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# Fusion of the Heart and the First Heartbeats

Cell differentiation occurs independently in the right and left heart-forming primordia (each containing first and second heart fields). As they migrate toward each other, the ventral splanchnic mesoderm cells of the primordia begin to express N-cadherin on their apices, sort out from the somatic (parietal) mesoderm cells, and join together to form an epithelial layer. This joining of the somatic mesoderm will lead to the formation of the pericardial cavity, the sac in which the heart is formed (Linask 1992). A small population of splanchnic mesoderm then downregulates N-cadherin and delaminates from the epithelium to form the endocardium, the lining of the heart that is continuous with the blood vessels. Indeed, these cells may already have been specified as endocardial precursors in the second heart field (Milgrom-Hoffman et al. 2011). The endocardial cells produce many of the heart valves, secrete the proteins that regulate myocardial growth, and regulate the placement of nervous tissue in the heart. The epithelial layer of splanchnic mesoderm forms the myocardium (Manasek 1968; Linask et al. 1997), which will give rise to the cardiac muscles that will pump for the lifetime of the organism.

Pulsations of the chick heart begin while the paired primordia are still fusing. Heart muscle cells develop an inherent ability to contract, and isolated heart cells from 7-day rat or chick embryos will continue to beat when placed in petri dishes (Harary and Farley 1963; DeHaan 1967; Imanaka-Yoshida et al. 1998). The pulsations are made possible by the appearance of the sodium-calcium exchange pump in the muscle cell membrane; inhibiting this channel's function prevents the heartbeat from starting (Wakimoto et al. 2000; Linask et al. 2001). Eventually, the rhythmicity of the heartbeat becomes coordinated by the sinus venosus. The electric impulses generated here initiate waves of muscle contraction through the tubular heart. In this way, the heart can pump blood even before its intricate system of valves has been completed.

Eventually, the rhythm of the heartbeat will be governed by the "pacemaker," the sinoatrial node of the right atrium. The pacemaker cells appear to be cardiac myocytes that have been transformed into electrical conductive tissue by Tbx3. Tbx3 appears to (1) repress those genes involved in atrial myocyte differentiation and those genes (such as those encoding gap junction proteins) that enabled the earlier contractions, and (2) activate those genes whose products increase the resting potential of the sinoatrial cells and their conducting network throughout the heart (Hoogaars et al. 2007; Bakker et al. 2012).

# Looping and formation of heart chambers

The direction of heart looping is dependent on the left-right patterning proteins (Nodal and Pitx2) discussed in Chapter 13. Within the heart primordium, Nkx2-5 regulates the Hand1 and Hand2 transcription factors. Both Hand proteins appear to be synthesized throughout the early heart tube, but as looping commences, Hand1 becomes restricted to the future left ventricle and Hand2 to the right. Without these proteins, looping is abnormal, since the ventricles fail to form properly[i] (Srivastava et al. 1995; Biben and Harvey 1997).

Cytoskeletal proteins and their adhesion to the extracellular matrices are very important in these turning events, suggesting that biophysical forces (mechanotransduction) also are important for the looping process (Hove et al. 2003; Garita et al. 2011). First, the myosin-like protein flectin regulates

the physical tension of the heart tissues differently on different sides of the heart (Tsuda et al. 1996; Lu et al. 2008). Second, the transcription factors Nkx2-5 and Mef2C activate the Xin (Chinese for "heart") gene, whose product binds to actin microfilaments and mediates cytoskeletal changes that are essential for heart looping (Wang et al. 1999; Grosskurth et al. 2008). Third, metalloproteinases—especially metalloproteinase-2 (MMP2)—are critical for remodeling the cytoskeleton. If MMP2 gene expression is blocked, the extracellular matrix fails to change, the asymmetric cell divisions (which cause the left dorsal side to grow faster than the right) fail to occur, and heart looping stops (Linask et al. 2005).

The formation of the heart valves—four leaflike flaps that must open and shut without failure once each second for the duration of an individual's life—is just starting to be understood. In mammals, endocardial cushions form from the endocardium and divide the tube into right and left atrioventricular channels. Meanwhile, the primitive atrium is partitioned by two septa that grow ventrally toward the endocardial cushions. The cushions are thought to serve as the "glue" for the normal septation of the mammalian heart into four chambers (Webb et al. 1998; Sizarov et al. 2012). Moreover, they are thought to serve as valves during early heart development (Lamers et al. 1988). As the heart enlarges, however, specialized valves develop to prevent the return of blood into the atria and to prevent the mixing of bloods from the two sides of the heart. These valves begin to form when cells from the myocardium produce a factor that causes cells from the adjacent endocardium to detach and enter the hyaluronate-rich "cardiac jelly" extracellular matrix between the two layers (Markwald et al. 1977; Potts et al. 1991). In mammals, BMP2 appears to be necessary for inducing this epithelial-mesenchymal transition (EMT) and for forming the endocardial cushions from cardiac myocytes (Ma et al. 2005; Rivera-Feliciano and Tabin 2006). BMP2 induces the transcription factors Tbx2 and Tbx3, which promote EMT by activating the genes encoding TGF-b2, Twist, and the enzymes that synthesize the hyaluronic acid that separates the cells and becomes a major part of the cardiac jelly (Shirai et al. 2009).

With the formation of the septa (which usually occurs in the seventh week of human development), the heart is a four-chambered structure with the pulmonary artery connected to the right ventricle and the aorta connected to the left ventricle. The septa between the fetal atria have openings in them, however, so blood can still cross from one side of the heart into the other. This crossing of blood is needed for the survival of the fetus before circulation to functional lungs has been established. Upon the infant's first breath, the septal openings close and the left and right circulatory loops are established.



[i] Zebrafish, with only one ventricle, have only one type of Hand protein. When the gene encoding this protein is mutated, the entire ventricular portion of the heart fails to form (Srivastava and Olson 2000). Nongenetic agents are also critical in normal zebrafish heart formation. In the absence of high-pressure blood flow, heart looping, chamber formation, and valve development are impaired (Hove et al. 2003).