

Development of the Endocardium

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Abstract The endocardium, the endothelial lining of the heart, plays complex and critical roles in heart development, particularly in the formation of the cardiac valves and septa, the division of the truncus arteriosus into the aortic and pulmonary trunks, the development of Purkinje fibers that form the cardiac conduction system, and the formation of trabecular myocardium. Current data suggest that the endocardium is a regionally specialized endothelium that arises through a process of *de novo* vasculogenesis from a distinct population of mesodermal cardiogenic precursors in the cardiac crescent. In this article, we review recent developments in the understanding of the embryonic origins of the endocardium. Specifically, we summarize vasculogenesis and specification of endothelial cells from mesodermal precursors, and we review the transcriptional pathways involved in these processes. We discuss the lineage relationships between the endocardium and other endothelial populations and between the endocardium and the myocardium. Finally, we explore unresolved questions about the lineage relationships between the endocardium and the myocardium. One of the central questions involves the timing with which mesodermal cells, which arise in the primitive streak and migrate to the cardiac crescent, become committed to an endocardial fate. Two competing conceptual models of endocardial specification have been proposed. In the first, mesodermal precursor cells in the cardiac crescent are prespecified to become either endocardial or myocardial cells, while in the second, fate plasticity is retained by bipotential cardiogenic cells in the

cardiac crescent. We propose a third model that reconciles these two views and suggest future experiments that might resolve this question.

Keywords Endocardium · Endothelial · Heart

Overview of Cardiogenesis

The cardiovascular system is the first organ system to form and function in the vertebrate embryo. The heart forms when regions of bilaterally symmetrical cardiac progenitor cells in the anterior lateral mesoderm fuse at the ventral midline to form a linear tube, which is continuous with the dorsal aorta anteriorly and the cardinal veins posteriorly [5]. The heart tube at the time of its formation is a two-layered structure, composed of an inner endothelial layer and an outer myocardial layer. The myocardial layer will form the muscular walls of the heart and the muscular portions of the interventricular septum. The endothelial layer comprises the endocardium [1, 4, 6, 54].

In addition to forming an endothelial layer within the heart that is contiguous with the rest of the vasculature, the endocardium has several other essential functions during heart development. For instance, the endocardium plays an important role in signaling to cardiomyocytes and is required for proper formation of trabecular myocardium [60, 65]. It is also involved in the signaling required for transdifferentiation of myocytes into the Purkinje fiber cells that comprise much of the cardiac conduction system [41, 42]. In addition, cells from specific regions of the endocardium will undergo endocardial-to-mesenchymal transformation (EMT) to form the endocardial cushions [12]. The endocardial cushions give rise to several important structures within the heart, including the valve

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leaflets, the membranous portion of the interventricular septum, and the atrial septum [51, 57]. In addition, endothelial-derived cells in the cardiac cushions function, along with cells of neural crest origin, to separate the common outflow tract (OFT) into the pulmonary artery and aorta [27, 38, 57]. Despite the many essential functions of the endocardium during development and in postnatal cardiac function, much remains to be learned about the development and embryonic origins of the endothelial progenitor cells that will give rise to the endocardium.

Specification of Endothelial Cells

Vasculogenesis, the de novo formation of blood vessels, occurs necessarily before the onset of blood circulation. In amniotes, endothelial and hematopoietic cells first appear at the same time in the extraembryonic yolk sac and are thought to arise from a common mesodermal precursor, the hemangioblast [48, 67]. Vasculogenesis begins when hemangioblasts coalesce to form blood islands. The outer cells in the blood islands flatten as they differentiate into endothelial cells, while the inner cells differentiate into hematopoietic progenitors [20, 50]. Ultimately, these cells will form the extraembryonic yolk sac vasculature and the blood cells contained within it [20].

De novo vasculogenesis also occurs at multiple discrete sites within the embryo proper, including the paired dorsal aortae, within the heart tube, in the neural plexus, and in the primary vascular plexus [14, 20, 50]. In the embryo, mesoderm-derived endothelial precursors called angioblasts aggregate to form the dorsal and ventral aortae and the vitelline vessels [20]. Endocardial precursors arise via a similar process of de novo vasculogenesis within the cardiac crescent and are subsequently arranged into a vascular tube during ventral morphogenesis [1, 28]. The precise timing of specification of these precursors remains incompletely understood and is discussed below.

Multiple studies in fish, birds, and mammals have suggested an intimate relationship between endothelial and hematopoietic lineages. Lineage-tracing studies performed in zebrafish have revealed that blood cells and endothelial cells are derived from common precursors termed hemangioblasts in the ventral marginal zone of the blastula [33, 58]. In the mouse, hemangioblasts have been identified by coexpression of the mesodermal gene *brachyury* (*Bry*) and the VEGF receptor, *Flk-1* [18]. The relationship between vasculogenic and hematopoietic precursors has recently been found to be even more complex: genetic fate-mapping studies in the mouse revealed that an endothelial-specific Cre transgene resulted in the genetic labeling of a large number of hematopoietic cells in the adult bone marrow [69]. These findings lend support to the concept, previously

suggested by work in avian embryos, of a population of hemogenic endothelial cells that have the capacity to give rise to blood [29, 69].

After formation of the embryonic and extraembryonic vasculature, the initial primitive vessels are extensively remodeled into mature vessels through a complex process termed angiogenesis [20, 49, 50]. In addition, smooth muscle cells or pericytes are recruited to the vessel walls from surrounding mesenchyme [24]. During angiogenesis and vascular remodeling, vessels expand, coalesce, branch, and become specialized as arteries, veins, and capillaries [20, 24, 25].

Embryonic Origin of the Endocardium and Its Relationship to Other Endothelial Cells

The unique location and specialized functions of the endocardium have led some investigators to question whether it represents a unique and molecularly distinct population of cells or rather simply a spatially restricted subpopulation of endothelium. Several genetic models have offered some insight into this question. *Tie* and *Tie2*, two structurally related receptor tyrosine kinases, are required throughout the vasculature during late organogenesis and during postnatal life in mice [63]. *Tie*^{-/-};*Tie2*^{-/-} embryos display normal extracardiac vasculogenesis but have profound cardiac defects, including deficient endocardium, and *Tie*^{-/-};*Tie2*^{-/-} endothelial cells contribute normally to all vascular structures except the endocardium [52]. Similar results were observed in *cloche* mutant zebrafish, which have structurally normal endothelium of the dorsal aortae and cardinal veins, despite the absence of the endocardium [59]. Notably, *cloche* is also required for formation of hematopoietic stem cells, as well as for formation of the endocardium [59]. Importantly, and in contrast to the above examples, no factors or genes have been identified to date to be dispensable for endocardial specification but to be required for endothelial specification elsewhere in the embryo, suggesting that endocardial progenitors comprise a spatially restricted subset of a larger endothelial precursor population.

The endocardium appears to be unique among endothelial tissues in that it arises through de novo vasculogenesis from a population of precardiac progenitors, marked by *Flk-1* expression in the late primitive streak, which also give rise to cardiomyocytes [30]. Recent work has refined our understanding of the clonal origins of the endocardium. Embryonic stem cells derived from an *Nfatc1-nuc-lacZ* bacterial artificial chromosome (BAC) transgenic mouse line were used to generate embryoid bodies, which could be separated into three distinct populations on the basis of expression levels of E-cadherin (ECD) and *Flk1*:

$ECD^{high}Flk1^{low}$, $ECD^{low}Flk1^{high}$, and $ECD^{low}Flk1^{low}$ [43]. The $ECD^{low}Flk1^{high}$ population represents the hemangioblast population [43]. Importantly, only the $ECD^{low}Flk1^{low}$ population demonstrated detectable X-gal staining (indicative of *Nfatc1* expression), suggesting that the endocardium is derived from this non-hemangioblast population [43]. Interestingly, the $ECD^{low}Flk1^{low}$ population also displayed markers of vascular differentiation, including *Cdh5* (VE-cadherin) and *CD31* (Pecam1) and markers of myocardial differentiation, including *Nkx2-5*, *β -MyHC*, *cTnT*, *GATA4*, and *Mef2c* [43], again supporting the notion that this population gives rise to both myocardial and endocardial cells. These data are consistent with the recent discovery that the hemangioblast and cardiac potential of embryonic stem (ES) cell-derived embryoid bodies segregated to two distinct populations, $Bry^{+}/Flk1^{+}$ and $Bry^{+}/Flk1^{-}$, respectively, based on cell surface marker expression [32]. It was later found that the $Bry^{+}/Flk1^{-}$ population generates a second $Flk1^{+}$ population with robust cardiac potential that contains progenitors of cardiomyocytes, vascular smooth muscle cells, and endothelial (endocardial) cells [17]. Taken together, these data suggest that endocardial cells arise, at least in part, from a cardiovascular progenitor population distinct from the hemangioblasts that give rise to other endothelial subtypes.

The endocardium is known to express lineage-specific markers in a pattern distinct from other endothelial cell types. For example, *Nfatc1* is specifically expressed in the endocardium, with transcripts detectable at the time of initiation of endocardial differentiation in the primary heart-forming field at E7.5 and intracellular protein detectable at the linear heart tube stage at E8.5 [13]. Interestingly, despite its intranuclear location during early endocardiogenesis, NFATc1 does not appear to be required for endocardial specification or proliferation, although its activity is critical during later cardiac development and valvulogenesis [13, 53]. Thus, all of the observations to date support a model in which the endocardium is a spatially restricted population of endothelium, arising as a result of de novo vasculogenesis from precursor cells present in the cardiac crescent. Furthermore, the observations to date suggest that highly related, but distinct, genetic programs, involving overlapping subsets of factors, control the development of the endothelial cells that will comprise the endocardium versus endothelial cells located elsewhere in the embryo.

Relationship Between Endocardium and Myocardium

The lineage relationship between endocardial and myocardial cells remains unresolved. Specifically, while it is clear that endocardial and myocardial cells share a

common progenitor, it is not clear whether these progenitors are present in the early heart field within the anterior lateral mesoderm. Two basic models of lineage determination in the heart have been proposed [16, 28, 35]. The first model is based primarily on observations in avian and fish embryos and postulates that cells in the cardiac mesoderm are prespecified in the primitive streak, prior to migration to the cardiac crescent, to become either myocardium or endocardium (Fig. 1) [11, 66]. The second model is largely based on work performed in mouse embryos or using cells derived from mammalian embryos and suggests that endocardial and myocardial cells share a common progenitor within the cardiac mesoderm and that these bipotential (or multipotential) cells maintain fate plasticity until a much later time in development than the prespecification model (Fig. 2) [7, 30, 39, 46].

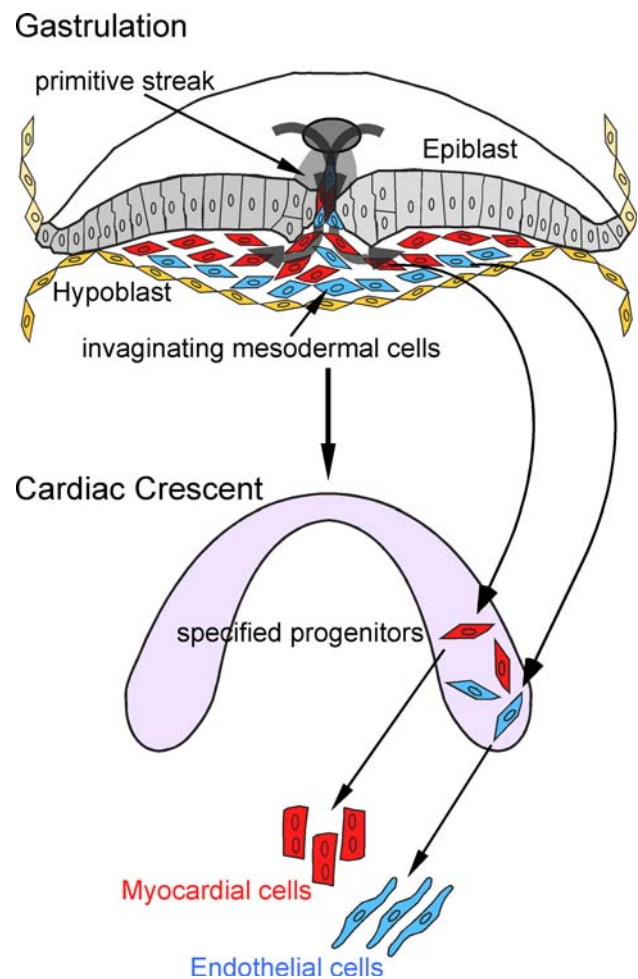


Fig. 1 A prespecification model for endocardial cells. Data obtained primarily from avian and fish models suggest that mesodermal cells are prespecified in the primitive streak before migration to the cardiac crescent to adopt either an endocardial or a myocardial fate. Endocardial precursors then receive inductive cues from the underlying endoderm to differentiate into cells with an endothelial phenotype

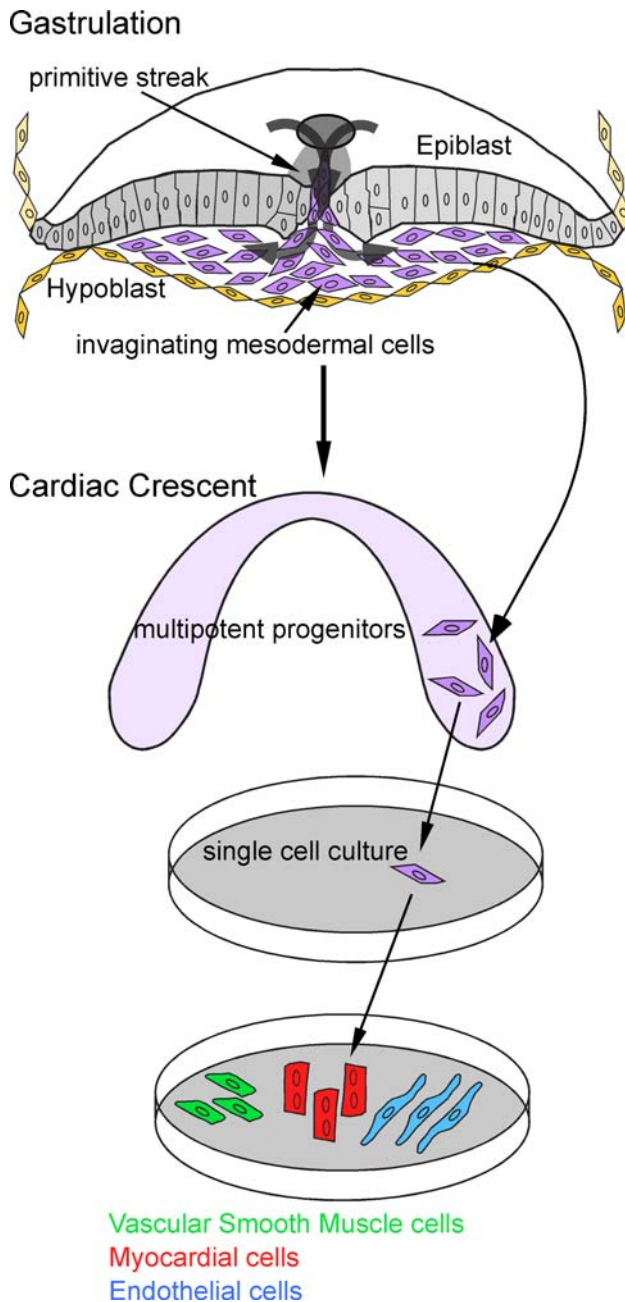


Fig. 2 A model for multipotent progenitor cells in the cardiac crescent. Data acquired primarily from mouse fate-mapping studies and ex vivo approaches using cells from mouse embryos suggest that there are multipotent progenitors in the cardiac crescent that can then be specified to adopt endocardial, myocardial, or vascular smooth muscle cell fates

Model 1: Cardiogenic Precursors Are Prespecified When They Migrate from the Primitive Streak to the Cardiac Crescent

In an elegant set of lineage-tracing experiments, Mikawa and coworkers introduced β -gal-expressing viral particles into the mesodermal layer of gastrulating chick embryos at

Hamburger-Hamilton (HH) stage 4 (11). When the embryos were examined at HH stage 10, β -gal⁺ cells were seen in both the endocardium and the myocardium. However, individual patches of β -gal⁺ cells, thought to have arisen from individual infected precursors, were seen to contain either myocardial or endocardial cells, but never both [11]. The latter result was observed even at viral titers that would only result in the infection of individual cells. From these results, Mikawa and coworkers concluded that individual cells in the cardiac mesoderm were already specified to give rise either to myocardial cells or to endocardial cells [11]. In a follow-up set of similar experiments, the same group infected cells in the primitive streak at HH stage 3 with a β -gal-encoding replication-deficient virus. Again, when they examined embryos at HH stage 10, they observed similar results as with the previous set of experiments. Individual β -gal⁺ patches of labeled cells, presumably derived from just a single infected progenitor cell, contained either endocardial cells or myocardial cells, but never both [66]. As with their previous study, these results support a model in which precardiogenic mesoderm cells are prespecified to either the myocardial or the endocardial lineage prior to migration through the primitive streak (Fig. 1).

Similarly, in a series of cell-labeling experiments in zebrafish, it was found that the majority of cells within the cardiogenic region did not give rise to endocardial cells. Rather, a subset of mesoderm precursors located in the ventral region of the heart field was found to give rise to endocardial cells [33]. As was observed in the chick experiments described above, individual cells labeled in the heart field never gave rise to both myocardium and endocardium.

Model 2: Precardiogenic Mesoderm in the Cardiac Crescent Contains Multipotent Cells That Can Give Rise to Both Endocardium and Myocardium

In contrast to the studies described above, in which endocardial cells are prespecified in the cardiac mesoderm, studies performed primarily in mouse embryos have suggested a different model in which mesodermal precursor cells in the cardiac crescent maintain fate plasticity and can give rise to both myocardium and endocardium [39]. Molecular marker analyses and Cre-based fate mapping approaches in mice have resulted in the identification of markers of the cardiac lineage that begin to be expressed in the mesoderm of the cardiac crescent after the cells have migrated through the primitive streak. Several of these markers label cells that give rise to descendants present in both the myocardium and the endocardium [9, 39, 64].

A lineage analysis of *Isl1*-expressing cells in developing mouse embryos demonstrated that *Isl1*^{Cre}-marked cells, which are first apparent in the cardiac crescent, give rise to both myocardial and endocardial cells [9]. This observation supports the notion that these two cell types arise from a common progenitor in the cardiac crescent, although it does not exclude the alternative hypothesis that *Isl1* may be simultaneously expressed in distinct progenitors for each of these lineages. Similarly, Verzi et al. examined the activity of a Cre-dependent reporter in which Cre was expressed under the control of anterior heart field (AHF)-specific regulatory elements from the *Mef2c* gene and found that both endocardial and myocardial cells were marked by the activity of the transgene [64]. Again, this is consistent with the notion that these two tissues share a common progenitor in the cardiac crescent but allows the possibility that the activity of the *Mef2c*-AHF-Cre transgene might also independently mark two discrete progenitor populations. Likewise, Chien and colleagues used genetic fate-mapping techniques in mice to identify a subset of multipotent cardiovascular progenitors with the transcriptional signature *Isl1*⁺; *Flk1*⁺; *Nkx2-5*⁺ that gave rise to multiple cell types in vivo and were able to generate cardiac muscle, smooth muscle, and endothelial cells in vitro (Fig. 2) [44].

More recent work has further elucidated the lineage relationships between myocardium and endocardium in the mouse. *Nkx2-5* is one of the earliest markers of the myocardial lineage, first expressed at the cardiac crescent stage [22, 23, 31, 36, 56]. Germline deletion of *Nkx2-5* results in profound cardiac defects, characterized in part by complete absence of the endocardial cushions [37, 62]. These observations, combined with the fate-mapping and lineage analyses discussed in the previous paragraph, provide further evidence for a model in which myocardial and endocardial cells arise from a common progenitor cell in the precardiac mesoderm [35]. Alternatively, these data are also consistent with the notion that *Nkx2-5*⁺ progenitor cells in the cardiac crescent do not represent a uniform population of bipotential cells but, rather, are composed of at least two distinct subpopulations that have been pre-specified to adopt myocyte and endocardial fates but that cannot be resolved on the basis of known markers. This alternative interpretation of the majority of these mouse experiments would be more consistent with the chick and zebrafish data supporting prespecification of endocardial and myocardial progenitors within the precardiac mesoderm, as discussed in the last section.

Further support for the notion that cardiac progenitor cells in the crescent remain multipotential and give rise to both myocytes and endocardial cells is provided by recent work examining the regulation of the Ets-related protein *Etv2* (*Etsrp71*, *ER71*) gene [19]. *Etv2* has been identified recently as a major master control gene for endothelial

specification [15, 19, 34, 61]. Interestingly, *Etv2* regulation in the endocardium appears to be under direct control of *Nkx2-5*, a cardiac master regulator [2, 19, 36]. Although these results do not directly address the potential or fate of progenitors within cardiogenic mesoderm, the observation that a cardiac master regulator activates an endothelial master regulator is consistent with the idea that an early program involving *Nkx2-5* sets up both the endocardial and the myocardial programs and suggests that the endocardium arises secondarily from a subset of cardiogenic progenitors via a cell-autonomous effect of *Nkx2-5*.

Because of the limited number and relative inaccessibility of cardiogenic mesoderm cells in the early mouse embryo, ES cell-based models have also been used extensively to investigate the early fate decisions of cardiogenic mesoderm. Kattman and colleagues examined in vitro differentiation of an ES cell line with a *GFP* reporter gene targeted to the mesoderm specification gene *brachyury* [18, 26, 30]. As ES cells were differentiated, some expressed GFP, indicating that they had adopted a mesoderm fate. These GFP-positive cells were all initially *Flk1*⁻, but a subset induced expression of the endothelial marker gene *Flk1* and this induction could be augmented by addition of the endothelial growth factor VEGF to the culture medium [30]. Importantly, some *Flk1*⁺ cells were observed to form aggregates of beating cells that expressed the cardiac-specific marker cTnT, indicating that these *Flk1*⁺ cells had the capacity to form either endothelial cells or cardiomyocytes [30].

In another study, Masino and colleagues used fluorescence-activated cell sorting (FACS) to isolate cells expressing YFP under control of an enhancer element from the *Nkx2-5* locus at the cardiac crescent stage, at the linear heart tube stage, and during cardiac looping, and they performed transcriptome-wide expression profiling [39]. These studies showed that these *Nkx2-5*⁺ cardiac precursor cells expressed markers of both cardiac myocyte differentiation and endothelial differentiation, lending credence to the notion that the precardiac mesoderm contains multipotential cells with the capacity to give rise to at least two descendent types, myocardial and endothelial cells [39].

It is important to note that cells with the capacity to give rise to both endothelial and myocardial cells are not restricted to mammalian systems. Several laboratories have also found evidence of bipotential mesoderm precursors in avian cells as well. The QCE-6 cell line, derived from explants of Japanese quail embryos collected at HH stage 4, can differentiate into both endothelial and myocardial cells [55], suggesting that a degree of plasticity exists in those progenitor cells as well. In addition, overexpression of the bHLH transcription factor *Scf/Tal1* together with the Lim-homeodomain transcription factor *Lmo2* in early zebrafish embryos has been shown to result in an expansion

of endothelial and hematopoietic cells, at the expense of myocardial differentiation, suggesting a shared precursor population [21].

Thus, it remains unclear whether mesodermal cells in the cardiac crescent represent a homogeneous population of bipotential cardiac progenitors or, rather, a heterogeneous population of progenitors that have been pre-specified to adopt either a myocardial or an endocardial fate. Future studies will be required to determine whether these two models can be reconciled and to clarify this issue.

Transcription Factors Involved in Endocardial Development

Regardless of whether endocardial progenitors are pre-specified at the time they enter the cardiac crescent or whether cells maintain significant plasticity until later in development, it is clear that the endocardium develops from a subset of cardiogenic precursors within the anterior lateral mesoderm [43]. In order to understand how these cardiogenic cells enter either the myocardial or the endocardial fate, it is critical to examine the transcriptional networks involved in these fate decisions. While endocardial cells arise from a distinct population of cardiovascular precursors, their specification appears to involve a genetic program shared with other endothelial populations. Several transcription factors have been shown to be required for both endothelial and endocardial specification. For example, *Etv2* and *Sc1/Tal1*, both key early regulators of endothelial development, are also required for endocardial specification [8, 14, 19]. These observations support the view that the endocardium is simply a spatially restricted subpopulation of endothelium. However, a limited number of transcription factors are expressed specifically in the endocardium, and examination of these and their relationship to established cardiac programs has led to an enhanced understanding of the unique nature of the endocardium.

The GATA family of zinc-finger proteins has been shown to be critical for cardiac development [10]. Al Moustafa and Chalifour found that mesodermal cells could be differentiated *in vitro* by treatment with retinoic acid [3]. Upon treatment, these cells attenuate expression of the pan-mesoderm markers *Brachyury* and *GATA4* and begin to express *GATA5* [47]. At the same time, the transcription factor *NFATc1* is translocated to the nucleus, where it can participate in transcriptional activation [47]. *In vitro* transactivation assays using a GATA-responsive reporter construct showed that *GATA5* and *NFATc1* synergistically activate endocardial gene expression [47]. This cooperative activation by GATA and NFAT factors lends support to the notion that the endocardium develops within a field of

cardiac progenitors as a molecularly distinct endothelial subpopulation in response to cardiac-specific cues.

Etv2 is an important endothelial and hematopoietic determination factor and has been shown recently to play a fundamental role in the transcriptional program controlling development of the endothelium, including the endocardium [15, 19, 34, 61]. *Etv2*-null embryos are normal at E8.5 but are nonviable between E9.0 and E10.0 and have no identifiable endocardium or endothelium [19]. Interestingly, *Etv2* appears to be activated directly by *Nkx2-5* in the progenitors that will give rise to the endocardium [19]. This result suggests that myocardial specification (or a more general specification of cardiac progenitors) may precede endocardial specification and *Etv2* activation. *Etv2*, in turn, bound to the promoter region of the *Tie2* gene *in vivo* and transactivated it in culture [19]. These data suggest that, while sharing a basic transcriptional network with other endothelial types, the endocardium is unique in that it arises from a distinct cardiac precursor population and that the genetic program controlling its specification is subordinate to a cardiogenic program in a transcriptional hierarchy responsible for cardiovascular development.

Future Directions

As outlined above, a central question in understanding endocardial development is whether the fate of precursor cells in the mesoderm is pre-specified by the time they arrive in the cardiac crescent or whether these precursors retain some fate plasticity. On the whole, *in vivo* avian models have supported the pre-specification model, while *in vitro* systems, primarily using mammalian cells, have lent support to the plasticity model [66]. A shortcoming of the existing *in vivo* work in mammalian systems is that it has failed to exclude the possibility that available markers might not be able to resolve potential component subpopulations within the cardiac crescent. A trivial explanation for discrepancies between the two models is that there may be inherent differences in the model organisms. However, another way of reconciling the two models is to postulate that cardiogenic precursors do indeed retain plasticity within the cardiac crescent, but that their fate decisions once they arrive in the crescent are constrained by positional cues. In this way, bipotential precursor cells labeled *in situ* will be seen giving rise to only one cell type, but can be induced to differentiate into both endocardium and myocardium when removed from the embryo (and the corresponding positional cues within the embryo) and cultured *ex vivo*.

To address the issue of whether bipotential (or multipotential) progenitor cells within the nascent heart field are restricted in their fate due to positional information,

cells from the ventral region of the heart field could be labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (diI) and transplanted to a more dorsal position in chick embryos. Subsequent identification of the label in myocardial cells would suggest that the cardiac crescent contains bipotential progenitors whose fate determination is constrained by positional cues. Additionally, the role of positional cues and possible species differences could be addressed in mouse embryos by retrospective fate mapping, as has been done so elegantly to map contributions of myocardial progenitors to the heart [40]. Specifically, an *nlaacZ* reporter construct could be targeted to the *Nkx2-5* locus in mouse. As has been shown previously, this reporter contains a *lacZ* reporter gene with a frameshift mutation (hence the name *laacZ*) and is inactive unless a rare intragenic recombination event occurs [40]. In the event of such a recombination, which restores functional *lacZ* expression, all progeny of the cell in which recombination occurred will be labeled [40]. Clonal labeling of both endocardial and myocardial cells in the neonatal heart would provide compelling evidence that individual *Nkx2-5*⁺ precursors in the cardiac crescent can give rise to both lineages.

Additionally, a genetic approach could be taken to address the possibility that a subset of cardiovascular progenitors becomes specified in the cardiac crescent to adopt an endothelial cell fate under the control of the cardiac regulator *Nkx2-5*. A transgenic mouse line has been published that expressed Cre recombinase under the control of an enhancer element from the *Nkx2-5* locus [45, 68]. Cre expression in these mice is first detected in cardiovascular progenitors of the cardiac crescent and is restricted to the myocardium after cardiac looping [68]. This Cre transgenic line could be used to create a conditional deletion of *Etv2*. If such a deletion resulted in disruption of the endothelial program in these cardiovascular progenitors and a defect in the endocardium, it would clarify two of the central questions of endocardial development. First, it would lend support to the notion that the cardiac master regulator *Nkx2-5* and the endothelial master regulator *Etv2* act in a hierarchical fashion to drive endocardial development. Second, it would shed light on the timing of endocardial specification, providing clear evidence that the endothelial program is not initiated until after *Nkx2-5* begins to function in the cardiac crescent.

Implications

The endocardium plays several well-established roles in the development of the heart. It serves as the source of mesenchymal cells in the endocardial cushions that give

rise to the structural elements of the cardiac valves as well as the atrial and membranous ventricular septa. It also participates in less well-appreciated ways in other aspects of cardiovascular development, including the formation of the cardiac conduction system and in proper myocardial trabeculation [41, 60, 65]. Given the toll of pediatric disease represented by valvular abnormalities, septal defects, conduction system abnormalities, and ventricular non-compaction, the role of the endocardium during development is critically important clinically. Thus, a more detailed appreciation of the molecular mechanisms of endocardial development is essential to gain a more nuanced understanding of human developmental defects and eventually may offer the hope of developing novel therapeutic strategies for congenital heart defects.

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