

## Cardiac Development: New concepts

Peter J. Gruber, MD, PhD<sup>a,b,\*</sup>

<sup>a</sup>*Cardiac Center, The Children's Hospital of Philadelphia,  
University of Pennsylvania School of Medicine, Suite 8527, 34th Street and Civic Center Boulevard,  
Philadelphia, PA 19104, USA*

<sup>b</sup>*The Molecular Cardiology Research Center, University of Pennsylvania School of Medicine,  
Suite 8527, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104, USA*

Congenital heart disease (CHD) is a devastating complex of diseases resulting from defects of development. It affects more than 1 of every 100 live births and is responsible for most prenatal losses [1,2]. Additionally, 3 per 1000 live births require an intervention (catheter based or surgical) during the first year of life. Despite its prevalence and severity, the causes of CHD are largely unknown. A clear molecular mechanism has been identified in only a small number of instances. In most of these situations, there is a vast gap between identifying the causative gene and understanding the mechanism by which structural or functional defects occur. Indeed, the ability to identify potential disease targets now far outstrips the capacity to test these hypotheses and define their mechanisms. What are the obstacles to unraveling the molecular controls of heart morphogenesis, and are there real-time solutions?

Presently, there is an acceleration of significant discovery that should reshape our understanding of congenital cardiac disorders. These advances include the description of an increasing number of gene-targeted mouse models of human cardiac disease; the availability of nearly complete genome sequence information for multiple organisms, including human beings; and increasingly sophisticated bioinformatics tools with which to use these data. The manipulation of gene expression is now possible not only in mouse models, where homologous recombination can be used to inactivate or modify a gene, but in organisms like zebrafish and *Xenopus* (frogs), where newer techniques can be used to analyze gene expression rapidly and efficiently. Increasingly, investigators are using

---

\* The Cardiac Center, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Suite 8527, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104.

*E-mail address:* pgruber@mail.med.upenn.edu

multiple organisms to model human disease because each provides distinct advantages. This review highlights the most recent and promising avenues of current research in cardiac development in terms of emerging molecular data and model systems.

### **Approaches to congenital heart disease**

How does one experimentally identify genetic associations with disease? Traditionally, these fall into two broad categories: forward or reverse genetic approaches. Historically, primitive model organisms, such as *Drosophila melanogaster* (fruit fly) or *Caenorhabditis elegans* (worm), were used in a classic forward genetic approach. In forward genetics, a genetic screen is used to identify mutants that produce a phenotype. Mutant organisms are isolated, and genes are identified by association of a genetic marker (a physical location on a chromosome) with a phenotype. The limiting factor in this type of study has been the ability to associate, or map, a gene to the mutant location (locus), a function of the density of the markers or locators on the DNA strand. In human beings, a similar approach is taken in which patients with a disease are examined for the presence of markers that cosegregate with the disease. In contrast to induced mutations in flies, a group of patients with a disease (natural mutants) are linked to genetic markers. With a close set of physical markers (usually single nucleotide polymorphisms [SNPs]), one can map the disease location to a fine resolution, such that candidate genes can be identified. Because most interesting cardiac morphologic abnormalities rare, informative CHD data sets are usually small, making this process difficult. In contrast, investigators studying oncogenesis frequently study patient data sets that number in the thousands [3]. This process, known as linkage analysis, was a formidable task, because markers were scarce. To overcome this problem, an increasingly dense physical map was required. Practically, with near completion of the Human Genome Project in the last 5 years, this hurdle of marker density has now become much easier. Now, it is often easier to find genes in human beings than it is in more primitive model organisms simply on the basis of sequence availability. The Haplotype Mapping (HapMap) project is a new initiative that should improve the speed and accuracy of these forward genetic approaches even further. The HapMap project endeavors to use haplotypes, or groups of SNPs that behave similarly, simultaneously to reduce the complexity of the number of markers as well as increase the power of genetic associations [4]. Certainly, classic forward genetic methods of gene identification are more powerful than ever before and ripe for further harvest.

A second method of gene identification is a reverse genetic approach. Instead of starting with a disease and finding the gene, one begins with a hypothesis in which a candidate gene is mutated in an animal model to produce a phenotype. This method is direct in that it not only associates a mutation with a phenotype but often provides powerful insights into its mechanism of action. Subsequently, human populations that mimic the animal model are examined for mutations in

the candidate gene. But how does one start? Which genes should one test? Increasingly, new technologies are making this approach less expensive in terms of time and money. Increasingly, techniques, such as conditional gene targeting, that incorporate temporal and spatial specificity to gene manipulation experiments are now routine. Forward and reverse approaches have been successful in identifying causative disease genes, although the most powerful studies use animal models to provide the mechanism and human genetics to validate animal hypotheses.

Yet an even more powerful (and considerably less expensive) way to improve the resolution of forward genetic experiments is to expand data sets through collaboration. Making rare samples available to multiple investigators should become a priority, using Comprehensive Cancer Center tissue banks as a paradigm for resource sharing. In 1992, the Department of Energy (DOE) and National Institutes of Health (NIH) published a series of guidelines on data sharing entitled *DOE-NIH Guidelines Policy for Sharing Data and Resources* [5]. Although these guidelines were intended to enforce timely sharing of resources generated by the Human Genome Project, the precedent was set for a broader application. Indeed, since 2002, 50% of program announcements (PAs) and 60% of requests for applications (RFAs) now require a data-sharing policy. Recent recommendations by the National Academy of Sciences denoted UPSIDE (uniform principle for sharing integral data expeditiously) [6,7] to emphasize the advantages of collaborative approaches. As further emphasis, over the past 3 years, the NIH has become explicit in its policy toward sharing of published publicly funded resources, with 50% of PAs and 60% of RFAs now requiring an explicit data-sharing policy; recent reports now suggest that this figure is becoming 100%. Considerable bioinformatic, logistic, and financial hurdles need to be addressed to ensure the wide adaptation of these principles, but political impediments should be abandoned. I next discuss examples of recent animal models that fulfill these criteria and phenocopy human disease. The critical next step is the difficult task of filling in the signaling details that should eventually allow one to manipulate the system to identify the actual mechanisms by which these diseases occur.

### ***TBX1***

DiGeorge syndrome is a complex of abnormalities that can be caused by a deletion in chromosome 22q11.2 and by a mutation in the gene *TBX1* (see the article on DiGeorge syndrome by Goldmuntz in this issue). It consists of thymic and parathyroid hypoplasia, resulting in hypocalcemia as well as defects in the outflow tract of the heart. These defects are all attributable to disturbances in the migration of neural crest cells into pharyngeal arches and pouches. The neural crest is a multipotential group of cells that migrate into the heart and are critical to the development of septae and outflow tract structures. An increasing number of genes affecting neural crest migration and function (eg, Pax3, semaphorins,

Notch) have been shown to produce cardiac defects when mutated [8–11]. DiGeorge syndrome may present as a number of overlapping phenotypes, including velocardiofacial syndrome (VCFS), conotruncal anomaly face syndrome, or isolated defects of cardiac outflow tract development [12]. *TBX1* itself is a gene that maps to the center of the DiGeorge chromosomal region of 22q11.2 and is a member of a set of phylogenetically conserved genes that share a common DNA binding domain, the T-box [13]. Although there are other conserved protein domains, they are considerably more divergent. At the current time, there are at least 20 distinct members, of which a number (*Tbx1*, *Tbx2*, *Tbx3*, *Tbx5*, *Tbx18*, and *Tbx20*) are expressed in regions that portend their clinical phenotype [14].

The identification of the role of *TBX1* in the formation of DiGeorge syndrome is an excellent example of the confluence of forward and reverse genetic techniques required to investigate complex phenotypes. As a first step, using classic forward genetic approaches, a 3-megabase critical chromosomal microdeletion of approximately 20 genes was associated with the clinical phenotype of DiGeorge syndrome. Subsequent mapping of the syndrome to an individual gene was confounded by the fact that the severity of the phenotype was not related to the size of the deletion, however. Further obscuring mapping attempts, several distinct nonoverlapping chromosomal microdeletions result in similar clinical pictures. By testing individual genes in the region identified through human forward genetic mapping approaches, patients with clinical phenotypes consistent with DiGeorge syndrome but lacking chromosomal deletions were examined for evidence of mutations. Despite considerable efforts, no convincing evidence of a single gene mutation was found [15]. Alternative approaches were required, and the focus shifted toward animal models.

Mice are similar to human beings in genetic and physical structure. Despite some differences, they are an excellent model to study cardiac development. One such difference is that the location of the DiGeorge region of human chromosome 22q11 is on mouse chromosome 16. Importantly, the relative genetic structure (composition and order of the genes) of the area is nearly identical, such that alterations of this region of mouse chromosome 16 should predict those of human chromosome 22 [16,17]. In a seminal experiment, mice engineered with deletion deficient for this homologous region displayed abnormalities similar to those of DiGeorge syndrome. Importantly, reintroducing individual genes located in this region corrected the cardiac defects, thus providing strong evidence for the direct involvement of these genes in the pathogenesis of DiGeorge syndrome [18]. Further microdeletions engineered in other laboratories narrowed down this region in a fashion identical to the original performed in human beings. These experiments showed that only abnormalities in *Tbx1* phenocopy the cardiac abnormalities seen in DiGeorge syndrome [19–21]. Importantly, further investigations using specific *Tbx1* gene-targeted mice conclusively demonstrated that an altered *Tbx1* dosage recapitulates the full spectrum of DiGeorge syndrome and VCFS defects [22]. Further mechanistic insight came from the zebrafish animal model, in which there is a mutation called *van gogh*. This mutant was originally

identified in a screen for jaw mutations in fish. During this mutational screen, a mutant was identified whose most prominent external feature was a small ear. Subsequently, the gene associated with this defect was found to disrupt the gene *tbx1*, and the corresponding embryos were further found to have defective development of pharyngeal neural crest derivatives [23]. Finally, moving back to human beings, mutations in *TBX1* were screened in 13 patients from 10 families that had clinical features of DiGeorge syndrome but harbored no microdeletions. Two mutations were found in 2 unrelated patients, and a third mutation was found in 3 patients in a family with the closely related VCFS [24]. With three animal systems (fish, mouse, and human) all supporting the hypothesis, *tbx1* (*Tbx1*, *TBX1*) can now be confidently described as the critical (although not only) gene involved in the pathogenesis of DiGeorge syndrome.

Regardless of the identification of *Tbx1* as the gene responsible for the cardiac defects of DiGeorge syndrome and closely related 22q11 syndromes, the mechanism responsible for the clinical phenotype remains obscure. How does a mutation in a single protein result in morphologic limb and cardiac defects? In fact, *Tbx1* alone cannot entirely account for the defects of DiGeorge syndrome, and other genes are likely to be important. It is largely through studies in mice and lower organisms that these few molecular insights have come. As mentioned previously, *Tbx1* is expressed in certain regions of the heart but also in areas surrounding the neural crest. With what proteins does *Tbx1* interact, and what genes does it regulate? Using genetic studies in fish, flies, and rodents, *Tbx1* has been found to regulate expression of fibroblast growth factors (Fgfs) 8 and 10, and deletion of *Fgf* “downstream genes” in the region of expression of *Tbx1* reproduces the defects seen in DiGeorge syndrome [25]. Other genes, such as *Vegf* and members of the *Forkhead* family, are now adding yet one more layer of details into the mechanism of these defects [26]. Importantly, none of the advances detailed here would have been possible without the cross-fertilization of human molecular genetics, genetic engineering of mice, large-scale genetic screens of lower organisms, and bioinformatics, and, increasingly, the pace of these changes is accelerating because of just this confluence of technologies.

## ***GATA4***

In contrast to *Tbx1*, where a classic forward genetic approach was the first clue toward identification of the molecular basis of DiGeorge syndrome, *GATA4* is a disease gene that was first examined in pure reverse genetics and then amplified in understanding with human studies. The *GATA* genes are a small family of evolutionarily ancient proteins that share a common DNA recognition core sequence (the nucleotides G-A-T-A) on DNA promoters and regulatory regions to which they bind and act as transcriptional regulators [27–29]. The proteins have two zinc-finger DNA binding motifs that mediate DNA-protein interactions as well as protein-protein interactions. First discovered in the early 1980s, there are two primary families, *GATA 1*, 2, and 3, which are important in various as-

pects of hematopoiesis, and *GATA 4, 5, and 6*, which are important for the development of mesodermal derivatives, including the gut, heart, liver, and gonads. Decades of experiments in tissue culture, rodents, chicks, and frogs all demonstrated that *Gata4* is important for cardiac differentiation, although the precise role of the protein in cardiac morphogenesis is not clear. Nearly 10 years later, chromosomal deletions in the region of 8p23.1 (where the human *GATA4* locus resides) were confirmed in patients with CHD, with a wide variety of CHD diagnoses (eg, atrial and ventricular septal defects, double-outlet right ventricle, complete common atrioventricular canal, pulmonary stenosis, hypoplastic left heart syndrome) suggesting that haploinsufficiency of *GATA4* may contribute to the formation of human CHD [30]. Further studies in nonsyndromic human beings expanded these findings, with the identification of *GATA4* point mutations in nonchromosomal patients [31]. Using a forward genetic approach with a large kindred spanning five generations, a whole-genome linkage analysis was performed, localizing the associated mutation to 8p22. By direct sequencing of *GATA4*, a single-base DNA change was identified that led to a single amino acid change in the GATA4 protein. In itself, this was a significant finding, although it was the pursuit of the mechanism that made this work more remarkable. Moving back again to in vitro model systems, identical mutant GATA4 was engineered and examined in tissue culture cells for its ability to activate genes important in cardiac development. Mimic mutations did indeed reduce the transcriptional activity of the GATA4. By what molecular mechanism does this take place, however?

GATA4 interacts with TBX-5 and NKX2-5, two molecules critical for cardiac morphogenesis. Did the identified human mutation alter the ability of GATA4 to interact functionally with these proteins or others important for cardiac development? Experiments in tissue culture cells using engineered proteins demonstrated that this was the case and additionally demonstrated a new interaction between GATA4 and TBX5. Additionally, *TBX5* mutations linked to similar cardiac abnormalities were examined and found to interrupt the GATA 4 interaction motif, providing reciprocal evidence for this important interaction. This combination of human genetics and biochemical analyses built on a molecular and bioinformatics background using multiple animal models demonstrates the power of current technology and also points toward a profound understanding of the mechanisms that produce CHD.

### **Cofactors and epigenetics**

Traditional approaches identifying transcription factor mutations and analysis have largely been mined. We can estimate that this is the case because of the redundancy we now see in mouse mutants: phenotypes now fall into a predictable set of subclasses, only a small fraction of which actually resemble clinical CHD. Rather than identifying new primary effectors, new insights are now coming from adaptor molecules or cofactors (connector molecules that transduce

or amplify signals). Myocardin is a member of the SAP family of transcription factors that associate with serum response factor (SRF), which is itself a widely expressed transcription factor that regulates the expression of many cardiac genes. Although the dynamics and signaling pathways of SRF function have been extensively probed in many animal model systems, new insights are being discovered by its interactions with other proteins, such as myocardin. Subsequently, other partners of myocardin named myocardin related transcription factor (MRTF)s have been uncovered. These demonstrate yet a third layer of complexity in this transcription factor network, and some mutants of these genes produce CHD in rodents [32–35]. Undoubtedly, this provided the heart with more ways to adapt to physiologic perturbations and provided a means for developmental and/or evolutionary complexity and robustness [36]. Comparison between primitive and complex developmental models may provide clues as to how these layers contribute to the above-mentioned attributes. The cross-talk between signaling systems is the next level that should reveal relations that can be harvested for basic biologic understanding as well as therapy.

A second example of these is the recent discovery of the profound effect of epigenetic modulators in development and disease [37]. Global regulators of transcriptional processes conceptually allow the simultaneous modulation of genetic loci. Sequence-specific DNA binding proteins recruit epigenetic modulators, such as histone deacetylases (HDACs), histone acetyl transferase (HAT), and DNA methyl transferases (DNMT). These, in turn, alter the conformation of the DNA strand and thus the activity of the genes contained in that location. HDACs are global regulators of transcriptional activity that have been identified to have profound effects on cardiac development and disease. The family of HDACs is composed of at least 11 family members with unique expression patterns. Seminal insights by Olson and others [36] have led to a generalizable model in which the recruitment of HDACs to a chromosomal locus results in the deacetylation and, in general, deactivation of transcriptional units. The critical role of class II HDACs, such as HDAC9, in the modulation of cardiac hypertrophy was elegantly demonstrated by overexpression and gene deletion studies in rodents [38]. Importantly, the activity of many of these proteins can be inhibited by small molecules and may be used to modulate cardiac function in a clinically relevant manner.

Again, to consider intervening in the process of CHD, understanding the mechanism—not just the gene responsible—is the key. Molecules that are critical to the initiation of cardiac morphogenesis have been studied in great detail (*Nkx2-5*, *Gata*, and *Tbx* family members), but only in rare cases has a partial mechanism for the defects been elucidated. This is an important point for cross-fertilization between the clinician and the scientist. For the most part, the molecular data to date are interesting to biologists and geneticists but not yet suitable for clinical consumption. From a human genetic perspective, despite advances in technology, it is not yet financially feasible (or scientifically plausible) to examine all patients with CHD for defects in candidate genes. We simply do not yet know enough about the molecular pathogenesis of these

diseases to provide useful information for families or physicians. From the developmental biologic perspective, knowledge of GATA4 interactions with a small number of other transcription factors does not yet tell us with sufficient depth why patients have holes in their heart, thickened leaflets, or altered conal muscular orientations. What is the mechanism by which the ventricular septal crest, atrial septum, and endocardial cushions are guided, meet, join, and differentiate into mature cardiac structures? Are these relatively late morphogenic events dictated directly by these same molecules, or are entirely different combinatorial sets of molecules still waiting to be discovered? How can these questions be approached?

It is a confluence of techniques, animal systems, and increasingly, a systems biologic approach that must be used to tackle complexity directly. It is likely that the further away from an early nodal point of cardiac differentiation one examines, the greater is the complexity of the interactions. The use of microarrays, or chips, especially those that evaluate global genomic information in terms of RNA production (expression arrays), location (BAC arrays), or function (chromatin immunoprecipitation on chips), is likely to be critical to generate large-scale simultaneous information. Importantly, these increasingly large data sets need to be processed, housed, and made available in such a format that they (the data sets) can all talk to each other. Through these naive-based approaches, novel hypotheses can then be generated to test in animal model systems. Three additional steps also have to be filled with respect to these ends. First, funding bodies have to recognize the value of the generation of these data sets outside the traditional paradigm of narrow hypothesis-driven investigation—what we are looking for is new hypotheses. Second, we need to evaluate these hypotheses rapidly, something for which lower animal systems, such as zebrafish, may be ideally suited. The ability to use knockdown (rather than the more precise but cumbersome knockout) technologies, such as RNA interference (RNAi), in a time-sensitive manner is providing a method to cull the pathways of interest, such that only the most promising need to be tested in expensive time-consuming mouse genetic models. Third, precise clinical phenotypes (in rodents and human beings) need to be integrated with molecular data. It is no longer appropriate for an article in the basic scientific literature to report a ventricular septal defect without specifying its specific subtype (eg, conoventricular, muscular, conoseptal). Similarly, an atrial septal defect must be identified by the specific location in the atrial septum and differentiated from a patent foramen ovale.

### **New approaches**

What systems provide the best hope for future progress? Despite the great success that organisms like worms, flies, and zebrafish have, mice have at least three distinct advantages. First, they are mammals and, despite some differences as outlined previously, provide an excellent animal model of cardiac develop-

ment. Second, more than 99% of mouse genes have homologues in human beings, and, third, the mouse genome supports targeted mutagenesis, allowing genes to be altered efficiently and precisely. These facts have catalyzed efforts to create large-scale mutation resources. Several large-scale mouse genetics programs are now fully operational. One such program, the N-ethyl-N-nitrosourea (ENU) Mouse Mutagenesis Program, uses genetic approaches to model human genetic disease and identify some key pathways [39]. The research program was aimed at generating large numbers of new mouse phenotypes, many of which are planned to carry disorders that model human genetic disease. Characterization of selected phenotypes using a positional candidate cloning approach should identify the underlying genes causing the phenotypes and yield information about the genetic pathways involved. Approximately 500 novel mouse phenotypes were identified from the mutagenesis screen in phase I, although screening for cardiac defects has not been a focus because morphologic defects are often lethal and therefore more cumbersome to study. The International Gene Trap Consortium has established banks of mutated embryonic stem (ES) cells with sequence-verified gene traps; however, to date, only approximately 10% of all genes have been deleted [40]. The Knockout Mouse project and the European Conditional Mouse Mutagenesis Program are now coordinating efforts in many areas to provide a complete catalog of gene mutations [41,42]. A promising new mode of gene interference is posttranscriptional gene silencing by RNAi. Introduction of RNA duplexes is an additional tool that can be used to reduce expression of a specific gene [43,44]. Indeed, RNAi technologies may provide many advantages over conventional gene targeting and should certainly complement classic gene deletion techniques. Largely unmined are the potentials of proteomics and epigenomics as well as epiproteomics. There is some evidence that protein modifications, such as sumoylation, glycosylation, and lipid modifications, such as myristylation and farnesylation, can profoundly influence later protein activity, with developmental and pathophysiologic ramifications [45–47]. These and other new approaches will lead to better research, diagnosis, and treatment of children with congenital heart disease.

## References

- [1] Hoffman JI. Incidence of congenital heart disease: I. Postnatal incidence. *Pediatr Cardiol* 1995;16:103–13.
- [2] Hoffman JI, Kaplan S. The incidence of congenital heart disease. *J Am Coll Cardiol* 2002; 39:1890–900.
- [3] Poynter JN, Gruber SB, Higgins PD, et al. Statins and the risk of colorectal cancer. *N Engl J Med* 2005;352:2184–92.
- [4] Foster MW, Sharp RR. Beyond race: towards a whole-genome perspective on human populations and genetic variation. *Nat Rev Genet* 2004;5:790–6.
- [5] Department of Energy/National Institutes of Health. DOE-NIH guidelines for sharing data and resources policy. *Human Genome News* 1992;4:4.
- [6] Czech T. Sharing publication-related and materials: responsibilities of authorship in the life sciences. Washington, DC: The National Academy Press. p. 1–16.

- [7] Cozzarelli NR. UPSIDE: uniform principle for sharing integral data and materials expeditiously. *Proc Natl Acad Sci USA* 2004;101:3721–2.
- [8] Brown CB, Feiner L, Lu MM, et al. PlexinA2 and semaphorin signaling during cardiac neural crest development. *Development* 2001;128:3071–80.
- [9] Feiner L, Webber AL, Brown CB, et al. Targeted disruption of semaphorin 3C leads to persistent truncus arteriosus and aortic arch interruption. *Development* 2001;128:3061–70.
- [10] Jarriault S, Brou C, Logeat F, et al. Signalling downstream of activated mammalian Notch. *Nature* 1995;377:355–8.
- [11] Li L, Krantz ID, Deng Y, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet* 1997;16:243–51.
- [12] McKusick V. DiGeorge syndrome. In: Online Mendelian inheritance in man. 2004. Available at: <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=188400>.
- [13] Tiller G, McKusick V. T-box 1. Online Mendelian inheritance in man. 2005. Available at: <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=602054>.
- [14] Plageman Jr TF, Yutzey KE. T-box genes and heart development: putting the “T” in heart. *Dev Dyn* 2005;232:11–20.
- [15] Epstein JA. Developing models of DiGeorge syndrome. *Trends Genet* 2001;17(Suppl):S13–7.
- [16] Galili N, Baldwin HS, Lund J, et al. A region of mouse chromosome 16 is syntenic to the DiGeorge, velocardiofacial syndrome minimal critical region. *Genome Res* 1997;7:399.
- [17] Lund J, Roe B, Chen F, et al. Sequence-ready physical map of the mouse chromosome 16 region with conserved synteny to the human velocardiofacial syndrome region on 22q11.2. *Mamm Genome* 1999;10:438–43.
- [18] Lindsay EA, Botta A, Jurecic V, et al. Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature* 1999;401:379–83.
- [19] Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, *Tbx1*. *Nat Genet* 2001;27:286–91.
- [20] Lindsay EA, Vitelli F, Su H, et al. *Tbx1* haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* 2001;410:97–101.
- [21] Merscher S, Funke B, Epstein JA, et al. *TBX1* is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell* 2001;104:619–29.
- [22] Liao J, Kochilas L, Nowotshin S, et al. Full spectrum of malformations in velo-cardio-facial syndrome/DiGeorge syndrome mouse models by altering *Tbx1* dosage. *Hum Mol Genet* 2004;13:1577–85.
- [23] Piotrowski T, Ahn DG, Schilling TF, et al. The zebrafish van gogh mutation disrupts *tbx1*, which is involved in the DiGeorge deletion syndrome in humans. *Development* 2003;130:5043–52.
- [24] Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet* 2003;362:1366–73.
- [25] Brown CB, Wenning JM, Lu MM, et al. Cre-mediated excision of *Fgf8* in the *Tbx1* expression domain reveals a critical role for *Fgf8* in cardiovascular development in the mouse. *Dev Biol* 2004;267:190–202.
- [26] Hu T, Yamagishi H, Maeda J, et al. *Tbx1* regulates fibroblast growth factors in the anterior heart field through a reinforcing autoregulatory loop involving forkhead transcription factors. *Development* 2004;131:5491–502.
- [27] Burch JB. Regulation of GATA gene expression during vertebrate development. *Semin Cell Dev Biol* 2005;16:71–81.
- [28] Peterkin T, Gibson A, Loose M, et al. The roles of GATA-4, -5 and -6 in vertebrate heart development. *Semin Cell Dev Biol* 2005;16:83–94.
- [29] Shimizu R, Yamamoto M. Gene expression regulation and domain function of hematopoietic GATA factors. *Semin Cell Dev Biol* 2005;16:129–36.
- [30] Pehlivan T, Pober BR, Brueckner M, et al. GATA4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease. *Am J Med Genet* 1999;83:201–6.
- [31] Garg V, Kathiriyai IS, Barnes R, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with *TBX5*. *Nature* 2003;424:443–7.

- [32] Wang D, Chang PS, Wang Z, et al. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. *Cell* 2001;105:851–62.
- [33] Wang DZ, Li S, Hockemeyer D, et al. Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc Natl Acad Sci USA* 2002;99:14855–60.
- [34] Li J, Zhu X, Chen M, et al. Myocardin-related transcription factor B is required in cardiac neural crest for smooth muscle differentiation and cardiovascular development. *Proc Natl Acad Sci USA* 2005;102:8916–21.
- [35] Niu Z, Yu W, Zhang SX, et al. Conditional mutagenesis of the murine serum response factor gene blocks cardiogenesis and the transcription of downstream gene targets. *J Biol Chem* 2005; 280:32531–8.
- [36] Wang DZ, Olson EN. Control of smooth muscle development by the myocardin family of transcriptional coactivators. *Curr Opin Genet Dev* 2004;14:558–66.
- [37] McKinsey TA, Olson EN. Toward transcriptional therapies for the failing heart: chemical screens to modulate genes. *J Clin Invest* 2005;115:538–46.
- [38] McKinsey TA, Zhang CL, Olson EN. Control of muscle development by dueling HATs and HDACs. *Curr Opin Genet Dev* 2001;11:497–504.
- [39] Nolan PM, Peters J, Strivens M, et al. A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat Genet* 2000;25:440–3.
- [40] Skarnes WC, von Melchner H, Wurst W, et al. A public gene trap resource for mouse functional genomics. *Nat Genet* 2004;36:543–4.
- [41] Austin CP, Battey JF, Bradley A, et al. The knockout mouse project. *Nat Genet* 2004;36:921–4.
- [42] Auwerx J, Avner P, Baldock R, et al. The European dimension for the mouse genome mutagenesis program. *Nat Genet* 2004;36:925–7.
- [43] Gura T. A silence that speaks volumes. *Nature* 2000;404:804–8.
- [44] Hammond SM, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double-stranded RNA. *Nat Rev Genet* 2001;2:110–9.
- [45] Haltiwanger RS, Lowe JB. Role of glycosylation in development. *Annu Rev Biochem* 2004; 73:491–537.
- [46] Wells L, Hart GW. O-GlcNAc turns twenty: functional implications for post-translational modification of nuclear and cytosolic proteins with a sugar. *FEBS Lett* 2003;546:154–8.
- [47] Takada Y, Khuri FR, Aggarwal BB. Protein farnesyltransferase inhibitor (SCH 66336) abolishes NF-kappaB activation induced by various carcinogens and inflammatory stimuli leading to suppression of NF-kappaB-regulated gene expression and up-regulation of apoptosis. *J Biol Chem* 2004;279:26287–99.