Molecular Pathways Controlling Heart Development

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Heart formation requires complex interactions among cells from multiple embryonic origins. Recent studies have begun to reveal the genetic pathways that control cardiac morphogenesis. Many of the genes within these pathways are conserved across vast phylogenetic distances, which has allowed cardiac development to be dissected in organisms ranging from flies to mammals. Studies of cardiac development have also revealed the molecular defects underlying several congenital cardiac malformations in humans and may ultimately provide opportunities for genetic testing and intervention.

Malformations of the heart and blood vessels account for the largest number of human birth defects, with a reported incidence of about 1% of live births, and among stillbirths the frequency has been estimated to be 10 times that (*1*). Formation of the heart requires migration, differentiation, and precise interactions among multiple embryonic cell types (*2*). The susceptibility of the heart to developmental anomalies reflects the complexity of these morphogenetic events. Although many human cardiac malformations are well characterized anatomically and physiologically, there is still little understanding of the genetic bases of these abnormalities. However, the recent identification of genes involved in early aspects of heart development and the realization that these genes are evolutionarily conserved have provided an impetus for studying the functions of cardiac regulatory genes in genetically tractable organisms such as flies and zebrafish. Elucidation of the role of these genes in heart development may lead to a better understanding of cardiovascular disease and form the basis for future therapeutic interventions. Here, we review current knowledge of the morphogenetic mechanisms controlling cardiac development as they relate to the anatomical and molecular abnormalities responsible for several congenital cardiac defects.

Morphogenesis and Looping of the Cardiac Tube

The heart is the first organ to form in vertebrates, and it arises through a complex series of morphogenetic interactions involving cells from several embryonic origins (Fig. 1). Beginning soon after gastrulation (about embryonic day 20 in humans), progenitor cells within the anterior lateral plate mesoderm become committed to a cardiogenic fate in response to an inducing signal thought to emanate from the adjacent endoderm (*3*). The specific signaling molecule or molecules responsible for cardiogenic commitment remains to be identified. Cardiac precursors form a bilaterally symmetric cardiogenic "field" that develops further into parallel cardiac primordia, which fuse at the midline to form the primitive cardiac tube. This straight heart tube contains an outer myocardium and an inner endocardium separated by an extracellular matrix known as the cardiac jelly. The tubular heart initiates rhythmic contractions at about day 23 in humans and then undergoes rightward looping, which is the first indication of left-right asymmetry in the embryo. The cellular mechanisms that drive cardiac looping remain poorly understood, but it has been postulated that differential rates of proliferation of cardioblasts, regional differences in intracardiac actin bundles, or altered cell adhesion across the heart tube may be involved. When considering the mechanisms for cardiac looping, it is important to distinguish between the process of looping and the directionality of looping (*4*). The directionality of looping reflects the overall asymmetry throughout the embryo, which is superimposed on the morphogenetic mechanisms for looping.

Looping of the heart to the right occurs in all vertebrate species, suggesting the existence of an evolutionarily conserved mechanism for this critical step in cardiac morphogenesis. Insight into the signals that initiate rightward looping of the heart tube has come from studies with chick embryos. Here the morphogen sonic hedgehog (Shh) is expressed on the left side and the activin receptor IIa (Act-RIIa) on the right side of the heart-forming region before looping (*5*). Activin or an activin-like molecule induces Act-RIIa and suppresses expression of Shh on the right side of the embryo, creating left-right asymmetry. Shh induces expression of the chick nodal-related morphogen (cNR1) on the left. Ectopic expression of Shh on the right side or Act-RIIa on the left side of the embryo randomizes the direction of looping, indicating that the normally asymmetric expression pattern of these morphogens directs rightward looping (Fig. 2).

The mechanisms that control the directionality of cardiac looping have also been explored by genetic analysis of two mouse mutants with abnormalities in left-right asymmetry. In mice homozygous for the *iv* (inversus viscerum) mutation, left-right orientation of the heart and viscera is randomized (*6*). The *iv* gene has been mapped to chromosome 12 but not yet cloned. In the *inv* (inversion of embryonic turning mutation) mouse, which arose as a result of a random transgene insertion mutation on chromosome 4, there is nearly a 100% reversal of left-right asymmetry and cardiac looping (*7*). Although the genes responsible for these mutant phenotypes are yet to be identified, the asymmetric expression of the morphogen nodal may provide some clues regarding the defects. Mice with the *inv* mutation express nodal along the right lateral mesoderm rather than the left, whereas homozygous *iv* mice exhibiting randomization of cardiac looping also have randomization of nodal expression, including reversal of asymmetry, symmetric expression, and bilateral lack of expression (*8*). Whether the *inv* and *iv* genes act in the same genetic pathway and how nodal acts within the molecular cascade of asymmetric signaling molecules remain to be determined.

Chamber Specification and Formation

After cardiac looping, the atrial and ventricular chambers of the heart become morphologically identifiable. Atrial and ventricular cardiac myocytes express distinct subsets of cardiac muscle genes that confer the contractile, electrophysiological, and pharmacologic properties unique to each chamber. Little is known of the mechanisms that control the diversification of cardiogenic precursors to atrial and ventricular phenotypes. Atrial and ventricular cells arise from separate lineages that are specified before looping occurs (*9*). These lineages differentiate according to their positions along the antero-

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posterior (A-P) axis of the embryo such that the heart tube can be divided into segments that give rise to the atrium, left ventricle, right ventricle, and the ventricular outflow tract (conotruncus) (Fig. 1A). Exposure of chick, frog, and zebrafish embryos to retinoic acid results in anterior truncations of the heart tube (*10*), suggesting that the heart acquires A-P polarity during or shortly after commitment to the cardiac lineage. Similar anterior truncations of the embryo have been observed in response to retinoic acid (*11*), suggesting that the heart-forming region is subject to the same A-P patterning mechanisms that specify the overall body plan. The effects of retinoic acid on patterning of the A-P axis of the embryo are associated with alterations in the expression of homeobox genes, which specify positional identity along the embryonic axes (*12*). One interesting possibility is that homeobox genes also regulate A-P patterning in the heart tube.

Most atrial and ventricular chamber-specific genes are expressed homogeneously throughout the cardiac tube and become regionally restricted later in development (*13*); however, chamber-specific myosin light and heavy chain genes show spatially restricted expression within the heart tube before the recognizable demarcation of chambers or the formation of septa (*14*). These genes are also expressed in different populations of cardiac myocytes derived from embryonic stem cells in vitro. These findings suggest that chamber specification precedes septation of the cardiac tube.

Separation of the cardiac tube into the atria, ventricles, and outflow tract is accomplished by mesenchymal outgrowths, known as cardiac cushions, that form as regional swellings of the cardiac jelly (*15*). Migration of endothelial cells to these cushions is accompanied by an epithelial-to-mesenchymal transformation. As the cushions expand, they fuse to form the anlage of the septal and valvular structures that demarcate the developing chambers. The common atrioventricular (AV) canal is septated into two separate left and right AV channels (Fig. 1C), while the conotruncus undergoes septation and spiraling, allowing proper orientation of the great vessels (aorta and pulmonary artery).

The swellings of the cardiac jelly in the cushion regions contain multiple extracellular (ECM) components that are believed to be secreted from the myocardial layer. In vitro assays for cushion formation indicate that endothelial cell transformation into cushion mesenchyme is dependent on secreted signals emanating specifically from the AV mesenchyme and that an ECM fraction from cultured cardiac myocytes can provide this signal (*15*). Cell-cell and cell-ECM interactions have been implicated in AV canal septation (*15*) and other aspects of cardiogenesis (*16*). The neural cell adhesion molecule NCAM is initially expressed throughout the endocardium, but it is dramatically down-regulated in the endocardial cushions. In contrast, the adhesion molecule tenascin is up-regulated in the same cells and is thought to disrupt cell-substrate adhesion, allowing cells to migrate through the ECM. Abnormalities or arrests in these processes may be responsible for some of the AV canal and conotruncal defects seen in infants.

Alterations in the expression of several genes have been demonstrated during AV valve morphogenesis (*15*). In particular, members of the transforming growth factor–β (TGF-β) family, including bone morphogenetic protein–4 (BMP4), TGF-β1, and TGF-β2, show restricted expression in the AV canal and conotruncal regions, as do the homeobox genes *msx1* and *mox1*.

Division of the common atria and common ventricle into left and right chambers occurs by formation of the interatrial and interventricular septa, respectively. A fenestrated wall of cardiac muscle arises between the left and right sides of the atria and ventricles that develops further into uniform septa. In addition, growth of the endocardial cushion in the rostral and caudal directions is responsible for formation of portions of the interatrial and interventricular septa, respectively (*17*).

Mutations of a wide variety of genes in mice result in hypoplasia of the muscular wall of the heart. Mice homozygous for a null mutation in the gene encoding retinoid X receptor-α (RXRα) display ventricular chamber hypoplasia and muscular ventricular septal defects (*18*). A similar phenotype is seen in mice carrying mutations in the N-*myc*, *TEF1*, Wilms tumor (*WT1*), and neurofibromatosis (*NF1*) genes (*19*). Deficiencies of the cell adhesion molecules α_4 integrin and VCAM (V

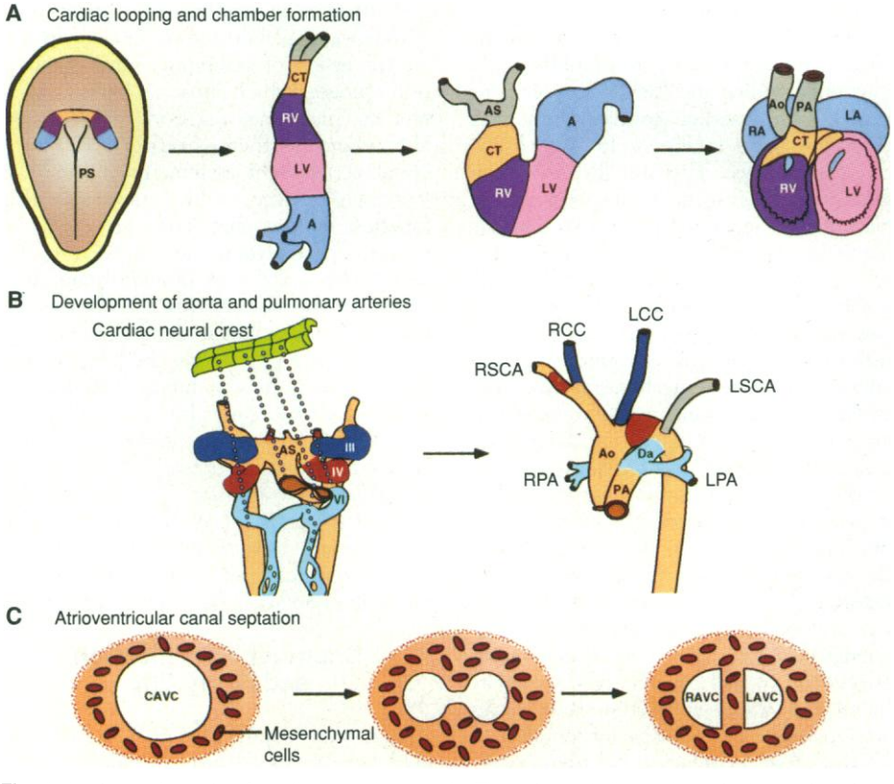

Fig. 1. Anatomical events of cardiac morphogenesis. The schematic illustrations depict cardiac development with color coding of morphologically related regions. (**A**) Cells of the precardiogenic mesoderm, shown in color, are specified at the embryonic primitive streak stage (leftmost panel) to form specific regions of the straight heart tube. The straight heart tube is specified in an A-P gradient to form the various regions and chambers of the looped and mature heart. PS, primitive streak; AS, aortic sac; CT, conotruncus; RV, right ventricle; LV, left ventricle; A, atria; RA, right atrium; LA, left atrium; Ao, aorta; PA, pulmonary artery. (**B**) Cardiac neural crest cells populate the bilateral aortic arches III, IV, and VI and the aortic sac. Extensive remodeling of the vasculature results in formation of the left aortic arch and proximal pulmonary arteries. Contributions of the aortic arch arteries to these regions are depicted in color. Da, ductus arteriosus; LSCA, left subclavian artery; LCC, left common carotid; RCC, right common carotid; RSCA, right subclavian artery; LPA, left pulmonary artery; RPA, right pulmonary artery. (**C**) Separation of the common atrioventricular canal (CAVC) into a right (RAVC) and left (LAVC) atrioventricular canal is accomplished by invasion of ectomesenchymal cells.

designates vascular) result in epicardial dissolution and subsequent myocardial thinning (*19*). The diversity of genes affecting myocardial growth suggests that this aspect of cardiac development is particularly sensitive to perturbations.

Signaling between the endocardium and the myocardium also appears to be important for ventricular growth. Neuregulin growth factors are expressed in the endocardium and are required for the development of trabeculae, the fingerlike projections of the ventricular myocardium. In mice deficient in neuregulin or its receptors, ERBB2 and ERBB4, the ventricular trabeculae fail to form, possibly as a result of decreased endocardial signals (*20*). Defects in myocardial formation and contractility are also observed in the *cloche* zebrafish mutant, which lacks endocardial cells (*21*).

Development of the Cardiac Conduction System

A unique and essential property of cardiac myocytes is their rhythmic and sequential contraction. Areas of spontaneous depolarization are initially seen in the caudal region of the straight heart tube even before contraction is visible (*22*). This region later forms the sinoatrial (SA) node, which serves as the pacemaker of the heart. Electrical impulses spread from the SA node throughout the atria and are initially propagated around the AV canal to the ventricles. As the atria and ventricles become electrically isolated by growth of the endocardial cushions, a region of the interventricular septum, known as the AV node, provides the only pathway from atria to ventricles for depolarization. The persistence of pathways of conduction around the AV canal may allow for the occurrence of some tachycardic arrhythmias. Depolarization in the ventricles occurs through specialized tissues known as the bundle of His and Purkinje fibers. The signals regulating the development of this conduction system from specialized cardiomyocytes have not been identified. The homeobox gene *msx2* is expressed in central portions of the conduction system, whereas the peripheral Purkinje fibers arise in association with the coronary arterial bed (*23*). Saturation mutagenesis of zebrafish has produced numerous mutants with visible conduction defects (*24*), and future identification of the mutant genes may provide important insight into this unique feature of cardiomyocytes.

The Neural Crest and Cardiovascular Development

Neural crest cells are a population of cells that migrate from the neural folds and differentiate into diverse cell types including melanocytes, neurons, connective tissue, and smooth muscle. Cardiac neural crest cells, originating from the midotic placode to somite 3, migrate through the circumpharyngeal region to populate the pharyngeal and aortic arches as well as the cardiac outflow tract and proximal great vessels (Fig. 1B) (*25*). The bilaterally symmetric aortic arches (III, IV, and VI), which arise from the aortic sac, undergo extensive remodeling during development and finally contribute to particular regions of the left-sided aortic arch and pulmonary arteries. Neural crest cells are also involved in the formation of the truncal cushion and membranous portion of the interventricular septum, proper separation and alignment of the great arteries, and development of the semilunar valves.

The role of the neural crest in heart development has been best demonstrated by studies with chick embryos. Neural crest ablation in these embryos produces a variety of cardiac defects, mostly of the conotruncus and aortic arch, including persistent truncus arteriosus and interrupted aortic arch (*25*). These abnormalities resemble those seen in DiGeorge syndrome, a human congenital syndrome believed to arise from neural crest defects (*26*).

Transcriptional Control of Cardiac Development

Cardiomyocytes are terminally differentiated cells and thus cannot be regenerated when the loss of heart muscle by myocardial cell death results in cardiac failure, as often occurs in acquired heart disease. An exception is the newt, in which terminally differentiated cardiomyocytes retain the ability to enter the cell cycle (*27*). An understanding of the molecular events associated with cardiac cell differentiation will be critical to future efforts to regenerate human myocardium by gene therapy. Thus far, these efforts have been hampered by the lack of cardiac cell lines. Recently, however, studies with the fruit fly *Drosophila* have begun to provide an understanding of the genetic pathways that regulate cardiogenesis and suggest that the basic mechanisms for cardiac development are highly conserved.

Fruit flies have a primitive heartlike structure known as the dorsal vessel (analogous to the straight heart tube of the vertebrate embryo) that contracts rhythmically and pumps hemolymph through an open circulatory system. Formation of the dorsal vessel is dependent on a homeodomain-containing transcription factor known as tinman (TIN), which is expressed in the early mesoderm and the dorsal vessel (*28*). Loss-of-function mutations of the gene *tinman* result in the complete absence of the dorsal vessel, indicating that *tinman* specifies the formation of cardioblasts. Searches for genes related to *tinman* led to the identification of a vertebrate gene known as *Nkx2.5* or *Csx*, which encodes a protein containing extensive sequence similarity with TIN in the homeodomain (*29*). *Nkx2.5* is initially expressed in cardiac progenitors and the pharyngeal endoderm, and it is the earliest known marker for the cardiogenic lineage in vertebrates. In mice homozygous for a null mutation in *Nkx2.5*, the heart tube forms and most cardiac contractile protein genes are expressed except for a

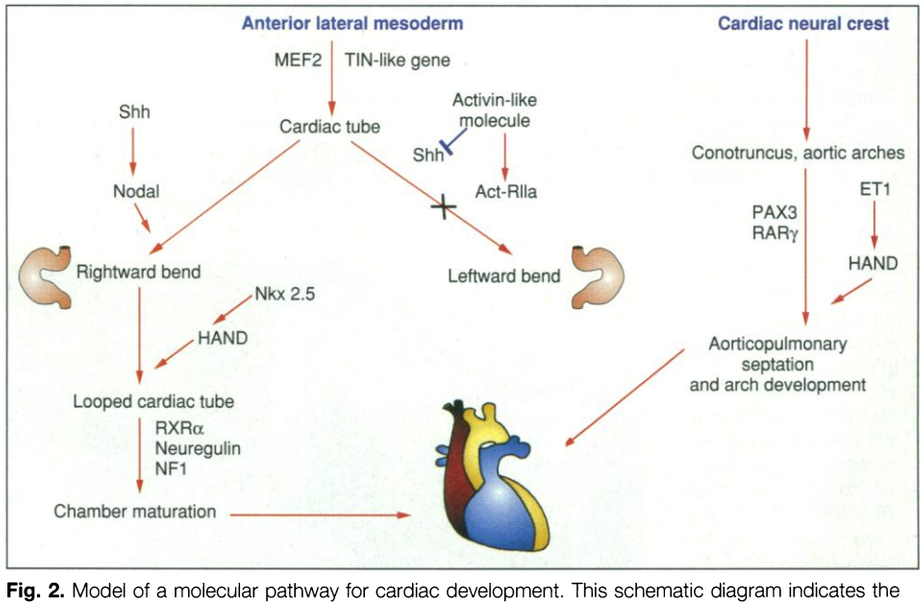

Fig. 2. Model of a molecular pathway for cardiac development. This schematic diagram indicates the possible positions of several proteins within a morphogenetic pathway that have been implicated in cardiac development.

ventricular-specific myosin light chain gene. The heart tube also bends correctly to the right but fails to undergo complete looping, possibly as a result of abnormal muscle growth (*30*). Although this phenotype demonstrates that the Nkx2.5 protein is required for heart development, the defect is manifested later than would be predicted from the *tinman* mutant phenotype in *Drosophila*, which is characterized by the complete absence of cardiogenic cells. It will be interesting to determine whether Nkx2.5 is indeed the functional equivalent of TIN and whether proteins encoded by other recently identified *Nkx2.5*-related genes (*31*) have overlapping functions in the cardiogenic pathway of vertebrates. Particularly important will be the identification of target genes for TIN and Nkx2.5 in the cardiac lineage.

Another transcription factor that appears to play a role in cardiogenesis in both *Drosophila* and vertebrates is myocyte enhancer binding factor–2 (MEF2), which belongs to the MADS (MCM1, agamous, deficiens, and serum response factor) box family of transactivators (*32*). The four *mef2* genes in vertebrates are expressed in precursors of the cardiac, skeletal, and smooth muscle lineages, as well as in certain other cell types (*33*). The MEF2 factors activate transcription through a conserved A-T–rich DNA sequence in the control regions of numerous muscle structural genes. A single *mef2* gene in *Drosophila*, known as *D-mef2*, is also expressed in precursors of myogenic lineages and their descendants (*34*). Loss-of-function mutations of *D-mef2* block differentiation of all muscle cell types in the embryo (*35*). However, *D-mef2* mutant embryos contain myoblasts that are normally positioned and specified. The dorsal vessel also forms normally and expresses *tinman*, but contractile protein genes are not expressed. This phenotype demonstrates that *D-mef2* controls a relatively late step in the muscle differentiation pathway and indicates that different muscle cell types share aspects of a common myogenic regulatory program under the control of MEF2 factors. The phenotypic consequences of *mef2* mutations on heart development in the mouse are not yet known, but the similarity in expression patterns, DNA binding, and transcriptional activation properties of the MEF2 factors from *Drosophila* and vertebrates suggest that the vertebrate MEF2 factors are likely to play a role in cardiomyocyte differentiation.

Certain members of the GATA family of transcription factors also appear to participate in cardiac muscle differentiation. GATA4 is expressed in the precardiac mesoderm and subsequently in the endocardial and myocardial layers of the heart tube and developing heart (*36*). Binding sites for GATA4 are found in the control regions of several cardiac muscle genes, and inhibition of GATA4 expression with antisense RNA blocks the differentiation of cardiac myocytes from the pluripotent P19 embryonal carcinoma cell line (*37*). GATA5 and GATA6 bind the same DNA sequence as GATA4, and their expression patterns overlap that of GATA4 in the cardiogenic lineage, raising the possibility that they perform similar functions (*38*).

Members of the basic helix-loop-helix (bHLH) family of transcription factors control the differentiation of skeletal muscle, neuronal, and hematopoeitic cells (*39*). The four skeletal muscle–specific (bHLH) transcription factors—MyoD, myogenin, Myf5, and MRF4—function within a regulatory pathway that establishes skeletal myoblast identity and controls the expression of muscle structural genes. In contrast to many other muscle genes, which are expressed in both cardiac and skeletal muscle, these myogenic bHLH factors are not expressed in cardiac muscle (*40*, *41*). Recently, two related bHLH factors, referred to as dHAND and eHAND, were found to be expressed in early cardiogenic progenitors and in the looping heart tube as well as in cardiac neural crest–derived cells (*42*, *43*) (Fig. 3). Inhibition of expression of these factors with antisense oligonucleotides in cultured chick embryos resulted in an arrest of cardiac morphogenesis at the looping stage, suggesting that these factors regulate a critical step in this morphogenetic pathway (*42*). Preliminary data indicate that eHAND expression in the heart is diminished in *Nkx2.5*-null mice (*44*), suggesting that these genes act in the same pathway to control cardiac looping.

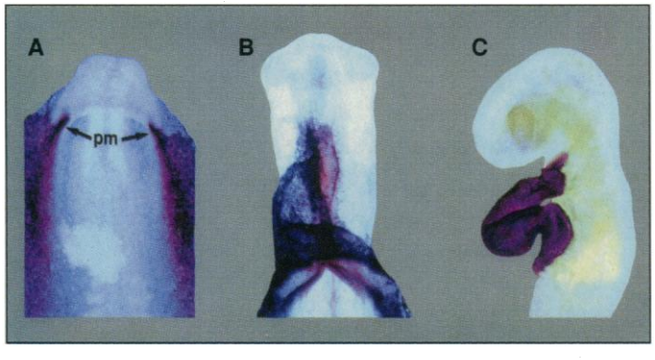

Fig. 3. Expression of dHAND transcripts during cardiac development in chick embryos, monitored by whole-mount in situ hybridization. The dHAND transcripts were first detected in the precardiogenic mesoderm (pm) in stage 8 embryos (**A**). Stage 10 embryos show expression of dHAND throughout the straight heart tube (**B**), and stage 16 embryos show expression of dHAND in the looped heart (**C**). Adapted from (*42*).

Congenital Cardiac Defects

Numerous congenital cardiac defects arise from anomalies in cardiac morphogenesis. Clinically described lesions represent a subset of defects compatible with fetal circulation, whereas the remainder result in embryonic lethality. Some of these defects are the result of arrest of cardiac development at early embryonic stages, and others represent aberrant development; hemodynamic alterations contribute to the phenotype of the developmental defects. Congenital heart diseases are usually classified according to their anatomical arrangements, but it may be more useful here to consider them according to their embryologic origins.

Cardiac looping defects. Looping of the straight cardiac tube is critical to proper orientation and alignment of the chambers and great vessels of the heart. Some defects in cardiac looping are associated with the congenital syndrome known as visceroatrial heterotaxy, in which there is a reversal of some or all of the visceral asymmetry (situs inversus, situs ambiguous), including leftward cardiac looping. Heterotaxy syndrome in humans is often associated with complex structural defects in the heart, including a common atrium, ventricular inversion, malalignment of the AV canal and the cardiac outflow tract, and abnormal venous and arterial vascular connections. Isolated defects that are not part of heterotaxy syndrome, such as ventricular inversion, are also likely to be related to looping abnormalities.

Recent molecular analysis suggests that cardiac looping defects and heterotaxy syndrome result from heterogeneous gene defects. A mutation in the connexin43 gap junction protein, which is critical for cell-cell communication, has been identified in children with heterotaxy syndrome (*45*). Inactivation of the connexin43 gene in mice produced a different cardiac phenotype, an obstruction in the right ventricular outflow tract (*46*). The differing phenotypes may be a consequence of the more global effects of a dominant negative mutant in the human and the potential for genetic redundancy in the null mouse model. It will be important to determine whether any components of the signaling pathways for left-right asymmetry are also altered in congenital heart diseases related to abnormal direction or alignment of the looped heart tube.

Defects in AV septation. The most com-

mon, though not necessarily the most severe, types of congenital heart defects in humans are those related to incomplete septation of the atria, ventricles, or AV canal. These can be divided into defects of the muscular septum and of the endocardial cushion separating the atria from the ventricles. Because the initial events in AV septation involve formation of a fenestrated wall between the left and right sides of the heart, AV septal defects may represent residual areas of fenestration between the chambers.

The etiology of AV septal defects is unknown. Insight into the genes involved may come from studies of families with Holt-Oram syndrome (heart-hand syndrome), which is characterized by cardiac and limb defects. The majority of cardiac defects are atrial septal defects and muscular ventricular septal defects. Linkage analysis has implicated a gene (or genes) at chromosome 12q2 (*47*), which remains to be identified.

Defects in the AV canal range in severity from complete connections between the four chambers to simple clefts in the AV valves. AV canal defects are common in children with Down syndrome (trisomy 21), and intensive efforts are under way to determine the critical gene or genes on chromosome 21q22.2-22.3. In a mouse model of Down syndrome, the trisomy 16 (Ts16) mouse, the critical region has been narrowed to a 6-Mb stretch of DNA that includes the collagen type VI genes, which are expressed in the human fetal heart, and the *ETS* oncogene and its relative *ETS2* (*48*). Like cardiac looping defects, AV canal defects are likely to be multifactorial in origin, as other loci outside of chromosome 21 have been implicated (*49*).

Neural crest defects. The CATCH-22 syndrome (named for cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcemia from deletions in chromosome 22) has been another exciting area of genetic research. This syndrome encompasses a wide spectrum of clinical manifestations, including those seen in DiGeorge syndrome and velo-cardio-facial syndrome where greater than 80% of affected individuals have microdeletions of chromosome 22q11 (*50*). The most common cardiac defects observed are persistent truncus arteriosus, which is caused by failure of the single primitive great vessel from the heart to septate into the aorta and pulmonary artery, and interruption of the aortic arch. Other cardiac defects include malalignment of the aorta with respect to the ventricular septum (tetralogy of Fallot, double-outlet right ventricle), and simple membranous ventricular septal defects arising from incomplete development of the conotruncus. About 25% of children with CATCH-22 syndrome have a right aortic arch rather than the normal left aortic arch. It seems likely that these clinically diverse abnormalities have a common etiology that is based on cardiac neural crest defects. The genetic locus responsible for CATCH-22 has been mapped to a 1.5-Mb region of chromosome 22q11 (*51*). It will be of great interest to determine whether the constellation of defects in CATCH-22 is due to mutation in one or multiple genes.

Many children with heart disease have isolated conotruncal or aortic arch defects without other features of CATCH-22 syndrome. Among these individuals, 20 to 30% have microdeletions of chromosome 22q11 (*52*). It is likely that genes on other chromosomes also contribute to some of the isolated defects observed (*53*). Insight into some of these genes may come from studies in other vertebrate models. The zebrafish mutant with an interrupted aortic arch, termed *gridlock*, provides a system for studying aortic arch anomalies (*54*). Genetically defined mice with cardiac neural crest defects may also be informative. The mouse mutant *Splotch*, which harbors a mutation in the homeobox gene *Pax3*, exhibits conotruncal or aortic arch defects, as do mice deficient in retinoic acid receptor (RARγ), the homeodomain protein HOXA3, and the *NF1* gene product (*55*). Endothelin-1 (ET1)–deficient mice also have a phenotype reminiscent of CATCH-22 syndrome, displaying conotruncal and aortic arch defects, cleft palate, and other craniofacial abnormalities (*56*). Recent evidence that the bHLH genes *dHAND* and *eHAND* are downregulated in neural crest–derived tissues in ET1-deficient mice (*57*) suggests that these transcription factors may regulate cardiac neural crest development.

Prospects

We are entering an exciting era for molecular cardiology in which studies of a variety of organisms are contributing to a detailed molecular understanding of normal and abnormal cardiac development. Genetic screening and counseling will become increasingly important for individuals with congenital heart disease, particularly as improvements in medical care allow a growing number of these individuals to reach reproductive age. Gene therapy for children with congenital heart disease remains a distant prospect. However, patients with acquired heart disease may benefit much earlier from the studies of cardiac development discussed here. For example, determination of the genes involved in proliferation, differentiation, and growth of embryonic cardiac myocytes will greatly facilitate efforts to regenerate heart muscle in patients with myocardial cell loss. Potential modes of such therapy include direct delivery of genes into the myocardium or introduction of genetically altered cells into the heart. The combined advances in molecular biology, genetics, and gene therapy will undoubtedly lead to improvements in both our understanding and our therapeutic interventions for cardiovascular disease.

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Molecular Genetics of Human Blood Pressure Variation

Richard P. Lifton

Hypertension is a common multifactorial vascular disorder of largely unknown cause. Recognition that hypertension is in part genetically determined has motivated studies to identify mutations that confer susceptibility. Thus far, mutations in at least 10 genes have been shown to alter blood pressure; most of these are rare mutations imparting large quantitative effects that either raise or lower blood pressure. These mutations alter blood pressure through a common pathway, changing salt and water reabsorption in the kidney. These findings demonstrate the utility of molecular genetic approaches to the understanding of blood pressure variation and may provide insight into the physiologic mechanisms underlying common forms of hypertension.

Elevated arterial blood pressure, or hypertension, affects 15 to 20% of the adult population in industrialized societies and is one of the principal independent risk factors for stroke, myocardial infarction, and end-stage renal disease. Because of this high prevalence, management of hypertension is thought to constitute the single largest health care expense in the United States.

Despite the public health importance of this trait, the pathogenesis of hypertension remains unknown in the overwhelming majority of patients. At face value, this ignorance may seem surprising, because the determinants of blood pressure are deceptively simple—blood pressure is the product of cardiac output and systemic vascular resistance to flow. However, although many of the physiologic systems involved in regulation of blood pressure have been identified, it has proved difficult to determine which systems have primary derangements in hypertensive patients, largely because of the complex interactions of these different physiologic pathways. As a result, we cannot reliably identify susceptible individuals in preclinical stages so that disease may be prevented, and our current treatment of hypertensive patients is necessarily empiric.

A variety of genetic, environmental, and demographic factors contribute to blood pressure variation, even in single individuals (*1*). The recognition that a substantial fraction of human blood pressure variation is genetically determined derives from studies comparing the blood pressures of monozygotic and dizygotic twins (*2*), from epidemiologic studies of familial aggregation of blood pressure (*3*), and from adoption studies comparing the blood pressure of biologic and adoptive siblings (*4*). These findings suggest the application of molecular genetic approaches to identify these underlying genetic components. The progress that has been made in this effort is reviewed here.

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Mendelian Forms of Hypertension

Recently, considerable effort has been devoted to the identification of genes responsible for mendelian, or single-gene, forms of severe hypertension. The reasons for this effort are several. First, the effects of segregation of single alleles in families can be discerned, thereby simplifying molecular genetic analysis. Second, because morbidity from hypertension is proportional to its severity, severe inherited forms may have particularly high attendant morbidity. Identification of the responsible genes may provide novel diagnostic tools as well as an opportunity for targeted therapeutic intervention. Third, the genes and physiologic pathways implicated in severe forms of hypertension may also be involved in the more modest forms of hypertension commonly seen in the general population. Finally, identification of these genes may provide insight into the pathogenesis of common forms of hypertension, which have been variously proposed to result from primary abnormalities in the brain, heart, vasculature, adrenal gland, liver, and kidney.

Glucocorticoid-remediable aldosteronism