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REVIEW ARTICLES

Transcriptional Regulation of Cardiac Development: Implications for Congenital Heart Disease and DiGeorge Syndrome

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ABSTRACT

In recent years, impressive advances have occurred in our understanding of transcriptional regulation of cardiac development. These insights have begun to elucidate the mystery of congenital heart disease at the molecular level. In addition, the molecular pathways emerging from the study of cardiac development are being applied to the understanding of adult cardiac disease. Preliminary results support the contention that a thorough understanding of molecular programs governing cardiac morphogenesis will provide important insights into the pathogenesis of human cardiac diseases. This review will focus on examples of transcription factors that play critical roles at various phases of cardiac development and their relevance to cardiac disease. This is an exciting and burgeoning area of investigation. It is not possible to be all-inclusive, and the reader will note important efforts in the areas of cardiomyocyte determination, left-right asymmetry, cardiac muscular dystrophies, electrophysiology and vascular disease are not covered. For a more complete discussion, the reader is referred to recent reviews including the excellent compilation of observations assembled by Harvey and Rosenthal (1). **(*Pediatr Res* 48: 717–724, 2000)**

Abbreviations

DGS, DiGeorge syndrome
SRF, serum response factor
TGF, transforming growth factor
ECM, extracellular matrix

Cardiac morphogenesis. Most forms of congenital heart disease result from aberrations in cardiac morphogenesis including errors in cardiac septation, valve formation and proper patterning of the great vessels. These defects can be related to specific stages of cardiac development and to specific molecular pathways (known and unknown) functional at each stage (for review, see (2, 3)). A schematic representation of early heart development is depicted in Figure 1. Early in gestation (day 18 in humans or day 7.5 in mouse), cardiac primordia can be identified as bilaterally symmetric components derived from the lateral plate mesoderm. By day 22 (day 8.5 in the mouse) these primordia migrate medially and fuse to form a single heart tube composed of two cell layers (myocardium and endocardium) separated by a vast extracellular matrix, the cardiac jelly. The midline heart tube folds upon itself (cardiac looping) and distinct regions corresponding to future chambers are discernible. By day 25 in humans (day 10.5 in mouse), localized swellings of the extracellular matrix are invaded by underlying endothelial cells to initiate the formation of the endocardial cushions that will later condense to form the mature cardiac valves. Thereafter, a series of complex septation events results in delineation of the cardiac chambers. By day 34 (day 11.5 in the mouse), neural crest cells have migrated from the dorsal neural tube along aortic arches 3, 4, and 6 and have invaded the outflow tract of the heart. These cells are required to induce septation of the single great vessel emerging from the embryonic heart (the truncus arteriosus), thus forming the aorta and pulmonary artery (4). They contribute to the mesenchymal elements of the ductus arteriosus and great vessels and initiate remodeling of the aortic arches. Cardiac neural crest differentiates into a subpopulation of arterial smooth muscle cells. At birth, the ductus arteriosus closes, thus completing the formation of separate pulmonary and systemic circulations.

Fusion of cardiac primordia. Specific transcriptional regulators have emerged as critical factors for many of these developmental events (see Fig. 1). For example, the GATA4 transcription factor appears to be required for midline fusion of

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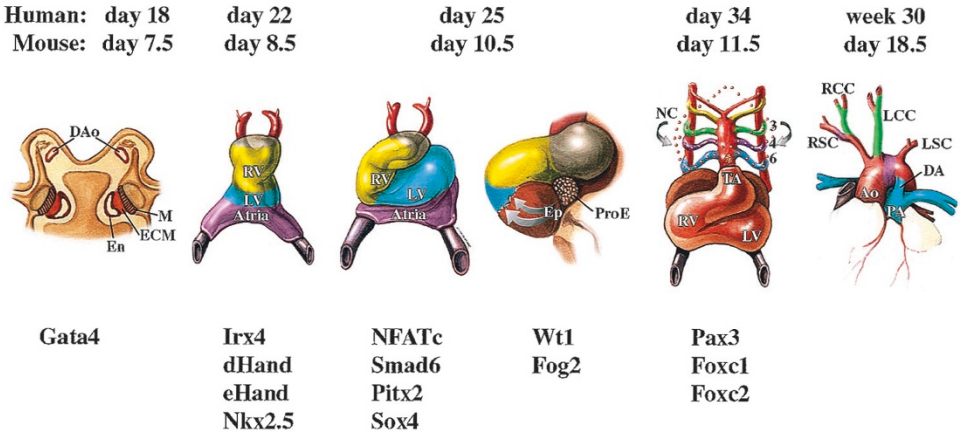

Figure 1. Schematic representation of cardiac development. Approximate human and murine gestational ages are indicated above the drawings and transcription factors discussed in the text are listed below. A schematic cross section through an early embryo is shown in the first panel to indicate the bilaterally symmetric cardiac structures composed of an endocardial tube (En) separated by an extracellular matrix (ECM) from the myocardial precursors (M). Bilateral dorsal aortae (DAo) are indicated. The second panel shows a frontal view of the midline cardiac tube. Gene expression analysis reveals early specification of chambers including right ventricle (RV) and left ventricle (LV). Panels 3 and 4 represent frontal and left lateral views, respectively, of a looped heart tube. The pro-epicardial organ (ProE) is located posteriorly and gives rise to epicardial cells (Ep, shown in brown) that migrate over the ventricles, as indicated by arrows. Panels 5 and 6 depict vascular remodeling. The aortic arches in panel 5 (numbered) are populated by neural crest cells (NC, arrows) and are color coded to match the mature arterial segments indicated in panel 6. TA, truncus arteriosus. RSC, right subclavian artery. RCC, right carotid artery. LCC, left carotid artery. LSC, left subclavian artery. DA, ductus arteriosus. Ao, aorta. PA, pulmonary artery. (Modified from (1, 111)).

the primitive bilateral heart tubes (5, 6). GATA transcription factors are defined by the presence of a zinc finger DNA binding domain that recognizes a "GATA" DNA sequence motif. GATA1, 2, and 3 are important during hematopoietic development (7, 8), while GATA4, 5, and 6 are expressed in the heart (9, 10). Inactivation of *GATA4* in the mouse results in early embryonic lethality. Cardiac progenitors are specified, and bilateral primordia can be identified, but a midline cardiac tube fails to form (5, 6). GATA factors may have later functions during cardiac development, and may be redundant with one another. Potential GATA binding sites have been found in many cardiac specific gene promoters, and GATA4 is capable of synergizing with other transcription factors such as Nkx2.5 and serum response factor (SRF) to activate cardiac-specific gene expression (11, 12). GATA4 may have critical noncardiac functions as well. GATA4 may be required in noncardiac cells (including endoderm) for midline fusion of cardiac primordia since chimeric rescue experiments in the mouse (in which the embryonic endoderm is derived from wild-type cells) rescues midline fusion even though the cardiac cells themselves are GATA4 deficient (13).

Elucidation of molecular pathways regulated by GATA4 in cardiomyocytes has led to important observations that may relate to our understanding and treatment of cardiac hypertrophy and heart failure (14). GATA-associated factors (such as Friend-of-GATA (FOG) or FOG2) may be involved in Tetralogy of Fallot, coronary vascular anomalies or other congenital defects. Both FOG2 and nuclear factor of activated T cells 3 (NFAT3) have been identified as potential heterodimerization partners for GATA4 (14). Since NFAT3 is activated by calcineurin in response to changes in intracellular calcium, it is possible that an NFAT3-GATA4 transcriptional complex mediates some aspects of the hypertrophic response triggered by rising intracellular calcium. Perhaps this pathway results in the well described re-initiation of the "fetal gene program" characteristic of cardiac hypertrophy and subsequent heart failure, though this model remains controversial (15–21).

Chamber specification. A growing number of transcription factors are expressed in chamber-specific patterns, and are likely to be responsible for specifying chamber identity. Cardiac chambers are morphologically distinct even at early stages of development (Fig. 1) (2, 3). It is becoming evident that these differences are unlikely to be explained by differences in hemodynamics. Rather, programmed differences in gene expression appear to determine cell fate and regional identity. This paradigm is reminiscent of neuronal cell fate determination mediated by the overlapping pattern of *Hox* gene expression (the "*Hox* code") along the anteroposterior neural axis of the embryo (for review, see (22)). Recently, a novel Iroquois-related homeobox gene, *Irx4*, has been described that exhibits ventricular-specific expression during development (23). Tissue-specific gene inactivation and transgenic over-expression experiments will be important to determine if *Irx4* is involved in ventricular specification.

The basic helix-loop-helix transcription factors dHand and eHand are expressed predominantly in right and left ventricles during mouse development (24). In chick embryos, their chamber-specific expression pattern is less distinct. *dHand* deficient embryos form a poorly developed right ventricle, though the tissue appears to be correctly specified (25). Hypoplastic right and left ventricle syndromes may be related to mutations in these or similar factors (26). In addition, *dHand* is expressed in regions populated by neural crest cells, and analysis of potential downstream genes regulated by dHand has provided candidate genes for DiGeorge syndrome.

Looping morphogenesis. Inactivation of the *Nkx2.5* homeobox gene in the mouse results in failure of looping morphogenesis (27, 28). This gene encodes a DNA binding protein containing a 60 amino acid helix-turn-helix motif related to homeobox-containing (*HOX*) genes that regulate early embry-

onic patterning. It is closely related to the *tinman* gene of the fruit fly *Drosophila melanogaster* (29). In the fly, *tinman* is required for formation of the dorsal vessel, a structure that appears to represent the evolutionary ancestor of the mammalian heart. Many related *Nkx* genes are expressed in mammals in overlapping patterns (30), but *Nkx2.5* appears to play a unique function in cardiac development. It is expressed from the earliest stages of cardiomyocyte determination. Although mutant embryos are able to form a primitive heart tube and express cardiac specific genes including myosin, they die during mid-gestation and the heart fails to loop normally (28). The transcriptional regulation of *Nkx2.5* expression is complex. A series of elegant reports, reviewed elsewhere (31), indicate that chamber-specific expression is regulated by distinct enhancer sequences. This suggests that chamber-specific upstream factors that mediate *Nkx2.5* expression exist in a region-specific fashion. These factors are also likely to participate in defining chamber-specific identity (*e.g.*, atrial *versus* ventricular, right ventricular *versus* left ventricular). Recently, hypomorphic alleles and heterozygous loss-of-function of *Nkx2.5* have been shown to result in atrial septation defects and conduction abnormalities in humans (32, 33).

Valvulogenesis. Endocardial cushion defects and congenital valvular abnormalities, including pulmonic and aortic stenosis, bicuspid aortic valve, mitral valve prolapse and cleft mitral valve, are common. Abnormalities of recently described molecular pathways are likely to account for at least some of these cases. Transcriptional regulators such as NFATc, the Sry-related homeobox gene *Sox4*, and the downstream modulator of TGFβ superfamily signaling Smad6 are required for proper endocardial cushion formation and maturation (34–37). Members of the TGFβ family of secreted growth factors, the type III TGFβ receptor, and the EGF receptor are also involved (38–40). These receptors may mediate intracellular signals through the small GTP binding protein ras, since mutation in the ras-GAP protein encoded by the Neurofibromatosis gene *NF1* disrupt valvulogenesis (41–43). Interestingly, there appears to be an increased incidence of valvular pulmonic stenosis in patients with von Recklinghausen Neurofibromatosis associated with mutations in the *NF1* gene (44–46). It will be of interest to determine whether somatic mutations in the *NF1* gene account for some sporadic cases of pulmonic stenosis.

Inactivation of the murine bicoid type homeobox gene *Pitx2* also results in enlargement of the endocardial cushions, though *Pitx2* itself is expressed in the myocardium overlying the cushion region (47–49). *Pitx2* plays an important role in left-right patterning and homozygous deficient mice also display a hypoplastic right ventricle and atrio-ventricular septal defects. Heterozygous mutations in the human homologue of *Pitx2*, *RIEG*, cause Rieger syndrome characterized by tooth and eye developmental defects (50). Cardiac and laterality defects are not commonly associated with Reiger syndrome.

Epicardial-derived cells contribute to the forming endocardial cushions and coronary vessels. The epicardium is derived from cells emerging from the pro-epicardial organ which is located posterior to the forming heart (Fig. 1, panel 4). Cells migrate from this embryonic structure and envelop the myocardium in a caudal-to-rostral direction. Some epicardial cells invade the myocardium and populate the endocardial cushions. Others contribute to the formation of intramyocardial capillaries (51). Signals mediated by retinoic acid (RA) are probably critical for this process as suggested by the expression of a critical enzyme in RA biosynthesis, retinaldehyde dehydrogenase type II (RALDH2) (52). The product of the Wilms' tumor gene *WT1*, a nuclear transcription factor, is also required for epicardial development (53) as is *FOG2* (54). An exciting area of active research relates to the role of epicardial cells in myocardial maturation, coronary artery development and valvulogenesis. This process, as well as cardiac neural crest function, may be altered by mutations in retinoic acid receptors in mice (55, 56), and by teratogens such as retinoic acid or by vitamin A deficiency in humans.

Outflow tract septation and patterning of the great vessels. Neural crest cells populate many regions of the developing embryo and differentiate into numerous cell types, forming the peripheral nervous system, melanocytes, and contributing to the thyroid, parathyroid and thymus glands. Classic studies performed in developing chick embryos demonstrated that neural crest cells migrate from the neural tube, along the aortic arches, and populate the outflow tract and outflow endocardial cushions during mid-gestation (4). Ablation of a discreet subset of cranial neural crest cells before emergence from the neural tube results in predictable cardiac malformations including persistent truncus arteriosus, double outlet right ventricle, interrupted aortic arch and related defects (4). In mice, mutations in the paired-box-containing gene *Pax3* result in similar defects (57). In both ablated chicks and Pax3-deficient (*Splotch)* mice, defects in thymus, thyroid and parathyroid derivatives are also apparent. This phenotype in *Splotch* mice is strikingly reminiscent of human patients with DiGeorge syndrome (see below). While mutations in *PAX3* have not been shown to cause DiGeorge syndrome in man, it is likely that similar molecular and developmental pathways are affected, making *Splotch* mice a potentially useful model for the study of neural crest related cardiac defects. Using a neural crest-specific element in the *Pax3* promoter to direct expression of Cre recombinase in transgenic mice, cardiac neural crest cells have been fate-mapped to the aorto-pulmonary septum, aortic arches, ductus arteriosus and outflow endocardial cushions (Fig. 2 (58), and JAE, unpublished results). Later in development, they differentiate into smooth muscle cells in the aortic arch and head vessels. These regions of the forming vasculature seem particularly sensitive to genetic perturbations. They are affected by several signaling cascades including those mediated by the endothelin receptor A (59), endothelin 1 (60), endothelin converting enzyme 1 (61) and by the winged helix transcription factors Foxc1 (Mf1) and Foxc2 (Mfh1) (62). Mutations in these genes in mice lead to interruptions of the aorta. Interestingly, mutation of the secreted semaphorin signaling molecule Sema3C also leads to interrupted aortic arch (L. Feiner, JAE and J. Raper, personal communication). Semaphorins act in the CNS to mediate axon pathfinding by causing growth cone collapse, and may similarly function during cardiac development to direct neural crest migration. A potential Sema3C receptor, PlexinA2, is expressed by cardiac neural crest cells (JAE, unpublished results). Future studies will be needed to

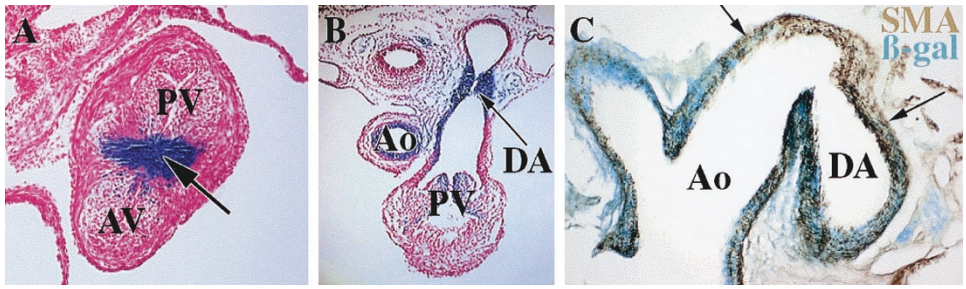

Figure 2. Fate-mapping of cardiac neural crest cells. Mouse embryos carrying Cre recombinase under the control of the neural crest-specific element of the *Pax3* promoter were crossed with transgenic mice carrying a Cre-reporter that expresses β-galactosidase (β-gal) in response to Cre activity. Once Cre recombinase is activated by the *Pax 3* promoter, β-gal is expressed. It will continue to be expressed constitutively by that cell and by all daughter cells. Hence, the fate of the Pax3 expressing neural crest precursors can be followed during cardiovascular development. (*A*) At embryonic day 12.5 neural crest cells (arrow) contribute to the forming aorto-pulmonary septum at the level of the aortic valve (AV) and pulmonic valve (PV). (*B*) At embryonic day 18.5, neural crest descendants contribute to the wall of the aorta (Ao), to the tips of the pulmonic valve and to the cushions of the ductus arteriosus (DA). (*C*) Immunohistochemistry using a smooth muscle actin (SMA) specific antibody demonstrates that β-gal positive neural crest descendants become smooth muscle cells in the aortic arch. β-gal positive cells do not contribute to the smooth muscle of the descending aorta beyond the ductus arteriosus. This transition point is indicated by arrows in *C*.

determine whether mutations in any of these genes account for interruptions or coarctations of the aorta in humans.

DiGeorge Syndrome. DiGeorge Syndrome (DGS), also known as velocardiofacial syndrome (VCFS), has received a good deal of attention in the past few years (for reviews see (63–68)). It is one of the most common congenital defects occurring with a frequency of 1/4000 live births. It is characterized by a constellation of abnormalities suggestive of defective cranial neural crest function particularly with respect to neural crest populating aortic arches 3 and 4. These include *c*ardiac outflow tract anomalies, *a*bnormal facies, *t*hymic hypoplasia, *c*left palate, *h*ypocalcemia and a microdeletion on chromosome *22*, hence the acronym CATCH22. The microdeletion can result in haploid insufficiency of up to 30 genes (69, 70).

Understanding the etiology of DGS has been confounded by several observations including the fact that the severity of the phenotype is not related to the size of the deletion. DGS patients can carry deletions of up to 3 megabases. However, there are patients with considerably smaller deletions with equally severe phenotypes. In fact, a balanced translocation between chromosome 2 and 22, ADU (t2;22) that did not involve a loss of DNA resulted in a mild DGS phenotype (71). Theoretically, this translocation should have resulted in the disruption of a single gene. This was not the case. The break point did not appear to affect any specific gene directly (72–74). These observations have led investigators to propose the existence of "functional architecture" within this region of chromosome 22, the disruption of which leads to aberrant regulation of a gene or genes required for cranial neural crest function. The complexity of the situation is further documented by reports showing DGS-like phenotypes resulting from haploid deletions involving chromosome 10p (75, 76). Clearly, the genetic control of outflow tract assembly is such that perturbations of the function of any one or combination of several genes can result in phenotypes associated with DGS (see previous discussion).

Several hypotheses have been put forth to explain the etiology of DGS. The simplest is that there is a "DiGeorge gene" within the deleted region of 22q11.2 whose function is dosage dependent. That is, loss or mutation of one copy of the gene changes the amount of product available adversely affecting a crucial step in cranial neural crest function. This has led to the search for patients with mutations in candidate genes. An interesting candidate gene was proposed by Yamagishi *et al.* (77). They identified *Ufd1L* as a probable downstream target of *dHand*, a gene required for early heart development (see above). *Ufd1L* is the mouse homolog of the yeast ubiquitin fusion degradation protein 1 gene. It encodes a protein possibly required for targeting proteins for degradation. Thus, a deficiency in this protein might lead to the accumulation of unwanted proteins resulting in cell death (apoptosis) or aberrant differentiation. A screen of 182 DGS patients revealed *UFD1L* was included in all deletions. Most interesting, one patient was discovered with a deletion of *UFD1L*, and while a portion of the gene *CDC45L* immediately proximal to *UFD1L* was also missing, *UFD1L* appeared the more likely causal target (77). In an attempt to confirm this observation, many groups have screened patients for single mutations in *UFD1L* (for example see (78)). To date, none have been found. Further, mice carrying a heterozygous deletion in this gene showed no evidence of a DGS phenotype despite a 50% reduction in message (79).

Another interesting candidate gene is *HIRA*, which encodes a transcription factor that interacts with Pax3 (80). Treatment of chick embryos with antisense ribonucleotides targeting *chHira* mRNA results in outflow tract abnormalities, particularly persistent truncus arteriosus, that resemble those accompanying DGS (81). However, no patients with point mutations in *HIRA* have been reported. A variety of other candidate genes are found within the 22q11.2 deletion (see Table 1). These include genes encoding putative transcription factors, extracellular matrix molecules, cell surface receptors, transport proteins, and protein kinases. Again, no single mutations leading to DGS have been identified. Further, where tested, mice heterozygous for mutations in genes homologous to those found within the DiGeorge region display no abnormal phe-

Table 1. *Genes within the region of chromosome 22q11 commonly deleted in DiGeorge syndrome listed in order from centromere to telomere (see Fig. 3)*

Gene	RNA Expression	*in situ* Expression	Function	References
IDD/LAN	All tissues		Adhesion receptor? SEZ-12 seizure protein	(72–74)
TSK1	Pseudogene in human	Adult testis	Serine/threonine kinase	(85)
TSK-2	Testis	Adult testis	Serine/threonine kinase	(69)
DGS-H	Skeletal muscle, heart		Unknown	(69)
DGS-I/ES2	All tissues		Homology to hypothetical C elegans protein	(69, 88)
GSCL		Brain, primordial germ cells	Member of GSC transcription factor family	(85, 89)
CTP	All tissues		Mitochondrial citrate transport protein	(90, 91)
CLTCL	All tissues, High in skeletal muscle		Clathrin-like heavy chain	(69, 92, 93)
DVL22	All tissues, high in skeletal muscle, heart		Homology to 3UTR of Dishevelled	(94)
22k48			Novel intercistronic transcript. Possibly involved in protein trafficking	(95)
HIRA/TUPLE	All tissues	Embryonic cranial neural folds, BA 1 and 2, neural crest, limb	Transcription factor, interacts with PAX3	(96, 97)
UFD1L	All tissues, high in skeletal muscle, heart, pancreas	E9.5-E11.5 brain, lungs cardiac outflow, otocyst, eye	Homology to yeast ubiquitin fusion degradation 1 protein	(98)
CDC45L			Homolog of yeast CDC45 involved in initiation of DNA synthesis	(99)
TMVCF	E9.5 mouse; adult lung, heart, skeletal muscle		Predicted transmembrane protein	(100)
CDCrel-1/H5			Cell division cycle related protein	(101)
GP1Bβ, H5			Part of platelet von Willebrand factor receptor complex	(102)
TBX1		BA 1–3, otic vesicle, jugular lymph sacs, lung, tongue	Homology to DNA binding domain of Brachyury T, transcription factor	(103)
T10	Low general expression, high in fetal liver		Serine/threonine rich, unknown	(104)
COMT	Neuronal, placental, and other tissues		Catechol-o-methytransferase	(105)
ARVCF	All fetal and adult tissues		Catenin family member	(106)
RANBP1			Ran GTPase receptor, nuclear transport	(82)
N41	All tissues		unknown	(107)
DGCR-6	All tissues, high in skeletal muscle, heart		Homology to Drosophila gdl, human LAMC1	(108)
LZTR-1	All tissues, fetal liver		Transcription factor	(109)
ZNF74	E10-E12 (mouse), not adult		12 zinc finger motifs	(110)

RNA expression by Northern blot analysis is summarized (column 2) in addition to RNA and/or protein expression as detected by *in situ* hybridization or immunohistochemistry (column 3). Known or presumed functional data is listed in column 4. BA = branchial arches.

notypes. Thus, to date, there is no strong evidence for the existence of a single "DiGeorge gene."

Another approach to understanding the etiology of DGS has been to create an animal model carrying deletions that involve clusters of genes similar to those deleted in DiGeorge patients. This approach depends upon the conservation of synteny between human and mouse chromosomes. The "DiGeorge region" of human 22q11 is conserved within mouse chromosome 16. The *relative* order of most genes within the syntenic region is conserved with certain blocks of genes being inverted in the mouse with respect to the centromere-telemere orientation (Fig. 3) (82–85). The function of genes within this complex has been tested in mice by deleting entire segments of chromosome 16 corresponding to those noted in DGS patients. In one case, mice carrying deletions of a region within the "minimal DiGeorge critical region" (MDGCR) were constructed (86). The MDGCR represents the smallest portion of the 22q11.2 deletion common to most DiGeorge patients. While animals homozygous for the 150-Kb deletion died early in gestation, heterozygous animals were without significant morphogenetic phenotypes. This is consistent with the recent identification of DGS patients with deletions completely outside the MDGCR (87).

In another study, animals carrying a larger 1.2 megabase deletion were constructed (Fig. 3). In this case, heterozygous deleted animals survived and displayed heart anomalies resulting from abnormal remodeling of the fourth brachial arch arteries reminiscent of vascular anomalies accompanying DGS (79). The phenotype was reversed by duplication of the deleted region proving that it was caused by haploid insufficiency of one or more genes within this deleted region and not by a position effect. Interestingly, in the genetic backgrounds reported, the defects were specific for aortic arch IV derivatives and did not include many OFT defects, facial dysmorphias, cleft palate, thyroid or parathyroid hypoplasias characteristic of DGS. Thus, while this deleted region includes genes required for certain aspects of aortic arch development, there must be additional genes residing outside this region that act as modifiers and are important to other aspects of early heart and facial morphogenesis relevant to the DGS. This presumption is sup-

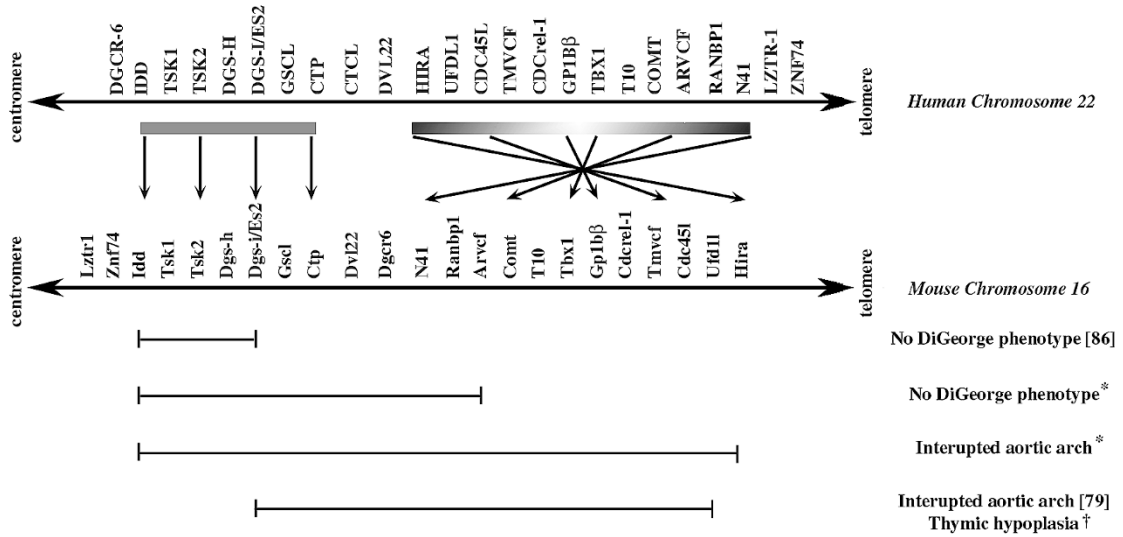

Figure 3. The DiGeorge region of human chromosome 22. The region of chromosome 22q11 commonly deleted in DiGeorge syndrome is shown schematically with the syntenic region of mouse chromosome 16 (modified from (79)). Genes within this region are indicated (spacing is not to scale). The expression pattern and function of these genes are summarized in Table 1. A segment of this genomic locus is inverted in the mouse compared with the human as indicated by the crossed arrows. Genomic deletions in the mouse have been engineered to produce mouse models of DiGeorge syndrome. The extent of each of four deletions is indicated below the mouse chromosome with the resulting phenotype indicated at the right. Taken together, these results suggest that a relatively small region between *Arvcf* and *Ufd1L* contains at least one gene required for aortic arch remodeling and neural crest development. (*A. Skoultchi and R. Kucherlapati, personal communication; †A. Baldini, personal communication).

ported by preliminary evidence that thymus hypoplasia becomes apparent when these mice are crossed onto different genetic backgrounds (A. Baldini, personal communication). Additional engineered deletions, in progress in several labs around the world, will continue to refine the region of mouse chromosome 16 containing genes critical for cardiovascular development and neural crest function (see Fig. 3). For instance, a group at Albert Einstein University has created a deletion that removes the genes between *Idd* and *Arvcf* that does not produce any DiGeorge-like characteristics in the heterozygous state. However, a larger deletion extending from *Idd* to *Hira* does produce interrupted aortic arch (A. Skoultchi, R. Kucherlapati, personal communication). Systematic complementation of genes across the deletion should lead to the identification of those critical to the DGS phenotype. Thus, while the origin of DGS remains a mystery, critical and informative experiments are underway. The ability to construct elegant animal models, the completion of the human genome project and our emerging understanding of chromosomal structure and gene regulation, suggest that we should soon begin to understand the molecular origin of dysmorphias characteristic of DGS.

In summary, transcription factors that play critical functions at specific stages of cardiac development are emerging from basic studies, providing excellent candidate genes responsible for various forms of congenital and adult cardiac diseases. While complete loss-of-function in animal models often results in severe cardiac morphogenetic defects, more subtle mutations are being found in humans with milder forms of structural heart disease. Molecular pathways deciphered from the study of developmental processes may be reiterated during pathologic adult cardiac conditions. The further study of molecular determinants of embryonic cardiac development offers a rational approach for the identification of disease causing genes.

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