacZ/nlaacZ Chimeric Hearts

Random clones of myocardial cells were generated spontaneously using the mouse α-cardiac actin/nlacZ transgenic line, as described by Meilhac et al. Briefly, a duplication of a portion of the coding region was introduced into the nlacZ gene, which interrupts the open reading frame. This construct was targeted to the α-cardiac actin locus and is expressed throughout the myocardium. Homologous recombination, which occurs at low frequency, restores the open reading frame of the nlacZ gene, resulting in chimeric hearts containing clonally related β-gal–positive cells that were revealed by X-gal staining as described.

Results

Early Transgene Markers of Right and Left Ventricular Myocardium

The Mlc1v-nlacZ-24 (Mlc1v-24) and Mlc3f-nlacZ-2 (Mlc3f-2) transgenes are expressed in complementary patterns in the midgestation mouse heart (Figure 1a and 1c). The Mlc1v-24 transgene is expressed in the embryonic right ventricle and outflow tract (Figure 1a) as a result of integration of this transgene upstream of the gene encoding fibroblast growth factor 10 (Fgf10), where it is probably the target of an enhancer for regulatory sequences normally acting on the Fgf10 promoter. In contrast, Mlc3f regulatory sequences drive transcription of the Mlc3f-2 transgene (Figure 1c) in the embryonic right atrium and left ventricle, whereas the Mlc3f-nlacZ-9 (Mlc3f-9) transgene (Figure 1b) is expressed in both embryonic ventricles but not in outflow tract myocardium.

The Mlc1v-24 transgene is expressed in pharyngeal mesoderm mainly situated dorsal to the heart tube at E8.75 (Figure 1g). Sagittal sections through an E8.75 heart, after looping has initiated, reveal transgene expression in arterial pole myocardium, which is immediately adjacent to a layer of pharyngeal mesoderm, corresponding to the dorsal mesocardium, where the transgene is also strongly expressed (Figure 1d, arrowhead). This layer of cells does not express the Mlc3f-9 or Mlc3f-2 transgenes (Figure 1e and 1f; arrowhead) and is in continuity with both the arterial and venous poles of the heart tube. These cells form the dorsal wall of the pericardial cavity (Figure 1d, arrowhead), which is contiguous with the heart tube before breakdown of the dorsal mesocardium. The Mlc3f-2 transgene is expressed throughout the heart tube with the exception of arterial pole myocardium (Figure 1i, arrowhead), in a complementary profile to the Mlc1v-24 transgene (Figure 1g). The Mlc3f-9 transgene is expressed throughout the linear heart tube at E8.75 (Figure 1h).

Pharyngeal Mesoderm Gives Rise to Myocardial Cells With Outflow Tract and Right Ventricular Identities

Cell-labeling experiments in chick and mouse embryos have shown that myocardial precursor cells derived from pharyngeal mesoderm participate in the formation of the myocardial wall of the outflow tract. In order to examine the myogenic potential of cells in the pharyngeal mesoderm, lying anteriorly and also dorsally to the early heart tube, we cultured this region from embryos of the different transgenic lines described earlier (Figure 1). The Mlc1v-24 transgenic line marks pharyngeal mesoderm as well as, at later stages, the future right ventricular and outflow tract myocardium. In contrast, the other two lines only mark cells in the heart; Mlc3f-9 expression indicates right and left ventricular identity, whereas that of Mlc3f-2 marks the left ventricle. Explants of pharyngeal mesoderm isolated from 6/7 somite stage embryos (E8.25), as shown in Figure 2a through 2c, contained beating foci of myocardial cells after 36 hours of culture (Figure 2d through 2f). To determine the identity of these myocardial cells, we assayed β-galactosidase activity in...
situ. Explants isolated from embryos carrying the Mlc1v-24 transgene were strongly positive. This transgene is already transcribed in pharyngeal mesoderm; however, β-galactosidase is also detectable in myocardial cells in the explant (Figure 2g). Cardiomyocytes in explants from the Mlc3f-2 transgene were β-galactosidase negative, whereas some cells were positive in the Mlc3f-9 explants (Figure 2h and 2i, Table 1). Because Mlc3f-9 and Mlc1v-24 transgenes are coexpressed in the future embryonic right ventricle, but differ in their expression in the outflow tract myocardium of the embryo (Figure 1), these observations suggest that the majority of myocardial cells observed in such explants have an outflow tract identity. Detection of a small number of β-galactosidase–positive cells in explants derived from Mlc3f-9 embryos (Figure 2h, arrowhead) suggests that a fraction of cardiomyocytes derived from pharyngeal mesoderm at the 6/7 somite stage (E8.5) have a right ventricular identity.

To examine this further, explants were excised at the 5 somite stage (E8.25), 4 hours earlier. When these earlier explants were stained after 36 hours of culture, equivalent β-galactosidase activity was observed in explants isolated from embryos carrying either the Mlc1v-24 or Mlc3f-9 transgenes (Figure 2j and 2k). No β-galactosidase activity was observed in explants isolated from Mlc3f-2 embryos at the 5 somite stage (Figure 2l). Thus, explants cultured from pharyngeal mesoderm isolated at this stage develop a right ventricular identity as indicated by robust expression of the Mlc3f-9 transgene. From these results, we conclude that pharyngeal mesoderm lying dorsal to the differentiated cells of the early heart tube can give rise to myocardial cells that have an embryonic right ventricular identity and that, subsequently, pharyngeal mesoderm is a source of cells with an outflow tract identity.

DiI-labeling experiments, where the dye was injected into pharyngeal mesoderm lying dorsal to the heart tube at E8.5, show that after embryo culture, cells in the outer curvature of the heart form a right ventricular identity. Retrospective clonal analysis was performed using nlacZ/nlaacZ reporter sequence was targeted to the cardiac actin gene. nlacZ is inactive unless it undergoes a rare intragenic recombination event to yield a clone of nlacZ cells, which will be scored as β-galactosidase positive.

Cell Lineage Analysis Reveals an Interventricular Boundary

Based on the observations shown in Figures 2 and 3, we conclude that right ventricular cardiomyocytes arise from the anterior heart field. As a result, it might be expected that the interventricular region represents a lineage boundary within the developing heart. Retrospective clonal analysis was performed using nlacZ/nlaacZ chimerae in which the nlacZ reporter sequence is targeted to the α-cardiac actin gene. nlacZ is inactive unless it undergoes a rare intragenic recombination event to yield a clone of nlacZ cells, which will be scored as β-galactosidase positive.
actin gene is expressed (Figure 4). This genetic approach has already contributed to the study of cell behavior in the developing heart. Two phases of growth have been identified: myocardial precursor cells undergo dispersive growth until the time of heart tube formation when myocardial cell growth becomes coherent. We used the nlacZ method to examine the distribution of β-galactosidase–positive cells in the interventricular region (Table 2).
the embryonic right and left ventricles can be distinguished morphologically by the interventricular sulcus, which marks the site of the forming interventricular septum (Figure 4, arrowheads). Four examples of large clusters in the right or left ventricle illustrate that clusters are typically elongated circumferentially in relation to the heart tube, parallel to the interventricular groove (Figure 4a through 4f). The labeled cells do not extend across the two ventricular territories, suggesting that growth of clusters is restricted with respect to the interventricular septum, as confirmed by sections (Figure 4c and 4d). This observation is consistent with the establishment of a clonal boundary between the two ventricles during the coherent phase of cardiomyocyte growth.

Linear Heart Tube Has a Mainly Left Ventricular Identity

Our observation that right ventricular cardiomyocytes are added to the linear heart tube from the AHF prompted us to investigate the identity of cardiomyocytes within the heart tube using the same transgenic lines. Explants were isolated from different domains of the heart tube. All cells in explants from the central region of the linear heart tube of Mlc3f-9 and Mlc3f-2 transgenic embryos (E8.5) are β-galactosidase–positive (Figure 5b and 5c), whereas cells in explants isolated from the same region of the heart tube of Mlc1v-24 embryos were β-galactosidase negative (Figure 5a). This result suggests that the central part of the linear heart tube displays a

<table>
<thead>
<tr>
<th>TABLE 2. No. of β-galactosidase Positive Clones, Examined at E10.5, Which Abut the Interventricular Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Ventricle</td>
</tr>
<tr>
<td>Large clusters (&gt;40 cells)</td>
</tr>
<tr>
<td>Inner curvature</td>
</tr>
<tr>
<td>Outer curvature</td>
</tr>
</tbody>
</table>

In this analysis where 1155 hearts were examined of which 734 had β-galactosidase positive cells, large clusters are the most informative because they extend into the ventricle on one side of the septum but do not traverse it. Small clones do not have the same extension, but also show oriented growth with respect to the sulcus.
future left ventricular identity. In contrast, explants isolated from the arterial extremity of the linear heart tube, which at this stage lies at right angles to the rest of the tube, when in fact it is at right angles to it, a through c, Explants were excised from the linear portion of the tubular heart at E8.5 (7 somites). β-galactosidase activity was assayed after 24 to 48 hours of culture. X-gal staining reveals that the linear portion of the embryonic heart tube has a left ventricular identity because β-galactosidase activity is observed in both Mlc3f-2 and Mlc3f-9 transgenic explants, whereas no activity is detected in explants from Mlc1v-24 embryos. d through f, Explants dissected from the most anterior portion of the tubular heart at the same stage, β-galactosidase activity is detected in explants derived from Mlc1v-24 and Mlc3f-9 embryos, but not in explants from Mlc3f-2 embryos, indicating a right ventricular identity. g through i, Explants dissected from the most anterior part of this region at the 5 somite stage. X-gal staining reveals a small proportion of cells with right ventricular identity because fewer β-galactosidase-positive cells are detected in explants derived from Mlc3f-2 embryos (i), compared with those with other transgenes (g and h). Note the smaller number of β-galactosidase-positive cells in Mlc1v-24 explants (g, arrowhead) compared with explants from Mlc3f-9 embryos (h), in which the transgene is expressed in both future right and left ventricles.

**Figure 5.** Identity of the linear heart tube. Diagrams on the left side illustrate the stage of the embryos used and the part of the heart tube taken for culture (black). The most anterior region (delimited by a dotted line) is shown in the same plane as the rest of the tube, when in fact it is at right angles to it. a through c, Explants were excised from the linear portion of the tubular heart at E8.5 (7 somites). β-galactosidase activity was assayed after 24 to 48 hours of culture. X-gal staining reveals that the linear portion of the embryonic heart tube has a left ventricular identity because β-galactosidase activity is observed in both Mlc3f-2 and Mlc3f-9 transgen explants, whereas no activity is detected in explants from Mlc1v-24 embryos. d through f, Explants dissected from the most anterior portion of the tubular heart at the same stage, β-galactosidase activity is detected in explants derived from Mlc1v-24 and Mlc3f-9 embryos, but not in explants from Mlc3f-2 embryos, indicating a right ventricular identity. g through i, Explants dissected from the most anterior part of this region at the 5 somite stage. X-gal staining reveals a small proportion of cells with right ventricular identity because fewer β-galactosidase-positive cells are detected in explants derived from Mlc3f-2 embryos (i), compared with those with other transgenes (g and h). Note the smaller number of β-galactosidase-positive cells in Mlc1v-24 explants (g, arrowhead) compared with explants from Mlc3f-9 embryos (h), in which the transgene is expressed in both future right and left ventricles.

To confirm these observations, based on transgene expression profiles, we performed a series of DiI-labeling experiments. The heart was labeled by DiI injection, followed by embryo culture for up to 40 hours, during which time cardiac looping took place (Figure 6). When the medial portion of the ventral myocardium of the cardiac crescent was injected with DiI at the E8.25 stage (Figure 6a and 6b), labeled cells were found in the left ventricle adjacent to the interventricular septum (Figure 6c). Interestingly, a similar result was obtained when the anterior region of the heart tube was labeled at the E8.5 stage (Figure 6d through 6f). When injections were made into the right side of the midportion of the heart tube, after looping has initiated at the E8.5 stage (Figure 6g and 6h), DiI-labeled cells were found in the outer curvature of the interventricular region (Figure 6i). This result shows that the major part of the linear heart tube is fated to give rise to the left ventricle and supports the conclusion, drawn from the explant experiments, that the main identity of the linear heart tube in the mouse is that of future left ventricular myocardium.

**Discussion**

We have investigated the extent to which the AHF contributes to the embryonic heart. Using explant culture coupled with regionalized transgene markers and DiI-injection, we have documented the progressive acquisition of myocardial cells with right ventricular and outflow tract identity as the heart tube, initially composed almost entirely of prospective left ventricular myocardium, elongates anteriorly. A major part of the embryonic heart, including the right ventricle as well as the outflow tract, is therefore derived from precursor cells situated in pharyngeal mesoderm, initially lying dorsally and then anteriorly to the cardiac tube.
This finding was suggested by the expression profile of the Mlc1v-24 transgenic mouse line, where insertion of the transgene adjacent to Fgf10, leads to transcription of the nlacZ reporter in Fgf10 expressing cells of the AHF, and detection of /H9252-galactosidase protein, but not transcript, in the myocardium of the right ventricle, as well as the outflow tract. 5 A recent publication on the Islet 1 mutant mouse shows expression of this gene in pharyngeal mesoderm, lying both anteriorly and dorsally to the developing heart tube, in a profile that overlaps with that of the Mlc1v-24 transgene. 16 A lineage tracing study with an Islet 1 Cre and the Rosa 26 lacZ mouse suggested that these cells contribute to both outflow tract and right ventricular myocardium. Interestingly, there was also a contribution to the venous pole.16 Our observation of nlacZ/nlaacZ chimeric hearts at E10.5 reveals that labeled clusters of clonally related cells are restricted to either left or right ventricular compartments. Furthermore, differential patterns of oriented clonal cell growth show that right and left ventricular cells display differential behavior probably in response to distinct developmental programs during heart formation.17 We have recently completed an extensive retrospective clonal analysis of myocardial cells at E8.5, which demonstrates the existence of two cell lineages: left ventricular myocardium is derived exclusively from the first lineage, whereas the outflow tract myocardium, together with part of the right ventricular (and atrial) myocardium is derived from the second lineage.17 This lineage analysis is in keeping with the results reported in this study on the predominantly left ventricular identity of the early heart tube. However, the observation that some clones span future right and left ventricular territories within the E8.5 heart tube suggests that these are not completely restricted during the earliest stages of heart development, and that the right/left ventricular boundary is established later. Some very large clones extend over the entire length of the cardiac tube pointing to an early common progenitor of the two lineages. The differences between progenitor cell populations that contribute to the heart thus may be more critically temporal, rather than spatial. This clonal analysis, together with the observation on islet 1 and the initial location of cardiac cell progenitors in pharyngeal mesoderm lying dorsal to the cardiac tube, will necessitate reconsideration of the AHF nomenclature.

Using in vivo labeling studies in the chick, de la Cruz and colleagues3,8 mapped the fate of the myocardial component of the linear heart tube. These studies demonstrated that it is composed of two regions, the primordium of the right ventricle, situated anteriorly and the primordium of the left ventricle situated posteriorly. This is in contrast to our results that show that the early heart tube in the mouse embryo is predominantly fated to give rise to the embryonic left ventricle, with the exception of a few cells in the most anterior region. This apparent discrepancy between birds and mammals may be due to differences in the timing of heart tube formation with respect to that of the intraembryonic coelom. In addition, looping is initiated later in the chick embryo than in the mouse and therefore the linear heart tube stage is more evident in the former. The contribution of pharyngeal mesoderm from the secondary,7 or the anterior heart field,6 to the avian cardiac tube is limited to outflow tract myocardium.

At the molecular level, the transcriptional regulation of the Mlc3f-2 transgene, and other transgenes such as Mlc2v-lacZ,18...
underlines the difference between left and right embryonic ventricles in the mouse embryo. In contrast, a gene such as *dHand*, which only becomes restricted to the right ventricle later, does not appear to reflect the different cellular origins of these compartments.\(^{19}\) The *Tbx5* gene, encoding a T-box transcription factor, is interesting in this context. It is initially expressed throughout the cardiac crescent and in the linear tube, with subsequent expression predominantly in the left ventricle (and atria).\(^{20}\) *Tbx5* deficiency in homozygous mice causes severe hypoplasia of posterior segments in the developing heart, with an apparently normal right ventricle and outflow tract.\(^{21}\) Furthermore, in chick or mouse embryos an ectopic expression of *Tbx5* in right ventricular precursor cells gives rise to a heart with a single ventricular chamber with left identity.\(^{22}\) These observations suggest that *Tbx5* is a gene required in the linear heart tube, which has an essentially left ventricular identity, but not in cardiomyocytes derived from the AHF, which contribute to the right ventricle. Expression of *Tbx5* in the atria and single ventricular chamber of the amphibian heart is essential for cardiogenesis.\(^{23}\) In species with a right ventricular chamber and separate pulmonary circulatory system, *Tbx5* function within the left ventricle (and atria) appears to be maintained.\(^{21}\)

The conclusion that the right ventricular, as well as outflow tract, myocardium is derived from pharyngeal mesoderm has implications for the understanding of congenital heart disease. The spectrum of congenital heart defects found in human patients illustrates the modular development of the heart, because a particular region, rather than the heart as a whole, is frequently affected.\(^{24}\) Ventricular hypoplasia of either ventricle is one of the more severe defects encountered in pediatric cardiology.\(^{25}\) Hypoplasia resulting in abnormal physiology of a single ventricle is typical and the unaffected ventricle in these cases is usually morphologically and physiologically normal. This observation underscores the differences in developmental program between right and left ventricular chambers and supports the hypothesis that right and left ventricular morphogenesis is regulated by different genetic pathways reflecting differences in their embryological origins. Defects in ventricular septation are a second class of severe congenital heart defects. Such defects can now be considered to arise at the interface between cardiomyocytes derived from the cardiac crescent and those derived from the pharyngeal mesoderm of the anterior heart field.

**Acknowledgments**

The laboratory of M.B. is supported by the Pasteur Institute and the CNRS and a grant from the ACI Integrative Biology Program of the French Research Ministry. S.M. was supported by a fellowship from the French Research Ministry and the University of Paris. R.K. is an INSERM research fellow. S.Z. is a CNRS research fellow and supported by the British Heart Foundation Program Grant RG/03/ 012. We are grateful to Dr Diego Franco for helpful discussions and Dr Andrew Munk for advice on explant culture. We thank Emmanuel Pecnard for technical assistance.

**References**

8. de la Cruz MV, Sanchez-Gomez C, Palominio MA. The primitive cardiac regions in the straight tube heart (stage 9) and their anatomical expression in the mature heart: an experimental study in the chick embryo. *J Anat.* 1989;165:121–131.
Right Ventricular Myocardium Derives From the Anterior Heart Field
Stéphane Zaffran, Robert G. Kelly, Sigolène M. Meilhac, Margaret E. Buckingham and Nigel A. Brown

Circ Res. published online June 24, 2004;
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/early/2004/06/24/01.RES.0000136815.73623.BE.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the
Editorial Office. Once the online version of the published article for which permission is being requested is
located, click Request Permissions in the middle column of the Web page under Services. Further information
about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/