Development of the Coronary Vessel System

David E. Reese, Takashi Mikawa, David M. Bader

Abstract—Formation of the coronary vessels is a fundamental event in heart development. Congenital abnormalities in the coronary system can have major deleterious effects on heart function. It is also possible that subtle variation in the patterning of coronary vessels has significant but uncharacterized effects on myocardial structure and function. In addition, generation of the coronary vascular system represents a complex system for analysis of regulation of cell fate determination, cell and epithelial migration, epithelial/mesenchymal transition, and patterning of a complex three-dimensional structure. In this review, we present the descriptive embryology of this process as well as the recent data that shed light on the unique developmental mechanisms underlying generation of coronary vessels. This review also attempts to identify areas where additional research is needed and highlights the questions that must be answered for a meaningful understanding of coronary vessel development. (Circ Res. 2002;91:761-768.)

Key Words: coronary vessels ● development

Problems of the coronary vascular system lead to major problems with the heart. Although the basic pathways taken by major coronary vessels over the surface of the heart are presented in cardiology textbooks, there is much variation in the pattern that is not well understood. Also, the enormity of the coronary system is not well appreciated as all, or nearly all, cardiac myocytes in mammalian hearts are in contact with a capillary and that the mammalian heart is one of the most vascularized organs of the body. Still, not a single cell that makes up the coronary system of the body. Not everyone has coronary vessels. First, before discussing the structure and development of the coronary system, it should be noted that not all organisms with a heart have coronary vessels. No invertebrates with hearts have coronary vessels. Among the vertebrates, mammals, reptiles, and avians have coronary systems complete with arterial output and venous return. What these three classes of vertebrates have in common is their dependence on pulmonic respiration and the lack of cutaneous respiration. Cutaneous respiration involves the exchange of gases through subcutaneous capillaries with the atmosphere. In this way, carbon dioxide is dissipated and oxygen is restored to venous blood. The hearts of these organisms may have complete unexplored area of vertebrate embryology that has important implications for human health.

Not Everyone Has Coronary Vessels

First, before discussing the structure and development of the coronary system, it should be noted that not all organisms with a heart have coronary vessels. No invertebrates with hearts have coronary vessels. Among the vertebrates, mammals, reptiles, and avians have coronary systems complete with arterial output and venous return. What these three classes of vertebrates have in common is their dependence on pulmonic respiration and the lack of cutaneous respiration. Cutaneous respiration involves the exchange of gases through subcutaneous capillaries with the atmosphere. In this way, carbon dioxide is dissipated and oxygen is restored to venous blood. The hearts of these organisms may have complete
anatomical separation of left (oxygenated) and right (deoxygenated) sides of the heart or have a physiological separation of oxygenated and deoxygenated blood. The use of circulating luminal blood to deliver oxygen to the right ventricle is impossible. In addition, these species have thick-walled ventricles that cannot be served by simple gas diffusion from the myocardium to the heart lumen. Thus, an alternative method of delivery, presumably the coronary system, was necessitated.

One of the remaining vertebrate classes, the amphibians, has cutaneous respiration. Most amphibians, such as newts, salamanders, and bullfrogs\textsuperscript{3,4} have no coronary vessels. In fish, the last vertebrate class, the presence of coronary vessels is variable. In general, larger, fast-swimming predatory fish have extensive coronary vessels, as do fish that live in poorly oxygenated environs. In these fish, coronary arteries penetrate the outer compact regions of the ventricles.\textsuperscript{5} Zebrafish also have coronary vessels on the surface of the heart and in the compact ventricle.\textsuperscript{6}

**Structure of the Coronary Vascular System**

A brief consideration of the adult structure is necessary before turning to a discussion of coronary development. For a detailed description of the anatomy and histology of coronary vessels, the reader may consult any major text in cardiovascular medicine. For purposes of describing the overall structure of the coronary system, we focus on studies of the human heart. When development and its genetic control are discussed, we turn to experimental models more aptly suited.

The blood supply to the heart of higher vertebrates actually originates outside the heart from the ascending aorta. The origins or ostia of the right and left coronary arteries are located in the sinuses of the aortic valves and thus are superior to the heart. This region is actually the first site of systemic oxygenated blood. (By comparison, the origins of coronary arteries in trout are the paired dorsal aortas distal to the ascending gill arteries, which by the way are the first sites of systemic oxygenated blood in fish.) These arteries travel well-defined routes along the surface of the heart in the epicardium and give rise to branches that penetrate the substance of the myocardium. (Many texts and articles refer to the “epicardium” as the “visceral pericardium.” For this review, we use the term “epicardium.”) Small muscular arteries are found throughout the myocardium that further branch into an extensive capillary bed that embraces all, or nearly all, cardiac myocytes. The venous return to the coronary sinus courses over the surface of the heart with accompanying arteries. The coronary sinus returns blood to the right atrium just inferior to the opening of the vena cava. Thus, although the major arteries and veins travel together over the surface of the heart, the origin of the coronary arteries and termination of the coronary sinus are different. This situation is very interesting given the mechanism of development detailed next. Epicardial lymphatics do not appear to travel with arteries and veins.

Histologically, the arteries of the coronary system are similar to arteries seen throughout the body. The tunica intima consists of a continuous endothelium and associated subendothelial connective tissue space bounded externally by an internal elastic membrane. Smooth muscle cells and elastic laminae occupy the tunica media, and the adventitia comprises connective tissue cells and fibers. Coronary arteries that run in the epicardium have been characterized as “elastic” arteries, although the number of smooth muscle cells is greater and the amount of elastic fibers is less than in other elastic arteries.\textsuperscript{7} Branches of the major epicardial arteries that penetrate the myocardial wall are classified as “muscular” arteries that in turn give rise to arterioles and eventually to the capillary bed.

Although the patterning of the coronary system is predictable, there is significant variation in the positioning of the larger vessels and their intramuscular branches. Normally, two coronary arteries arise from the aorta, but in many cases (50% of humans)\textsuperscript{8} a third artery is present. These major arteries course over the surface of the heart in the subepicardial connective tissue. Right and left coronary arteries contribute to an arterial ring that encircles the atrioventricular sulcus (Figure 1). In many human hearts, the major source of this arterial ring comes from the left circumflex artery as a branch of the left coronary artery with the remaining contribution originating from the right coronary artery. Still, significant variation in the relative contributions from right and left sides is common. Thus, it is clear that there is much play in the system and that the overriding developmental and physiological pressures are supplying major arteries to encircle the heart without reference to their origins.

Major epicardial arteries “descend” from this atrioventricular circle formed from branches of the right and left main coronary arteries. Again, variation in the main branches is fairly common, and absence of a major branch of the coronary arteries leads to extensive collateral branching from other arteries to “fill the breach.” For example, four major descending arteries are seen along the posterior surface of the ventricles (Figure 1). In the slight majority of human hearts (54%), three vessels arise from the left coronary and one from the right. The other 46% of cases have variation in contribu-

**Figure 1.** Diagram of the major coronary arteries on the surface of the heart. The positions of the ventricles (darker red) and atria (lighter red) are given. A, Anterior view. Two coronary arteries take origin from the ascending aorta (the pulmonary trunk is cut away). The right coronary courses in the anterior atrioventricular groove and provides many branches to the posterior surface. The left coronary divides into the left circumflex (not seen in this diagram) and diagonal and anterior descending branches. B, Posterior view. The right coronary continues from the anterior surface in the atrioventricular groove. The left circumflex artery is also seen in this groove and provides a major surface vessel at its termination.
vessel anomalies but also may reflect the catastrophic nature of valve, muscle, and great arteries, but it is helpful to understand that the pattern of branching. These arteries are highly variable when visualized by plastic casting or ink injection. Indeed, when reviewing these studies, summarized in Reference 7, one is struck by the total lack of pattern in the arteries that penetrate the myocardium, that is, except for two important things: (1) There appears to be a fairly consistent spacing between these penetrating muscular arteries. (2) The branching of these arteries leaves no space untouched. Thus, it seems that the overriding issue in coronary artery patterning is unimpeded delivery of blood to the capillaries rather than the particular route that the blood takes. The effect that subtle variations in the branching pattern of small coronary vessels have on heart function, especially myocardial performance, is not currently understood. Variation in the origin, number, and patterning of the major coronary arteries is far greater than variation seen in valves, myocardium, and/or great vessels. This may reflect the catastrophic nature of valve, muscle, and great vessel anomalies but also may reflect the “latitude” or “play” in the system of generating coronary arteries. In addition, the complex or even dynamic nature of coronary vessel development may lead to wide variation in the adult structure, as we will review next. Thus, the representations seen in texts of cardiology may be the most frequently observed arterial pathways, but it is helpful to understand that the pattern of coronary arterial structure can vary greatly.

**Development of the Coronary Vessels**

Although interest in coronary arteries is primarily focused on the debilitating effects disease has on their function, the generation of these vessels is truly a complex story in developmental biology and clearly plays a central role in the function of the adult structure. Cell lineage commitment and diversification, directed cell migration, control of epithelial/mesenchymal transition, and cell differentiation are some of the hallmarks in the development of coronary arteries. For this focused review, a description of heart development and its relationship to coronary vasculogenesis will begin with a consideration of the origin of the cell types that make up the heart.

**Origin of Cells That Make Up the Heart**

The principal cell types that make up the heart are cardiac myocytes, endocardial endothelium, fibroblasts, vascular smooth muscle, and vascular endothelium. These cells have different origins, and a key to understanding coronary vessel development is knowing their embryonic history.

Although many critical events in cardiogenesis occur before the formation of the heart, the present discussion of coronary vessel development will begin at the tubular heart stage. The earliest stages of cardiogenesis have been reviewed by Fishman and Chien. The tubular heart is formed as an endothelial tube within a muscular tube (Figure 2). Both the endothelium and the muscle layers develop as epithelia from lateral plate mesoderm. These are the only two cardiac cell types generated by lateral mesoderm. After establishment of the heart tube, complex morphogenetic events such as looping, diversification of atria and ventricles, and valve formation take place. During this process, the basic epithelial nature and positioning of the endothelial and muscular tubes are maintained with one exception: delamination of endothelial cells in the atrioventricular canal and conus to form the connective tissue of the valves. In the anterior region of the heart tube that will become ventricle, myogenic cells proliferate and form extensive trabeculae while myocytes in the posterior, atrial-forming regions proliferate but do not extensively trabeculate. During this period of development (stage 14 in chickens, day 9.5 in mice, and about 24 days in humans), the heart has an endocardium and a myocardium but lacks an epicardium, as first described by Manasek. These early morphogenetic processes also occur in the total absence of blood vessels.

Cells that generate the epicardium arise from a different origin and in a very different manner from cells that generate the myocardium and endocardium. Although the earliest location of these progenitors is still in debate, they appear to arise from an epithelium associated with the septum transversum (and references within). The septum transversum is an outgrowth or diverticulum from the dorsal body wall that subdivides the embryonic coelom, the cavity that lies between the developing body wall and the forming organs, into an anterior pleuropericardial cavity and a posterior peritoneal cavity. (The pleuropericardial cavity is quickly subdivided into pleural and pericardial cavities.) The septum transversum extends downward from the dorsal body wall to close off the
pericardial and peritoneal cavities and forms the resulting embryonic structure that will give rise to the diaphragm. Thus, the heart has become ensaced in its own space, the pericardial cavity, and separated from the peritoneum by the diaphragm. At this point, the pericardial cavity and heart still lack the epithelial surface that is so critical for development and cardiac function.

The First Directed Migration: Formation of the Epicardium

Generation of the epicardium plays an essential role in the development of the coronary vasculature. The formation of the continuous, simple squamous epithelium that constitutes the epicardium and pericardium begins as an extension or outgrowth from the septum transversum and becomes visible near the sinoatrial pole of the heart; this structure is called the proepicardial organ (PEO). At or around stage 18 in the chick and 10.5 days postcoitum (dpc) in the mouse, the PEO makes contact with the surface of the developing heart tube. It is composed of epithelial cells, a true epithelium in chicks and groups of epithelial cells in mice that extend and contact the heart near the future atria. In the chick, when the advancing PEO makes contact with the myocardium, the advancing proepicardium envelops the heart principally in different directions (Figure 2). Epicardial migration proceeds over the atria and somewhat later over the ventricles. The advancing epithelium spreads out over the heart, eventually covering the entire myocardium and pericardial cavity. It should be noted that an epithelial contribution to this process from the bulboventricular pole of the heart has been observed. In mice, groups of epithelial cells move over the heart and eventually form a continuous epithelial sheet. At present, detailed studies of epicardial movement in other species are somewhat limited and would be fruitful areas for future study. In addition, it is very interesting to speculate on the signaling mechanisms that regulate this first “directed” migration of epithelial cells to and over the heart. This epithelial migration is highly ordered in the chick, where this process is best described, suggesting a tightly regulated developmental process. It is quite possible that diffusible factors from the myocardium act as chemoattractants during these events.

This embryonic movement of cells results in the formation of a continuous simple squamous epithelium that covers the heart and body wall. This continuous epithelium creates a body compartment, a space connecting the heart and components of the dorsal body wall. Communication between the heart and the rest of the body is conducted through this body compartment. Thus, such structures as nerves from the CNS and blood vessels coursing to and from the heart can have protected space in which to travel.

Formation of the epicardium is a relatively rare example of epithelial sheet migration during embryogenesis. This form of cell movement is the migration of an intact epithelium with a free edge over the surface of an organ. There are several components of this process that must be tightly regulated in order to generate the epicardium. First, proepicardial cells must be able to move but not completely lose their adhesive behavior. While the PEO of the chick moves as an intact sheet and that of the mouse moves as groups of cells, both move in a directed manner to and then over the heart. Genetic models of mouse development have revealed interesting concepts in epicardial migration and cell adhesion. VCAM-1- and α-integrin–null mice lack an epicardium in the hearts of mice that survive into the second week of development. This strongly suggests that disruption of cell adhesion systems regulating matrix interactions impedes epicardial cell migration. In addition, embryonic lethality in these models occurs before the onset of normal coronary blood flow, suggesting a critical function of the epicardium in cardiac development before the advent of vasculogenesis. The work of Manner has shown the requirement of epicardium for sustained development of the myocardium. Clearly, the regulation of epicardial movement and the factors or signals from the epicardium that participate in the regulation of myocyte proliferation, growth, and patterning of myocardium are potentially exciting areas of study.

Epithelial to Mesenchymal Transition in Coronary Vasculogenesis

As stated previously, the initial epicardium is a simple squamous epithelium that completely envelops the heart by stage 26 (chick) and 12.5 dpc (mouse). Concurrent with epithelial migration, the epicardium thickens and some cells lose contact with the epicardial epithelium. These cells become freely migratory mesenchyme and move into the forming subepicardial connective tissue space. Production of these freely migratory cells is an example of epithelial-to-mesenchymal transition (EMT). EMT plays an essential role in the formation of other organs during embryogenesis. For example, generation of the endocrine pancreas and formation of kidney are processes that are dependent on EMT. In each case, unique cell types are generated from the mesenchyme, producing islet cells, nephric epithelium, or connective tissue.

Morphologically, epicardial EMT does not appear to be fundamentally unique or different from other examples of EMT. Still, the cells generated from the resulting mesenchyme, namely vasculogenic endothelium and smooth muscle, have not yet been identified in other examples of EMT, which suggests that there are unique aspects to this particular developmental process. Thus, it would seem necessary to determine the precise molecular regulation of epicardial EMT and to determine whether this is a truly unique form of EMT. Studies to elucidate the factors, presumably from the myocardium that regulates the production and migration of epicardial mesenchyme, are just underway. In addition, recent studies using genetic approaches have shown that EMT from the epicardium is dependent on a FOG-2–(friend of GATA) regulated signaling system from the myocardium that has not been described in other tissues. FOG-2–null mice die at embryonic day 12.5 of multiple cardiac abnormalities including overriding aorta, subpulmonary stenosis, and ventricular septal defects. In the absence of FOG-2 transcriptional regulation, an epicardium is present but no mesenchyme is generated and thus no blood vessels are
formed. Presumably, a FOG-2–dependent signaling pathway is disrupted in the myocardium that regulates EMT from the epicardium. Although the molecular targets of the myocardially expressed FOG-2 pathway in the epicardium are now just being identified, it is certain that interaction between FOG-2 and GATA factors is essential for progression of cardiac myocyte differentiation and signaling to the developing coronary system. Clearly, the identification of myocardial factors regulating the delamination of vasculogenic precursors is essential for an understanding of coronary vessel development and should provide new concepts for the study of EMT in various embryonic systems. To summarize, few molecular determinants of EMT during coronary vasculogenesis are known at the present time. It is not clear whether a new group of signaling molecules, receptors, and signal transducers will be identified or that the molecular regulators already seen in other examples of EMT will be conserved here.

The epicardium with its newly formed connective tissue space covering the atria is thick whereas the ventricular epithelium is relatively thin. The epicardium of the atrio-ventricular sulcus, the region where the first coronary vessels form, is particularly thick and contains an abundance of mesenchymal cells. In this region, some of the proepicardial mesenchyme coalesce to form channels within the connective tissue space and become the endothelium of the coronary vessels. Other mesenchymal cells take up positions adjacent to this endothelium and differentiate into arterial smooth muscle. These channels then fuse to form blood vessels. While there is no blood flow and hence no blood pressure at this time, the forming epicardial arteries are large, much larger than arteries that penetrate the myocardium. It is interesting to note that the initial size of the proximal and distal coronary arteries is set in the absence of blood flow. The mechanisms that govern the patterning and regulation of coronary vessel size are largely unknown but certainly an area that will draw interest in the future.

The Second Directed Migration: Movement of Mesenchyme Through the Myocardium

Several studies have shown that the mesenchyme that migrates into spaces is formed in the developing myocardium and is solely produced from the epicardium. These mesenchymal cells will produce all the coronary vasculature of the adult heart. Several lines of evidence show that cells of the PEO give rise to the epicardium, cardiac fibroblasts, vascular endothelium, and vascular smooth muscle. First, ablation of the PEO or obstruction of PEO migration results in the absence of cells in the heart. Culture of the PEO and analysis of the resulting cell phenotypes show the presence of the predicted cells after a period of growth and differentiation. Further, analysis of protein expression such as Bves, a putative cell adhesion molecule expressed in coronary vessel precursor cells, in epicardially derived cells has shown the pervasive nature of mesenchymal migration in the myocardium. Finally, lineage analysis with chick/quail chimeras, vital dyes, or retroviral marking detects the presence of PEO-derived cells in all regions of the heart in the coronary vascular bed and within the substance of the myocardium as fibroblasts.

The migration of cells into newly created spaces is pervasive, as every myocyte in the adult heart is in contact with PEO-derived endothelial cells of the coronary vasculature. The spaces in the myocardial wall in which these cells move never come into contact with the lumen of the heart. Coronary vessels do not receive blood from the heart but from the aorta, and opening blood flow from the heart lumen into the developing myocardial wall in the absence of a continuous vasculature would be disastrous. After these cells migrate throughout spaces in the developing myocardium, they link to establish blood vessels through a vasculogenic process.

Regulation of this second mesenchymal migration during coronary vasculogenesis is also an open question. First, this migration generates a huge number of cells. The massive nature of this migration can be easily appreciated by looking at the pictures in Manner, showing the movement of quail PEO cells into a chick heart. The magnitude of this cellular invasion should not be surprising, considering that all myocytes of the adult heart are in contact with cells of the coronary vasculature. Next, this migration is pervasive, with cells reaching positions throughout the heart. Indeed, Gittenberger-de Groot and Poelmann and their colleagues have shown that PEO-derived cells penetrate all the way through the myocardium to the endocardial space. This delaminated mesenchyme must travel through spaces generated in the developing myocardium. Remember that the ventricles first expand in size by the growth of trabeculae from the outer wall. While it is clear that vasculogenic mesenchyme travels through these spaces, the cellular and molecular mechanisms used to generate them is not presently understood. Are matrix-digesting processes used by the developing heart to provide a pathway for these cells to travel? Additionally, all this probably takes place without ever disrupting the endocardial endothelium. Cell migration, matrix remodeling, and production of the intact coronary vasculature must take care to leave the endocardial endothelium alone.

Another point to consider in this phase of coronary vasculogenesis is the potential interactions of cells during epithelial and mesenchymal migration. Our understanding of vasculogenesis in general is that angioblasts/endothelial cells induce local mesenchyme to commit to the smooth muscle/pericyte cell lineage. At present, there are no data to suggest that molecular regulation of this process described for other sites of vasculogenesis, namely the TIE-1/TIE-2 angiopoietin pathway, is variant in the coronary system. If we are to believe that there is no “local mesenchyme” in heart development other than those cells derived from the epicardium, it would mean that the cells that will eventually differentiate into smooth muscle are derived from the same epicardial epithelium. These mesenchymal cells are traveling in the same spaces that the endothelial cells are moving in and they are really not “local.” Although the underlying molecular signaling mechanisms used in other vasculogenic systems may well be used in this case, it appears that coronary vasculogenesis has clear differences from other systems studied thus far in the production or movement of vasculo-
genic cells. An understanding of the interplay between these two cell types during intracardiac migration is needed.

The Decision to Make Arteries and Veins, Remodeling, and Making the Final Connection to Systemic Circulation
Emerging data have identified several molecules that play a role in the diversification of vessels into arteries and veins. At present, there are no published studies regarding the decision of coronary vasculogenic cells to differentiate into arteries or veins. Indeed, there is almost no information in the literature about the differentiation and potential remodeling of coronary veins. Except for the apparently unique mechanism for distributing coronary vasculogenic cells throughout the heart, there is also no clear reason to postulate that these decisions will be significantly different from those revealed in other developmental systems. The same situation holds for the remodeling process during coronary vasculogenesis. Several gene products have been implicated in this process. Coronary vessels are extensively remodeled during development, and the degree of variation in the adult structure suggests that there is much latitude in the production of the adult structure.

One unique aspect of coronary vasculogenesis is the final connection of the coronary arteries to the aorta. First, it is necessary to note that the initial phases of coronary vasculogenesis, like other forms of vasculogenesis, proceed in the absence of blood flow and that the caliber of proximal and distal arteries is governed by forces other than blood flow. Although intermittent flow via transient connection to the aorta may provide sporadic blood to the forming vessels, the caliber of developing arteries and veins is most likely driven by other mechanisms. Next, the proximal end of the coronary arteries actually grows into the aorta, penetrating the tunica media and finally the intima. Manner’s study shows the ends of the coronary arteries moving toward the aorta within the epicardial space. The final connection to the aorta involves local apoptotic events that eventually lead to the remodeling of coronary endothelia with that of the aorta. From these studies, one might suggest that the circuitry of the coronary vascular system is well established or even completed before the system “taps into” the systemic circulation. Still, it should be noted that small channels from the forming coronary system penetrate the substance of the aorta and are thought to provide intermittent blood flow before the establishment of the final connections of the definitive coronary arteries. Thus, the cellular and molecular details of the final connection to systemic circulation are unresolved. Very little is understood about processes that regulate the final connection of the venous return via the coronary sinus to the right atrium. As mentioned in a previous section (Structure of the Coronary Vascular System), the origins of the coronary arteries to the aorta and the connection of the coronary sinus to the right atrium are in different regions of the heart. This brings up an interesting developmental question, namely, how do these two vessel systems run parallel on the surface of the heart but diverge to make connections to the systemic circulation?

Considering the acrobatic nature of this developmental system, it should not be surprising that errors occur. Misconnections to the aorta, such as coronary connection to the pulmonary artery, are almost already “radial” not “longitudinal.” That is, this means malpositioned coronary vessels are not observed up the ascending aorta, but they are observed at the correct level and are radially misplaced on the aorta or pulmonary artery. Why mistakes tend to be radial and not longitudinal is not currently known. While the basic morphological events that lead to the completion of the coronary circuit are known, the underlying cellular and molecular regulation of these processes is unresolved.

Cell Lineage Diversification During Coronary Vessel Development
The generation of diverse cell types during coronary vessel development may be the most confounding problem in this complex developmental puzzle. Although it is abundantly clear that different cell phenotypes are generated in the PEO, the timing and mechanism of cell lineage diversification are largely unknown. Retroviral marking experiments from the Mikawa laboratory show that clones of cells derived from the marking of an individual cell within the PEO give rise to a single phenotype (eg, epicardial cells, endothelial cells, smooth muscle cells, or fibroblasts). It should be noted that these experiments are fate-mapping experiments and do not test whether these cells retain a latent pluripotency that is not actuated during normal development. Although these experiments do not prove that clonal diversification has taken place, they strongly suggest that factors regulating these events exert their effect before or at the time the PEO is formed.

During the migration of PEO-derived mesenchyme throughout the spaces in the developing heart, there is ample time for cell-cell interaction. Many studies have demonstrated that the interaction between angioblasts/endothelial cells with mesenchyme is essential for the commitment and differentiation of smooth muscle. While the expression patterns of the main signaling molecules and transcription factors governing smooth muscle development in the coronary system are now being completed, it would seem unlikely that novel regulatory mechanisms would be identified in coronary vasculogenesis. Still, the apparently novel production and migration of mesenchyme for vasculogenesis during coronary vessel development make for interesting areas of exploration.

Finally, it appears that coronary vasculogenesis uses a unique mechanism in the generation of vasculogenic cells. Our current understanding is that progenitors that later become the endothelium and smooth muscle of the coronary system are part of the advancing PEO, migrating epicardium, and delaminated mesenchyme. Certainly, this arrangement has not been described in other models of vasculogenesis. Still, it is interesting to consider that many other organ systems including the entire gut, are covered by a mesothelium like the epicardium, have their major blood supply delivered in the submesothelial space like the epicardium, and originate from the aorta as unpaired arteries like the coronar-
ies. Could this embryonic mechanism for generating blood vessels to organs be not so unique?

Some Questions
We have tried to present a succinct picture of coronary vessel development. Obviously, there are many topics that could be discussed in greater detail, and we may have omitted specific issues and important references inadvertently. We hope that this review will serve as a starting point for some investigators who wish to pursue work in this field. We have compiled a short list of questions or areas of interest that we believe would be important and interesting to answer. There are many other questions and ideas that our colleagues in the field may present, but here is our list.

Concerning vasculogenesis as a whole:

Is coronary vasculogenesis really different from other examples?
Are the same molecular signals used here?
Does the mesothelial origin of coronary vascular precursors result in the propagation of different signals?

Concerning epithelial movement of the PEO to the heart:

What is/are the signal(s) that attract/induce the movement?
What are the molecules that mediate this movement?

Concerning production of mesenchyme:

What is/are the signal(s) that initiate delamination?
Are these signals similar to other systems where EMT takes place?
How are the numbers of mesenchymal cells regulated?

Concerning migration of mesenchyme within the myocardium:

What is the attractant, and is there only one? Is the attractant to invade the myocardium the same as the one that draws it to developing blood vessels?

Concerning lineage diversification:

Are all progenitors truly part of the advancing epicardium?
When is lineage diversification determined?
What are the regulators of lineage determination?
How are the numbers of progenitors regulated?

A Final Thought
Coronary vessel development is essential for the generation of most vertebrate hearts. The basic mechanisms used by nature to construct these vessels in these organisms are most likely conserved. Still, it is interesting to think about the origin of this process. How did nature couple the generation of the epicardium with the production and distribution of vasculogenic cells? Hopefully, we can work together to figure this out.

Acknowledgments
This work was supported by an NIH grant (PO1-HL67105). We would like to thank Drs Tom Kume and Jon Backstrom for their helpful comments. Also, we thank members of the Mikawa and Bader laboratories for their critical reading of the manuscript and Brian Robertson for his assistance in preparing the figures.

References
26. Tevosian SG, Deconinck AE, Tanaka M, Schinke M, Litovsky SH, Izumo S, Fujiwara Y, Orkin SH. FOG-2, a cofactor for GATA transcription...


Development of the Coronary Vessel System
David E. Reese, Takashi Mikawa and David M. Bader

Circ Res. 2002;91:761-768
doi: 10.1161/01.RES.0000038961.53759.3C
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 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/91/9/761

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/