DEVELOPMENT

A crowning achievement for deciphering coronary origins

Postnatal maturation of the heart spurs coronary vessel development from an unanticipated source

By **C. Geoffrey Burns**[1,2,3] *and* **Caroline E. Burns**[1,2,3]

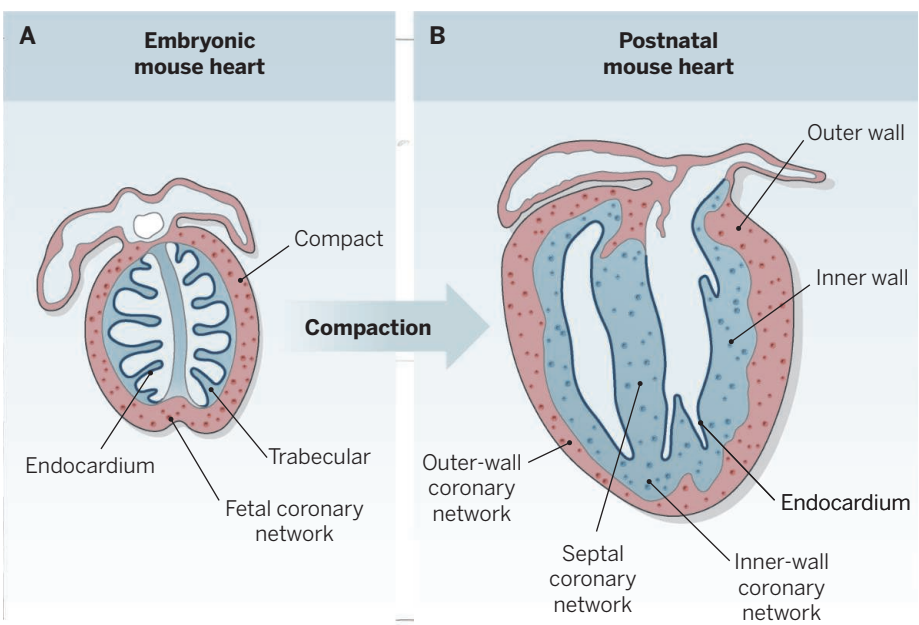
Named for its crownlike appearance (*1*), the coronary circulation comprises arteries and veins that transport blood to and from the heart's muscle, respectively. When a large coronary artery becomes obstructed, a downstream segment of cardiac muscle dies from oxygen deprivation in a pathologic condition called acute myocardial infarction (*2*), or heart attack. Over time, the injured heart replaces damaged muscle with scar tissue (*3*) that maintains cardiac wall integrity but also adversely affects pump function, which often leads to heart failure (*4*). Therapeutic interventions that stimulate the growth of new cardiac muscle to supplant scar formation would substantially improve heart attack outcomes (*5*). The success of any such strategy would also rely on concomitant growth of new coronary blood vessels. Therefore, a comprehensive understanding of how this specialized vascular bed develops is a prerequisite for regenerative strategies designed to reduce the morbidity and mortality caused by heart failure after myocardial infarction. On page 90 in this issue, Tian *et al.* (*6*) demonstrate that a large fraction of coronary vessels in the mouse heart form during postnatal life through a unique mechanism.

Formation and maturation of mammalian heart muscle (myocardium) is a dynamic process that begins during embryogenesis and continues after birth (*6*, *7*). The primitive embryonic heart is composed of a compact layer of muscle lined internally by specialized endothelial cells, the endocardium. At this stage, the walls of the embryonic heart provide a smooth surface for blood flow. As heart development proceeds, a second layer of cardiac muscle, with meshlike tissue architecture, emerges between the compact muscle layer and endocardium (see the figure). This trabecular layer comprises a collection of thin, interconnected strands of muscle (trabeculae) derived from, and anchored to, the compact layer. Beginning prior to birth and ending in the postnatal period (*6*, *7*), the trabecular layer of myocardium undergoes "compaction," in which its meshlike muscle densifies onto the compact layer, causing the latter to thicken. As a result, the adult myocardial wall contains an outer segment derived from embryonic compact muscle and an inner segment that forms through trabecular compaction.

The two embryonic myocardial layers have differential requirements for a coronary circulation. The compact myocardium receives oxygen from a fetal coronary network (*8*–*12*). By contrast, the trabecular layer receives oxygen by diffusion from chamber blood (*13*). As compaction of trabecular muscle ensues, new coronary vessels emerge in this region. Until this study by Tian *et al.*, the inner-wall coronary vessels were thought to arise by angiogenic invasion of the fetal coronary network (*7*). However, upon lineage tracing the endothelium of this fetal coronary system, Tian *et al.* discovered that inner-wall coronary vessels remained unlabeled, thereby highlighting an alternative source.

Tian *et al.* turned their attention to the endocardium. In addition to its close association with trabecular muscle, the endocardium decreases in surface area as the result of compaction, thereby creating excess endothelial cells potentially available for repurposing. The authors generated a mouse strain to exclusively label endocardial cells prior to coronary vessel formation. In the mature heart, a majority of inner wall vessels were labeled by the lineage trace, demonstrating that a subset of endocardial cells originally functioning as the endothelial lining of the trabeculae become repurposed for transporting blood to heart muscle. This mechanism for generating coronary vessels in the inner wall also establishes the circulation of the muscular wall separating the right and left sides of the heart (interventricular septum) that forms prior to birth also by compaction (*6*).

Tian *et al.* provide evidence that coalescence of trabecular myocardium traps endocardial cells, causing them to break through their basement membrane prior to forming a vascular plexus that matures into the inner-wall vasculature. When compared to the fetal coronary lineage trace, the endocardial trace was a near perfect mirror image, demonstrating that the mature coronary circulation is constructed



Heart vessel development. (**A**) The compact layer of the embryonic heart is irrigated by a fetal coronary circulation. Meshlike muscle (the trabecular layer), lined by specialized endothelial cells (endocardium), develops on the chamber walls. (**B**) During the perinatal period, the trabecular muscle condenses and traps a subset of endocardial cells that seed formation of a substantial fraction of cardiac vessels. This observation contradicts the prevailing notion that sprouting of the fetal coronary network generates all of the heart's vessels.

ILLUSTRATION: ADAPTED FROM C. GEOFFREY BURNS

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de novo from two mutually exclusive sources. In the adult mouse, 60% of the heart muscle is irrigated by vessels formed from endocardial cells during trabecular muscle compaction. Tian *et al.* speculate that delayed de novo creation of such a large proportion of the coronary network serves to meet the rapidly increasing metabolic demands of the thickening myocardial wall that would otherwise be unattainable by angiogenic expansion of the fetal coronary circulation.

Future studies will be required to delineate the molecular mechanisms that regulate postnatal development of the inner-wall vasculature and how they compare with those regulating establishment of the fetal coronaries. Tian *et al.* provide preliminary evidence that the former involves hypoxia and vascular endothelial growth factor, both implicated in fetal coronary development (*12*). An issue that remains to be resolved is the degree of interdependence between trabecular compaction and inner-wall vessel formation. If compaction is the driving force for vessel formation, then mutant mice that fail to undergo compaction (*7*) should lack inner-wall vessels. Alternatively, inner-wall vessel assembly might actively stimulate compaction. In this regard, it would be informative to analyze compaction in a mouse incapable of the endocardial to coronary endothelial lineage conversion. Another open question is whether this lineage conversion involves dedifferentiation to an angioblast state. It will also be interesting to identify the source of additional cell types, such as smooth muscle, presumed to associate with inner-wall coronary vessels. Ultimately, a complete understanding of this process will inform the development of therapeutic strategies to stimulate regeneration of coronary vessels from the endocardium of an infarcted heart. ■

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MICROBIOLOGY

The birth of cooperation

Mutualistic symbiosis can arise without prior coevolution

By **Duur K. Aanen**[1] *and* **Ton Bisseling**[2,3]

Mutually beneficial associations between individuals of different species, called mutualistic symbioses, have enabled major ecological innovations and underlie some of the major transitions in evolution (*1*). For example, the ancestor of plants domesticated endosymbiotic photosynthetic bacteria, today's chloroplasts, for carbon fixation. This association dramatically increased the habitat of these photosynthetic bacteria from the sea to terrestrial ecosystems. However, the colonization of land by plants required an additional symbiotic association, with fungal root symbionts that facilitate nutrient uptake (*2*). Yet, surprisingly little is known about how mutualistic symbioses evolved and persist. On page 94 of this issue, Hom and Murray show how mutualism may arise without prior coevolution (see the photo) (*3*).

Mutualism in the lab. *Chlamydomonas reinhardtii* grown alone (**left**) or in coculture with a nitrite-deficient mutant of *Neurospora crassa* (**middle** and **right**, two replicates). For further details, see (*3*).

For a mutualistic association to form between different species, the costs of the interaction must be outweighed by the benefits individuals receive from their partner symbiont (*4*). However, how this condition can be fulfilled has remained enigmatic. According to one model, mutualism follows coevolution (see the figure, panels A, B, and C), for example, after a start as parasitic interactions (*5*) or commensalism (*6*). Under certain conditions, for example, if the reproductive interests between the two partners become united via joint reproduction, several coevolutionary responses may favor mutualistic interactions (*1*, *7*, *8*). However, Janzen has argued that an organism may appear to have coevolved with a different organism but is in fact just using the ecological opportunity, a hypothesis known as ecological fitting (see the figure, panel D) (*9*).

Hom and Murray now provide experimental evidence for ecological fitting. They first tested whether a mutualistic interaction between two species—the yeast *Saccharomyces cerevisiae* and the alga *Chlamydomonas reinhardtii*—could be established without introducing any genetic modifications. The authors created an ecological setting in which the species can only survive when they establish a reciprocal carbon (C) and nitrogen (N) exchange. The yeast, but not the alga, can use glucose as a C source, whereas the alga can use the waste product of glucose metabolism, carbon dioxide (CO_2), in photosynthesis. On the other hand, the alga, but not the yeast, can use nitrite as a N source, but the yeast can use the conversion product of nitrite metabolism, ammonia (NH_4). When the two species are cocultured with glucose as the sole C source and nitrite as the sole N source, the alga depends on CO_2 provided by the yeast and the yeast on NH_4 produced by the alga.

Hom and Murray show that under these conditions, the two species become completely dependent on each other: They become obligate mutualists. Surprisingly, the morphology of the algal symbionts differs from that of their free-living forms. This was particularly obvious in the interaction with the filamentous fungi *Neurospora crassa* and *Aspergillus nidulans* (relatives of lichenous fungi). Hom and Murray show that these fungi can also form an obligate mutualism with *C. reinhardtii*, but only if they have lost the ability to reduce nitrite via genetic engineering. The interaction of these filamentous fungi with *Chlamy-*

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