Perspectives Series: Molecular Medicine in Genetically Engineered Animals

Genes and Physiology: Molecular Physiology in Genetically Engineered Animals

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Recent advances in transgenic and gene-targeting approaches, mouse genetics, and microsurgical technology are initiating a revolution that has led to the unexpected coupling of in vivo molecular physiology with genetically engineered mice (for a brief review, see references 1, 2). This perspective covers problems and prospects for using the engineered mouse as a model system to dissect complex, in vivo physiological traits, using the cardiovascular system as a paradigm for other complex organ systems (e.g., pulmonary, gastrointestinal, neural, and renal).

Monitoring complex in vivo cardiovascular phenotypes

The careful classification and characterization of the clinical phenotype is critical in mapping human cardiovascular disease genes, as the inclusion of false positives or negatives can undermine attempts to obtain a refined linkage analysis. Establishing strict phenotypic criteria for the long QT syndrome (3, 4) and hypertrophic cardiomyopathy (5, 6, 7) were essential in establishing genetic linkage and led to the subsequent identification of the disease genes by the candidate gene approach. Assessment of the cardiovascular phenotype in genetically manipulated mice has taken on an equally important role in developing murine models of human disease, determining the role of single genes in complex physiological traits, and in ascribing the lack of a phenotype to gene redundancy. Compounding the difficulty, the investigators having the skills required to generate transgenic or gene-targeted mice often do not have the expertise necessary to quantitatively monitor a complex physiological phenotype, that encompasses changes in diastolic or systolic function, neural control of integrative cardiovascular function, exercise-induced cardiorespiratory endpoints, or other complex parameters. Given the diminutive size of the mouse heart and associated vessels (the diameter of the adult mouse aorta is 1 mm in its widest distribution), a heart rate in excess of 450 beats per minute, the dearth of baseline parameters describing murine cardiovascular function and physiology, and the lack of widely available miniaturized technology to assess in vivo physiological phenotypes, until recently, most of the analysis of potential cardiovascular phenotypes in mice have been confined to simple histological assessments. Needless to say, since the usual classifications of many clinical diseases are not based on such parameters, the potential for making the wrong "diagnosis," missing a major phenotype, or wrongly concluding the absence of a phenotype are substantial. In the latter case, it may be premature to conclude that a lack of a phenotype in a gene-targeted animal is a true reflection of the functional redundancy of members of a given gene family, as this may simply reflect the inability to

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precisely assay a phenotype in a given tissue. For example, in the case of the myogenic determination genes MyoD and Myf-5, it is clear that these two genes are redundant in their ability to initiate skeletal myogenesis (8). However, it is by no means certain that the MyoD and Myf-5 null phenotypes are identical at the level of muscle function, physiology, and fiber-type specificity. Analyzing the phenotypes of these gene-targeted mice in more detail may become of interest, particularly in regard to physiological assays of muscle performance and adaptation to an increased workload, which requires the coordinate transcriptional activation of a panel of muscle genes.

In a few instances, identical phenotypes have been interpreted very differently by independent laboratories. Recently, two groups reported that embryos which are homozygous deficient for NF-1, the neurofibromatosis gene, displayed prominent defects in the cardiac outflow tract (9, 10). However, the cardiac phenotype was reported by one laboratory as persistent truncus arteriosus (10) and by the other as double outlet right ventricle (9). Upon review of the micrographs in the relevant manuscripts, the morphogenic defect is clearly double outlet right ventricle, which places the NF-1 defect in the region of the conotruncal cushions (below the aortic valve), as opposed to the aortic sac which lies above the aortic valve. Since persistent truncus arteriosus is associated with neural crest defects while double outlet right ventricle is not, the discrimination of the correct morphogenic phenotype has major mechanistic implications. Of course, there is a significant possibility that histological examination alone may not be sufficiently sensitive to detect many of the cardiovascular morphogenic defects of the most interest. Recently, a rapid throughput microdissection protocol, coupled with scanning electron microscopy, has led to the uncovering of a wide spectrum of congenital heart disease phenotypes in RXRα homozygous and heterozygous deficient mice (11), not revealed by standard histological examination (12, 13), underscoring the importance of the systematic quantitation of the severity and complexity of morphogenic defects. One can anticipate further advances in accurate phenotype assignment based upon development of new technology for three-dimensional magnetic resonance microscopy (which will allow high resolution tomographic analysis of embryonic morphogenesis) (14, 15), and miniaturized ultrasound imaging (Turnbull and Baldwin, personal communication; 2). In addition, microsurgical advances and the development of miniaturized technology/transducers/catheters should prove useful, as these approaches now allow the assessment of cardiac ventricular pressures, volumes, function, and shunt detection in the living murine embryo with placental circulation intact (see cover figure) (16). A partial listing of these recent developments towards molecular cardiovascular physiology have been provided in Table I. Variations of these ap-

Table I. In Vivo Approaches to the Assessment of Murine Cardiovascular Phenotypes

Modality	Technique	Procedure	Advantages	Disadvantages	References
Non-invasive imaging	Echocardiography	2D-guided M-mode and Doppler	Rapid screening for chamber size; wall thickness; cardiac function; Serial observations	Limited resolution*	(Tanaka, 55; Gardin, 56)
	Magnetic resonance imaging	Expensive	Longitudinal examination vascular wall; High resolution	Limited experience in mouse	(Libby, 57)
	Fetal echocardiography	Ultrasound backscatter microscopy	High resolution	Highly specialized equipment. Low frame rate (5–10 images/second)	(D. Turnbull and S. Baldwin, Personal Communication; 2)
	Magnetic resonance microscopy	High magnetic fields Expensive	Tomographic 3D images with high resolution	Pre-clinical; Motion artifacts	(Jacobs, 14; Smith, 15)
Invasive imaging	Contrast ventriculography	Densitometric method; Digital data collection. First pass RV; levophase LV images	Accurate measurement LV/RV volumes and function	X-ray based*	(Rockman, 42; Chien, 1)
	Intra-vital microscopy	Intact placental circulation; microinjection fluorescein tagged albumin; high-speed video microscopy	Fetal heart imaging (chamber size, function; detection malformations).	Terminal experiment	(Dyson, 16)
Hemodynamics	Micro-manometers	High-fidelity	Assessment systolic and diastolic function dp/dt estimations	Load-dependent*	(Milano, 35; Hunter, 45)
	Catheter-based; telemetry	Chronic instrumentation	24 hour blood pressure monitoring Conscious measurement	Further miniaturization needed for other phenotypes	

*Anesthetic administration required (see e.g., Milano, 35; Tanaka, 55 for details).

Table II. Examples of Murine Transgenic Models of Atherosclerosis‡*

Intervention	Phenotypic features
	ATHEROSCLEROSIS-PRONE
Human apo-A-II overexpression	Apo-AII 20% HDL protein content. Phenotype has no elevation in HDL-C. Despite normal HDL-C, risk atherosclerosis ↑ with fatty streak development even on low-fat diet. Indicates qualitative features HDL-C also important.
Human C-III overexpression	First animal model of primary hypertryglyceridemia. Triglyceride level proportional to C-III expression. Primary abnormality decreased VLDL fractional catabolic rate.
Human A-I, CIII, CETP overexpression	High triglyceride, low HDL-C phenotype. Comparable to most common lipoprotein disorder conferring susceptibility to CAD in humans.
Human-apo (a) overexpression	Apo (a) mice developed 20 times greater area lipid-rich lesions than control mice (outbred genetic background 3 months on atherogenic diet). < 5% apo (a) associated with lipoprotein; suggested apo (a) produces pathology independently of [LDL].
Apo E deficient	See text
LDL-receptor knockout	Homozygous mice have T_{chol} > twice normal litter mates with 7–9 times in ↑ LDL and IDL. Normal triglycerides. Correction with adenovirus-mediated gene transfer LDL-R protein. Increased atheroclerosis.
	ATHEROSCLEROSIS-RESISTANT
ApoE overexpression	See text
Human LPL overexpression	LPL directed by the chick β-actin promoter produced accelerated VLDL clearance and resistance to diet-induced hypercholesterolemia
Human LDL receptor overexpression	Radio-labelled LDL clearance ↑ 8–10 times compared to control mice. Resistant to high-fat high-cholesterol diet.
Human apo-AI overexpression	Major (> 70%) HDL protein. Selective doubling HDL-C. Useful model for examining effects of diet and drugs on HDL-C/apoA-I. Resistant to fatty streak development induced by atherogenic diet.

*∼ 20 genes involved in human lipid transport have been overexpressed or knocked out in transgenic mice. (Data from Breslow, 17; Ishibashi, 58; Lawn, 59). ‡For a more complete listing of mouse models with cardiovascular disease phenotypes, see 60.

proaches should now allow a wide range of in vivo phenotypic monitoring of both the embryonic and the adult mouse in organ systems that were heretofore difficult to approach (e.g., pulmonary function, GI motility, neural reflexes, renal blood flow and function). In this regard, it will be particularly critical to develop technology to monitor these physiological variables in the awake, unanesthetized animals, since anesthesia can induce physiological artifacts. Developing new approaches to allow repeated, in vivo sampling of small blood samples from the mouse is also likely to be valuable.

Species variability

Although mice and men share a subset of highly conserved genes that regulate fundamental aspects of cardiovascular morphogenesis, their inherent physiological, dietary, and environmental differences suggest that species variability may ultimately become a critical consideration in engineering mouse models of human cardiovascular disease. The pharmaceutical industry is well aware of the variability in physiological responses between closely related mammalian species, fueling a continued interest in using primates in the late stages of clinical drug development. For example, the electrophysiology of the rat heart is quite distinct from that of the human. As a result, rodents have served as relatively poor model systems to study electrophysiological phenotypes, particularly as they may relate to arrhythmogenesis. Likewise, coagulation and thrombosis cascades in the mouse are quite variable from the human counterparts, and the marmoset is the preferred small experiment animal for questions in this field. The coronary circulation of small animals is also not optimal for exploring coronary angiogenesis and revascularization, due to extensive collateralization. The availability of genetically inbred hypertensive rat strains has led to their utility for mapping quantitative trait loci in the setting of hypertension, and will continue to have a major advantage over mouse model systems of hypertension for many years. Further underscoring this point, a number of single gene knockouts in mice have yielded distinctly different phenotypes from the corresponding human monogenic disease phenotypes. Given the large potential for species variability, it is likely to become increasingly important to clearly define whether the mouse displays fidelity to the clinical phenotype of interest and/or displays a conserved response to the genetic manipulation of interest. If not, then the possibility exists of humanizing the mouse to display the response or phenotype of interest, and then evaluating the concomitant effects of a given genetic manipulation in this more appropriate genetic background. Perhaps the most clear example of this point is the beautiful body of work from a number of independent laboratories that has led to the engineering of mouse model of atherosclerosis and lipoprotein metabolism. The wild type mouse, with some exceptions (e.g., strain C57BL/6), is generally resistant to an "atherogenic" diet, lacks several of the critical genes which control lipoprotein metabolism. Nevertheless, due to the creation of models with powerful analogies to human atherogenesis (Table II), the mouse has become an important system for increasing our understanding of the human disease (17, 18). The generation of mouse models has been facilitated by the fact that human genes involved in lipoprotein metabolism are usually single-copy and have often been sequenced and mapped. The most atherogenic mouse

Table III. Examples of the Modification of Cardiac Structure and Function in Transgenic and Gene-targeted Mice‡

Principal phenotype	Mutation in mouse; Transgene/targeted allele	Transgenic/targeting strategy	Specific features*	Reference
Cardiac hypertrophy	p21^{ras}	MLC-2v promoter Ventricular specific expression p21^{ras}	Homozygotes: ↑ LV mass (∼ 60%); myocyte hypertrophy; myofibrillar disarray; selective diastolic dysfunction; normal systolic function; Heterozygotes: ↑ LV mass (∼ 8%)	(Hunter, 45)
	α-MHC	Point mutation α-MHC Arg403Gln, exon 13), "Hit and Run" technique	Myocyte hypertrophy, fibrosis, myofibrillar disarray, diastolic dysfunction	(Geisterfer-Lowrance, 61)
	IL-6 IL-6R	Two transgenic lines crossed (IL-6 and IL-6R)	Constitutive tyrosine phosphorylation gp 130/STAT 3; Concentric LV hypertrophy; myocyte hypertrophy; no disarray	(Hirota, 62)
	α_{1B} adrenergic receptor	αMHC promoter, Cardiac-specific expression constitutively active α_{1B} R	↑ α_{1B} R (3 times control); LV hypertrophy (↑ 20% heart/body weight ratio; myocyte hypertrophy; ↑ ANF expression)	(Milano, 63)
Congenital heart disease	hox-1.5	Homeobox gene (*hox-1.5*) replaced with *neo*	Homozygotes (−/−): craniofacial, pharyngeal and widespread cardiac and large vessel abnormalities. See DiGeorge syndrome	(Chisaka, 64)
	RXRα	Part of B/C1 exon and adjacent intron RXRα gene replaced with PGK-*neo* cassette	Homozygotes (−/−): intrauterine lethal; atrial-like ventricular phenotype; VSD (100%); AVSD (85%); DORV (60%); PTA (8%); Heterozygote: intermediate phenotype	(Sucov, 13; Dyson, 16; Gruber, 11)
	Endothelin-1	Edn-1 locus disrupted with exon 2 replaced with *neo*	Homozygotes (−/−): craniofacial and pharyngeal abnormalities with interrupted (2.3%) or tubular hypoplasia (4.6%) aortic arch; aberrant right subclavian artery (12.9%); ventricular septal defect (48.4%); Heterozygotes: hypertension	(Kurihara, 65)
	Connexin43 (Cx43)	Promoterless *neo* delete all transmembrane regions Cx43 (*Gjal*)	Homozygotes (−/−): mutants die at birth; gross enlargement conus RVOT (cf. pulmonic stenosis)	(Reaume, 66)
Enhanced contractility	β_2 adrenergic receptor	αMHC promoter with cardiac-specific expression human β_2AR	↑ β_2AR (55–195 times control); ↑ basal adenylate cyclase activity; no gross phenotype; ↑ LV function; inverse agonism demonstrated with β_2AR ligand ICI-118,551	(Milano, 35; Bond, 36)
	βARK inhibitor	αMHC promoter; Peptide COOH-terminus βARK	No gross phenotype; enhanced contractility (±isoprenaline)	(Koch, 37)

**VSD*, ventricular spetal defect; *AVSD*, atrioventricular cushion defect; *DORV*, double-outlet right ventricle; *APW*, aorticopulmonary window; *RVOT*, right ventricular outflow tract; *LV*, left ventricle. ‡For a more complete listing of mouse cardiac models, see reference 60.

strain is the apolipoprotein E (apoE)-deficient mouse, where the apoE gene has been disrupted by homologous recombination (19, 20). apoE is the ligand that is responsible for LDL and chylomicron remnant uptake, playing an important role in the clearance of lipoprotein particles from the circulation (17). The knockout of the gene for apoE results in a phenotype that is a true null mutation, with no expression of apoE, but has the considerable advantage that the homozygotes are both viable and fertile (19, 20). The pattern of disease is diet-responsive and the homozygotes fed a standard laboratory chow diet (0.01% cholesterol, 4.5% fat) have a serum cholesterol concentration of 400–500 mg per deciliter with the development of foam cells in the aortic sinus at 10 wk, whereas on a diet similar to that consumed in the United States (western diet, 0.15% cholesterol and fat 20%) serum cholesterol rises to ∼ 1800 mg per deciliter, this being mostly in the VLDL and IDL fractions

with triglycerides being minimally raised (19, 20). Animals consuming the western diet develop advanced lesions of atherosclerosis with their distribution and histology being almost indistinguishable from human disease (21, 22). These mice also provide a model for the study of lipoprotein oxidation with lesion oxidation-specific epitopes and antibodies to malondialdehyde-lysine in serum (23). Heterozygotes have diminished apoE with normal fasting lipids and slightly delayed postprandial lipid clearance consistent with half normal levels of E2 being sufficient to maintain normal serum lipids (19). Interestingly, crossing these animals with mice which display overexpression of human transgene AI, leads to an elevation of apo-AI, and HDL, and apparently alleviates atherogenicity, as it does also with apo(a) over-expressing mice (24). The physiological role of apoE is exemplified in two other models. The human apoE3 Leiden variant (tandem duplication amino acids 120–126) leads to type III hyperlipoproteinemia (25, 26); overexpression of this gene in so-called E3 Leiden mice leads to a phenocopy of the human disease (25). The apoE lipoprotein has also been over-expressed in transgenic mice leading to fourfold increase in apoE levels. These animals have a severalfold increase in radio-labeled VLDL/LDL clearance and are resistant to diet-induced hypercholesterolemia (27). In addition to increasing our understanding of the underlying processes (17), these mice can also be used in the development of drugs directed against the atherosclerotic process that can be used to predict efficacy in clinical trials (28); and also for testing the applicability of other interventions directed at these processes. In addition to serving as a model of atherogenesis, the mouse has now also been proven to be a valid model system to study a number of other cardiovascular phenotypes directly relevant to cardiovascular disease states in humans, including hypertension, cardiac contractility, hypertrophy, and morphogenic defects that are phenocopies of congenital heart disease phenotypes in man (see Table III).

Distinguishing developmental versus adult phenotypes

The breathtaking advances in the arena of genes and development underscores the value of using the mouse to dissect complex, polygenic phenotypes. The unraveling of molecular determinants of mammalian embryonic patterning, morphogenesis, and migration provide further support for the feasibility of attacking biological complexity at the molecular level in genetically manipulated mice. In this regard, discrete signaling pathways have been implicated in specific steps of cardiac morphogenesis, from establishment of left-right asymmetry in the primitive heart tube (activin), looping morphogenesis and ventricular specification (Nkx2.5), trabeculation (Neuregulin, erb B2, erb B4) outflow tract septation (Endothelin-1, PAX-3, RXRα/RAR), conotruncal defects (NF-1, RXRα), aortic arch anomalies (endothelin-1, RAR), ventricular maturation and expansion of the compact zone (RXRα, NF-1, TEF-1, N-MYC, WT-1), and endocardial cushion formation (RXRα, NF-1), thereby providing a host of candidate genes for analysis in sporadic cases of human congenital heart disease phenotypes (see Table III). The absolute requirement of the cardiovascular system for embryonic variability and growth has led to a rapidly growing list of genes that have an unsuspected, but very important role in cardiovascular development, and that are now announcing themselves by the crude, but definitive, phenotype of embryonic lethality. With well-characterized monitoring systems for murine congenital heart phenotypes at the molecular, morphological, and physiological levels, it should be relatively straight-forward to fully characterize these cardiovascular defects, which will facilitate connections with human congenital heart disease. However, defining where and when these molecules exert their critical function, as well as deriving the mechanistic pathway for these events, will ultimately require strategies for defining the spatial and temporal requirements for a given gene of interest, as well as approaches for obtaining the downstream target genes in the signaling pathways for defined morphogenic steps. In certain cases, obtaining the cell types of interest will also be challenging (e.g., cardiac neural crest), but knock-in strategies (29) using green fluorescent protein and cell-sorting could offer new opportunities. The temporal and spatial control of gene targeting through a variety of different approaches (Tet repressor/activator [30], interferon receptor reconstitution (31), estrogen/ecdysone receptor modulation) (32, 33) are clearly within reach, and should eventually elucidate where and when a given molecule of interest is required for a particular step of cardiovascular morphogenesis. Clearly, the availability of well-characterized tissue-restricted promoters that can drive high level, uniform, and cell type-specific expression of recombinase will become critical in the future. These promoters could also be valuable for attempting tissue specific rescue of deficiencies in ubiquitously expressed genes, which represents an alternative and complementary approach to the tissue-specific gene targeting strategy. Developing these new tricks of the trade, coupled with our existing wealth of knowledge of cardiovascular embryology, morphogenesis, and fetal physiology, will undoubtedly lead to a more in-depth understanding of the molecular framework of embryonic patterning during cardiovascular development, and should lead to major advances in identifying the key molecular determinants for complex cardiovascular defects. Similar approaches should be useful for approaching cardiovascular physiological phenotypes in another "developmental" window in the post-natal adult context, i.e., genes and physiology (34). With the above mentioned miniaturized technology for assaying complex cardiovascular physiological phenotypes growing on a routine bases, the era of a single gene physiology may be at hand. However, the "single gene" physiology approach will undoubtedly require the temporal and spatial control of the onset of the targeting event, to minimize the secondary effects on the postnatal phenotype that are likely to occur as a result of the loss of function during embryonic development. Discriminating between cell–cell, cell–matrix, and cell–autonomous events during in vivo signaling pathways for important adaptive physiological responses will also be critical, which should be approachable in genetically mosaic mice, or by cellular transplantation. The inherent cost limitations of breeding and maintaining a sufficient number of animals to monitor chronic adult physiological phenotypes point to the value of developing surrogate in vivo model systems that can uncover candidate genes worthy of further analysis in genetically manipulated mice.

In vivo mapping of cardiovascular signaling pathways

For the past two decades, molecular biology has employed reductionist approaches in the simplest in vitro, prokaryotic, and eukaryotic systems to identify genes which encode proteins of universal biological importance to all living cells. Until recently, the molecular dissection of in vivo phenotypes has been largely restricted to the study of cells and organisms with in-

herent genetic advantages, such as yeast (*S. cerevisiae*), worms (*C. elegans*), and fruitflies (*Drosophila*). Given the advent of the new mouse genetics, the opportunity exists to dissect signaling pathways for complex in vivo phenotypes.

One of the examples of the value of this approach is provided by recent molecular dissection of the proximal β-adrenergic receptor signaling pathways which control cardiac contractility by Lefkowitz and colleagues (35, 36, 37). β-adrenergic receptor (β-ADR) signaling pathways are critical in the regulation of cardiac contractility in response to sympathetic activation in normal physiological states, such as exercise. To better understand the relationship between individual molecules involved in β-ADR signaling and cardiac function, Lefkowitz and co-workers created a series of transgenic mice with cardiac targeted overexpression with either the β_2-ADR, βARK, or an inhibitor of βARK, documenting the power of combining molecular engineering with in vivo physiology to dissect and better understand complex mechanisms of cardiac function. Initial experiments showed that overexpression of the human β_2-ADR at high levels in the murine heart can lead to a two-fold higher level of myocardial cAMP (the effector molecule in the β-ADR signaling cascade) and a remarkable near doubling in contractility in vivo, in the intact mouse (35). These experiments led to the observation that activation of the β-ADR signaling pathway can occur even in the absence of agonist, supporting the concept of a two-state model of receptor activation in which receptors are in equilibrium between an inactive conformation and a spontaneously active conformation that can couple to G proteins in the absence of ligand (35, 36). These observations were extended to show that compounds previously thought to function as classic β receptor antagonists (i.e., antagonizing the action of an agonist such as norepinephrine), can actually "turn off" precoupled receptors and function as *inverse agonists*, a phenomenon not previously shown to occur in vivo (36). With this new information it will now be possible to identify compounds with previously unrecognized inverse agonist activity that may be exploited for potential therapeutic purposes.

During chronic congestive heart failure, agonist stimulated adenylyl cyclase activity is reduced (receptor desensitization) that is due, in part, to the impaired function of remaining receptors (receptor uncoupling). This condition is associated with an increase in activity and level of the enzyme responsible for β receptor uncoupling, the β-adrenergic receptor kinase (βARK). To determine the importance of βARK in the regulation of in vivo cardiac function, transgenic mice with cardiac overexpression of either βARK or a peptide inhibitor of βARK have been produced (37). Mice overexpressing βARK demonstrate attenuation of the isoproterenol stimulated increase in in vivo contractility, whereas the opposite phenotype is observed with the βARK inhibitor with significant enhancement in resting cardiac function. These findings document the importance of βARK as a critical modulator of cardiac function in vivo, and suggest the value of exploring further the pathophysiologic importance of changes in these molecules in experimental models of heart failure. In addition, since chronic exposure to β-adrenergic agonists, such as catecholamines, can be associated with cardiac injury, careful examination of the cardiac phenotype as a function of adult development is warranted.

While the above example underscores how changes in specific genes can affect cardiac function, it has also been appreciated for many years that changes in form and function can trigger specific changes in the gene programs of many cell types, including the heart. In response to mechanical stimuli, the myocardium adapts to increased workloads through the hypertrophy of individual muscle cells (for reviews see 38–40). Because the adult myocardial cell is terminally differentiated and has lost the ability to proliferate, cardiac growth during the hypertrophic process results primarily from an increase in protein content per individual myocardial cell, with little or no change in muscle cell number. Although this process is initially compensatory, there can be a pathological transition in which the heart becomes irreversibly enlarged and dilated, with the accompanying onset of dysfunction at the myocyte level. The central biochemical features of the myocardial hypertrophic response are an increase in contractile protein content and the re-expression of embryonic markers, both of which appear to be largely due to the transciptional activation of the corresponding genes that encode these proteins. The scientific challenge has been to identify the signaling pathways which mediate the complex hypertrophic response in vivo and the associated induction of a subset of cardiac muscle genes. One potential difficulty in defining the signaling mechanisms in vivo models of hypertrophy has been the limited ability to manipulate or control the complex in vivo physiology of hypertrophy. Ultimately, it will be necessary to document how mechanical cues orchestrate a specific set of genetic events in the adult cardiac muscle cell. Ideally, one would like to activate the expression of a dominantly acting gene product, or neutralize the activity of a specific signaling molecule, without interfering with the highly integrated physiology of hypertrophy in response to mechanical loading. Subsequently, an assessment could be made of the effects of the genetic alteration on the acquisition of specific molecular and cellular features of hypertrophy (increase in contractile protein content, activation of embryonic gene expression, increase in muscle cell size, etc.), as well as hallmarks of the physiological phenotype. Although inhibitors of various receptors and enzymes have been used to study the development of myocardial hypertrophy, in many cases the inhibitors are not specific and lead to secondary physiologic effects that confound the interpretation of the results. Thus, the ability to genetically manipulate an in vivo animal model would represent a significant advantage in the study of myocardial hypertrophy.

To capitalize on the advances, in mouse genetics and transgenic/gene-targeting technology, a major effort has been made to establish the mouse as a model system to study cardiac hypertrophy and failure (41–43). Since relatively little is known regarding the cardiovascular pathophysiology of the mouse, it first became necessary to directly examine the fidelity of the responses of the murine heart to a *bonafide* physiological stimulus for hypertrophy. Using microsurgical approaches to circumvent the diminutive size of the mouse heart and great vessels, a reproducible model of pressure-overload hypertrophy has been developed in the mouse (43). In short, the morphological and hemodynamic response of the murine heart to pressure overload is indistinguishable from that seen in larger mammalian species, including man (38, 39, 43). The ultimate utility of the mouse as a model system to study hypertrophic heart disease and heart failure rests upon the clear documentation that these various physiological phenotypes (diastolic function, compliance, systolic function, basal and agonist mediated increases in cardiac contractility and relaxation, global

ejection fraction, ventricular volumes, etc.) can be quantitatively assayed in the in vivo context in the mouse (for a review see 44). Miniaturized catheterization (1, 35) and microangiography technology (42) for quantitatively assaying these complex physiological phenotypes is now available, and has been routinely applied to identify physiological phenotypes in both transgenic and gene-targeted mice (45).

Previous studies have implicated *ras* dependent signaling pathways in the activation of features of hypertrophy in an in vitro model system (46). Using transgenic strategies, recent studies have provided direct evidence that *ras* is sufficient to activate a hypertrophic response in cardiac muscle in the in vivo context (45). Activation of the hypertrophic response has been monitored by several independent criteria, including an increase in LV mass/body weight ratio, increased myocardial cell size, and increased expression of an embryonic genetic marker of the hypertrophic response, ANF. The increase in these structural, morphological, and genetic markers of hypertrophy achieved by targeting oncogenic *ras* expression to the ventricular muscle cells are qualitatively similar to those seen in hypertrophy due to pressure overload in murine myocardium, but they occurred in the absence of concomitant valvular disease, hypertension, or other systemic effects. These mice exhibited a functional phenotype that is qualitatively similar to that seen in compensated human hypertensive heart disease. The findings in this murine model suggest a role for *ras*-dependent pathways in the genesis of cardiac hypertrophy, as well as in the transition between compensatory hypertrophy and the onset of cardiac muscle dysfunction and the development of myocyte disarray. These mice should be valuable in genetic-based approaches to identify further downstream signaling pathways which mediate this form of cardiac muscle dysfunction, using molecular physiological analysis to characterize the functional phenotype. As a genetically based model of cardiac muscle disease, these mice should now permit dissection of the interaction of *ras* with retinoids and other signaling pathways, through genetic crosses with other transgenic and gene-targeted strains, as well as physiological and pharmacological manipulations which induce or impair the development of hypertrophy. Furthermore, they identify *ras* and subsequent downstream signaling pathways as potential targets for interrupting the pathological process of hypertrophy in the in vivo context. In fact, based on these observations, we have recently demonstrated that agents which can block the oncogenic effects of *ras*, such as retinoids, can serve as suppressers of myocardial cell hypertrophy in an in vitro model system (47), supporting the potential therapeutic importance of these observations. A similar transgenic strategy is now being taken to examine the role of GP130 (40, 48, 49), G_q (50), and retinoid-dependent signaling pathways (47) in the control of this adaptive physiological response.

Genes and environment

In many cases, complex physiological phenotypes not only reflect genetic background but also are under the control of dietary and other environmental stimuli. Another fascinating example of the fruitful marriage of genes and physiology has been provided by the work of Oliver Smithies, who has systematically targeted key components of the renin-angiotensin system and related pathways, to evaluate their potential role in the control of resting blood pressure in the mouse (51–53). Knockouts of the ANP gene give rise to a salt-sensitive form of hypertension (51),while increasing the gene dosage of the angiotensinogen gene (one through four copies) results in a quantitative increase in resting blood pressure (52), providing credence to the concept of hypertension as a quintessential complex, quantitative trait. Mice carrying one normal and one disrupted copy of the ACE gene display a gender-dependent hypertensive phenotype (53). For all of these studies, blood pressure was monitored in the conscious, awake, unrestrained mouse. Since blood pressure is under diurnal control, it will be valuable to develop new approaches for blood pressure monitoring continuously throughout a 24-h period. Further mechanistic insights have been provided by another recent study that has convincingly shown that endothelial nitric oxide synthase is an important determinant of resting blood pressure, as gene-targeted mice lacking this enzyme display elevated blood pressure (54). In the future, it will be of interest to determine if the hypertensive phenotype in the above animals occurs as a result of changes in the systemic renin-angiotensin and nitric oxide synthetase signaling pathways, or reflect localized changes in tissue-specific regions (e.g., peripheral vascular smooth muscle, kidney, CNS, etc.)

Summary

Molecular medicine is quickly moving beyond the cloning and expression of human genes and toward the design of systems to assess unequivocally the function of a given gene and/or to identify genes that are required for the maintenance of a given in vivo phenotype. This movement toward coupling genetic and functional analysis is beginning to bridge the generation gap between molecular biologists and physiologists. As noted in the current Perspective, the cutting edge of the field of cardiovascular biology is beginning to gravitate back towards the integration of genes and physiology. In short, molecular technology has paved the way for the evolution of molecular physiology in transgenic and gene-targeted animals, which should lead to the identification of the genetic determinants for complex, integrative heart diseases.

References

1. Chien, K.R. 1993. Molecular advances in cardiovascular biology. *Science (Wash. DC)*. 260:916–917.
2. Lin, M.C., H.A. Rockman, and K.R. Chien. 1995. Heart and lung disease in engineered mice. *Nature Med.* 1:749–751.
3. Curran, M.E., I. Splawski, K.W. Timothy, G.M. Vincent, E.D. Green, and M.T. Keating. 1995. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell*. 80:795–803.
4. Sanguinetti, M.C., C. Jiang, M.E. Curran, and M.T. Keating. 1995. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell*. 81:299–307.
5. Geisterfer-Lowrance, A.A., S. Kass, G. Tanigawa, H.P. Vosberg, W. McKenna, C.E. Seidman, and J.G. Seidman. 1990. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell*. 62:999–1006.
6. Tanigawa, G., J.A. Jarcho, S. Kass, S.D. Solomon, H.P. Vosberg, J.G. Seidman, and C.E. Seidman. 1990. A molecular basis for familial hypertrophic cardiomyopathy: an alpha/beta cardiac myosin heavy chain hybrid gene. *Cell*. 62:991–998.
7. Thierfelder, L., H. Watkins, C. MacRae, R. Lamas, W. McKenna, H.P. Vosberg, J.G. Seidman, and C. E. Seidman. 1994. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell*. 77:701–712.
8. Rudnicki, M.A., P.N. Schnegelsberg, R.H. Stead, T. Braun, H.H. Arnold, and R. Jaenisch. 1993. MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell*. 75:1351–1359.
9. Jacks, T., T.S. Shih, E.M. Schmitt, R.T. Bronson, A. Bernards, and R.A. Weinberg. 1994. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. *Nat. Genet*. 7:353–361.
10. Brannan, C.I., A.S. Perkins, K.S. Vogel, N. Ratner, M.L. Nordlund,

S.W. Reid, A.M. Buchberg, N.A. Jenkins, L.F. Parada, and N.G. Copeland. 1994. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. *Genes Dev.* 8:1019–1029.

11. Gruber, P.J., S.W. Kubalak, H.M. Sucov, R.M. Evans, T. Pexieder, and K.R. Chien. 1995. RXRα deficiency confers genetic susceptibility to aortic sac, conotruncal, atrioventricular cushion, and ventricular morphogenic defects. *Circulation*. 92:I368. (Abstr.)

12. Kastner, P., J.M. Grondona, M. Mark, A. Gansmuller, M. LeMeur, D. Decimo, J-L. Vonesch, P. Dollé, and P. Chambon. 1994. Genetic analysis of RXRα developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell.* 78:987–1003.

13. Sucov, H., M.E. Dyson, C.L. Gumeringer, J. Price, K.R. Chien, and R.M. Evans. 1994. RXRα mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. *Genes Dev.* 8:1007–1018.

14. Jacobs, R.E., and S.E. Fraser. 1994. Magnetic resonance microscopy of embryonic cell lineages and movements. *Science (Wash. DC)*. 263:681–684.

15. Smith, B.R., G.A. Johnson, E.V. Groman, and E. Linney. 1994. Magnetic resonance microscopy of mouse embryos. *Proc. Natl. Acad. Sci. USA.* 91: 3530–3533.

16. Dyson, E., H. Sucov, S.W. Kubalak, G. Schmid-Schönbein, F. Delano, R.M. Evans, J. Ross, Jr., and K.R. Chien. 1995. Atrial-like phenotype is associated with embryonic ventricular failure in RXRα−/− mice. *Proc. Natl. Acad. Sci. USA.* 92:7386–7390.

17. Breslow, J.L. 1993. Transgenic mouse models of lipoprotein metabolism and atherosclerosis. *Proc. Natl. Acad. Sci. USA*. 90:8314–8318.

18. Stoltzfus, L., and E.M. Rubin. 1993. Atherogenesis: insights from the study of transgenic and gene-targeted mice. *Trends Cardiovasc. Med.* 3:130–134.

19. Plump, A.S., J.D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J.G. Verstuyft, E.M. Rubin, and J.L. Breslow. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* 71:343–353.

20. Zhang, S.H., R.L. Reddick, J.A. Piedrahita, and N. Maeda. 1992. Spontaneous hyperchloesterolemia and arteriallesions in mice lacking apolipoprotein E. *Science (Wash. DC)*. 258:468–471.

21. Nakashima, Y, A.S. Plump, E.W. Raines, J.L. Breslow, and R. Ross. 1994. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler. Throm.* 14:133–140.

22. Reddick, R.L., S.H. Zhang, and N. Maeda. 1994. Atherosclerosis in mice lacking apoE: evaluation of lesional development and progression. *Arterioscl. Thromb.* 14:141–147.

23. Palinski, W., V.A. Ord, A.S. Plump, J.L. Breslow, D. Steinberg, and J.L. Witzum. 1994. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. *Arterioscler. Thromb.* 14:605–616.

24. Paszty, C., N. Maeda, J. Verstuyft, and E.M. Rubin. 1994. Apolipoprotein AI transgene corrects apolipoprotein E deficiency-induced atherosclerosis in mice. *J. Clin. Invest.* 94:899–903.

25. Van der Maagenberg, A.M.J.M., M.H. Hofker, P.J. Krimpenfort, I. de Bruijn, B. van Vlijmen, H. van der Boom, L.M. Havekes, and R.R. Frants. 1993. Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. *J. Biol. Chem.* 268:10540–10545.

26. Fazio, S. 1993. Recent insights into the pathogenesis of type III hyperlipoproteinemia. *Trends Cardiovas. Med.* 3:191–196.

27. Shimano, H. N. Yamada, M. Katsuki, M. Shimada, T. Gotoda, K. Harada, T. Murase, C. Fukazawa, F. Takaku, and Y. Yazaki. 1992. Overexpression of apolipoprotein E in transgenic mice: marked reduction in plasma lipoproteins except high density lipoprotein and resistance against diet-induced hypercholesterolemia. *Proc. Natl. Acad. Sci. USA*. 89:1750–1754.

28. Tangirala, R.K., F. Casanada, E. Miller, J.L. Witzum, D. Steinberg, and W. Palinski. 1995. Effect of antioxidant N., N′-diphenyl 1,4-phenylenediamine (DPPD) on atherosclerosis in ApoE-deficient mice. *Arterioscl. Thromb.* 15: 1625–1630.

29. Hanks, M., W. Wurst, L. Anson-Cartwright, A.B. Auerback, and A.L. Joyner. 1995. Rescue of the En-1 mutant phenotype by replacement of En-1 with En-2. *Science (Wash. DC)*. 269:679–682.

30. Gossen, M., S. Freundlieb, G. Bender, G. Muller, W. Hillen, and H. Bujard. 1995. Transcriptional activation by tetracyclines in mammalian cells. *Science (Wash. DC)*. 268:1766–1769.

31. Kuhn, R., F. Schwenk, M. Aguet, and K. Rajewsky. 1995. Inducible gene targeting in mice. *Science (Wash. DC)*. 269:1427–1429.

32. Metzger, D., J. Clifford, H. Chiba, and P. Chambon. 1995. Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. *Proc. Natl. Acad. Sci. USA*. 92:6991–6995.

33. No, D., T-P. Yao, and R.M. Evans. 1996. Ecdysone inducible gene expression in mammalian cells. *Proc. Natl. Acad. Sci. USA*. In press.

34. Chien, K.R. 1995. Cannon Award Lecture. Cardiac muscle diseases in genetically engineered mice: the evolution of molecular physiology. *Am. J. Physiol.* 269:H753–H766.

35. Milano, C.A., L.F. Allen, H.A. Rockman, P.C. Dolber, T.R. Mcminn, K.R. Chien, T.D. Johnson, R.A. Bond, and R.J. Lefkowitz. 1994. Enhanced myocardial function in transgenic mice overexpressing the β_2-adrenergic receptor. *Science (Wash. DC)*. 264:582–586.

36. Bond, R.A., P. Leff, T.D. Johnson, C.A. Milano, H.A. Rockman, T.R. McMinn, S. Apparsundaram, M.F. Hyek, T.P. Kenakin, L.F. Allen, and R.J. Lefkowitz. 1995. Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β_2-adrenoceptor. *Nature (Lond.)*. 374: 272–276.

37. Koch, W.J., H.A. Rockman, R. Samama, R.A. Hamilton, R.A. Bond, C.A. Milano, and R.J. Lefkowitz. 1995. Reciprocally altered cardiac function in transgenic mice overexpressing the β-adrenergic receptor kinase or a βARK inhibitor. *Science (Wash. DC)*. 268:1350–1353.

38. Chien, K.R., H. Zhu, K.U. Knowlton, W. Miller-Hance, M. van Bilsen, T.X. O'Brien, and S.M. Evans. 1993. Transcriptional regulation during cardiac growth and development. *Annu. Rev. Physiol.* 55:77–95.

39. Morgan, H.E., and K.M. Baker. 1991. Cardiac hypertrophy. Mechanical, neural, and endocrine dependence. *Circulation.* 83:13–25.

40. Wollert, K.C., T. Taga, T. Kishimoto, C.C. Glembotski, A.B. Vernallis, J.K. Heath, D. Pennica, W.I. Wood, and K.R. Chien. 1996. Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy: assembly of sarcomeric units in series via gp130/leukemia inhibitory factor receptor dependent pathways. *J. Biol. Chem*. In press.

41. Rockman, H.A., K.U. Knowlton, J.R. Ross, Jr., and K.R. Chien. 1993. In vivo murine cardiac hypertrophy: a novel model to identify genetic signaling mechanisms that activate an adaptive physiologic response. *Circulation.* 87:14–21.

42. Rockman, H.A., S. Ono, R.S. Ross, L.R. Jones, M. Karimi, V. Bhargava, J. Ross, Jr., and K.R. Chien. 1994. Molecular and physiological alterations in murine ventricular dysfunction. *Proc. Natl. Acad. Sci. USA.* 91:2694–2698.

43. Rockman, H.A., R.S. Ross, A.N. Harris, K.U. Knowlton, M.E. Steinhelper, L. Field, J. Ross, and K.R. Chien. 1996. Segregation of atrial specific and inducible expression of an ANF transgene in an *in vivo* murine model of cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA.* 88:8277–8281.

44. S.W. Kubalak, P.A. Doevendans, H.A. Rockman, J.J. Hunter, N. Tanaka, J. Ross, Jr., and K.R. Chien. 1996. Molecular analysis of cardiac muscle diseases via mouse genetics. In *Methods in Molecular Genetics*, K.W. Adolph, editor. Academic Press, Orlando, Florida. In press.

45. Hunter, J.J., N. Tanaka, H.A. Rockman, J. Ross, Jr., and K.R. Chien. 1995. Ventricular expression of a MCL-2v-*Ras* fusion gene induces cardiac hypertrophy and selective diastolic dysfunction in transgenic mice. *J. Biol. Chem.* 270:32173–23178.

46. Thorburn, A., J. Thorburn, S-Y. Chen, S. Powers, H.E. Shubeita, J.R. Feramisco, and K.R. Chien. 1993. HRas dependent pathways can activate morphological and genetic markers of cardiac muscle cell hypertrophy. *J. Biol. Chem.* 268:2244–2249.

47. Zhou, M.D., H.M. Sucov, R.M. Evans, and K.R. Chien. 1995. Retinoid dependent pathways suppress myocardial cell hypertrophy. *Proc. Natl. Acad. Sci. USA.* 92:7391–7395.

48. Pennica, D., K.L. King, K.J. Shaw, E. Luis, J. Rullamas, S-M. Luoh, W.C. Darbonne, D.S. Knutzon, R. Yen, K.R. Chien, J.B. Baker, and W.I. Wood. 1995. Expression cloning of cardiotrophin-1, a cytokine that induces cardiac myocyte hypertrophy. *Proc. Natl. Acad. Sci. USA*. 92:1142–1146.

49. Sheng, Z., D. Pennica, W.I. Wood, and K.R. Chien. 1996. Cardiotrophin-1 displays early expression in the murine heart tube and promotes cardiac myocyte survival. *Development.* In press.

50. LaMorte, V.J., J. Thorburn, D. Absher, A. Spiegel, J.H. Brown, K.R. Chien, J.R. Feramisco, and K.U. Knowlton. 1994. G_q and *ras* dependent pathways mediate hypertrophy of neonatal rat ventricular myocytes following α_1-adrenergic stimulation. *J. Biol. Chem.* 269:13490–13496.

51. John, S.W.M., J.H. Krege, P.M. Oliver, J.R. Hagaman, J.B. Hodgin, S.C. Pang, T.G. Flynn, and O. Smithies. 1995. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science (Wash. DC)*. 267:679–681.

52. Kim, H.S., J.H. Krege, K.D. Kluckman, J.R. Hagaman, J.B. Hodgin, C.F. Best, J.C. Jennette, T.M. Coffman, N. Maeda, and O. Smithies. 1995. Genetic control of blood pressure and the angiotensinogen locus. *Proc. Natl. Acad. Sci. USA.* 92:2735–2739.

53. Krege, J.H., S.W.M. John, L.L. Laugenback, J.B. Hodgin, J.R. Hagaman, E.S. Backman, J.C. Jennette, D.A. O'Brien, and O. Smithies. 1995. Male-female differences in fertility and blood pressures in ACE deficient mice. *Nature (Lond.)*. 315:146–148.

54. Huang, P.L., Z. Huang, H. Mashimo, K.D. Bloch, M.A. Moskowitz, J.A. Bevan, and M.C. Fishman. 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature (Lond.)*. 377:239–242.

55. Tanaka, N., N. Dalton, H.A. Rockman, K.L. Peterson, J.J. Hunter, K.R. Chien, and J. Ross, Jr. 1996. Transthoracic echocardiography in the normal and abnormal mouse heart. *Circulation.* In press.

56. Gardin, J.M., F.M. Siri, R.N. Kitsis, J.G. Edwards, and L.A. Leinwand. 1995. Echocardiographic assessment of left ventricular mass and systolic function in mice. *Circ. Res.* 76:907–914.

57. Libby, P. 1995. Lesion versus lumen. *Nature Med.* 1:17–18.

58. Ishibashi, S., M.S. Brown, J.L. Goldstein, R.D. Gerard, R.E. Hammer, and J. Herz. 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J. Clin. Invest.* 92:883–893.

59. Lawn, R.M., D.P. Wade, R.E. Hammer, G. Chiesa, J.G. Verstuyft, and

E.M. Rubin. 1992. Atherogenesis in transgenic mice expressing human apolipoprotein(a). *Nature (Lond.).* 360:670–672.

60. Chien, K.R., and A.A. Grace. 1996. Principles of cardiovascular molecular and cellular biology. In *Heart Disease*, Fifth Edition, E. Braunwald, editor. W.B. Saunders Company, Orlando, Florida. In press.

61. Geisterfer-Lowrance, A., M. Christe, D. Conner, D. Ladd, S. Ingwalls, F. Schoen, C. Seidman, and J.G. Seidman. 1995. A targeted missense mutation in alpha-myosin heavy chain gene leads to alterations in physiologic phenotype in the mouse. *Circulation.* 92:I–233.

62. Hirota, H., K. Yoshida, T. Kishimoto, and T. Taga. 1995. Continuous activation of gp130, a signal transducing receptor component for interleukin 6-related cytokines causes myocardial hypertrophy in mice. *Proc. Natl. Acad. Sci. USA.* 92:4862–4866.

63. Milano, C.A., P.C. Dolber, H.A. Rockman, R.A. Bond, M.E. Venable, L.F. Allen, and R.J. Lefkowitz. 1994. Myocardial expression of a constitutively active alpha 1β-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA.* 91:10109–10113.

64. Chisaka, O., and M.R. Capecchi. 1991. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene hox-1.5. *Nature (Lond.).* 350:473–479.

65. Kurihara, Y., H. Kurihara, H. Oda, K. Maemura, R. Nagai, T. Ishikawa, and Y. Yazaki. 1995. Aortic arch malformations and ventricular septal defect in mice deficient in endothelin-1. *J. Clin. Invest.* 96:293–300.

66. Reaume, A.G., P.A. de Sousa, S. Kulkarni, B.L. Langille, D. Zhu, T.C. Davies, S.C. Juneja, G.M. Kidder, and J. Rossant. 1995. Cardiac malformations in neonatal mice lacking connexin43. *Science (Wash. DC).* 267:1831–1834.