Timing Is Everything in Life
Conditional Transgene Expression in the Cardiovascular System

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Abstract—Manipulation of the mouse genome by traditional transgenic approaches has facilitated studies of gene function within the context of the intact organism and allowed for the creation of useful animal models of human disease. However, the timing of gene activation or repression is a critical determinant of phenotype, and the ability to regulate the temporal profile of transgene expression remains an important experimental goal. In this Mini Review, we describe the current status of systems to tightly regulate target gene expression in vivo, focusing on binary systems using chimeric transcription factors. Although experimental difficulties persist, regulated expression systems are beginning to produce conditional phenotypes with exciting experimental implications. We review the experience to date and examine the potential utility of these approaches within the context of cardiovascular medicine. (Circ Res. 1998;82:837-844.)

Key Words: mouse genome ▪ gene expression ▪ chimeric transcription ▪ transgenic ▪ conditional

Overview
Phenotype reflects the precise hierarchical control of gene expression. From a purely descriptive point of view, gene expression in multicellular organisms may be defined in terms of its spatial and temporal characteristics. Modulation of either parameter may lead to phenotypic sequelae of varying importance. Although considerable progress has been made identifying and characterizing the cis- and trans-acting factors that regulate transcription, our present understanding of gene expression is in general inadequate to functionally separate those elements that individually control the timing and location of gene expression.1 Pragmatically, then, the experimentalist typically selects regulatory elements with appropriate tissue specificity and learns to live, often unsatisfactorily, with the magnitude and timing of expression within the tissue of interest.

A detailed review of the many approaches available for conditional transgene expression has recently appeared.2 Therefore, in this Mini Review, we first focus on a consideration of experimental scenarios that provide the impetus for the continued development of conditional transgenic systems. We then describe the experience to date with several regulated systems, highlighting the tetracycline-regulated binary system that has emerged as the present system of choice for a number of investigators.3

Experimental Paradigms
We can envision models of at least four types in which regulated transgene expression may be desirable, ie, models in which (1) toxicity is observed with constitutive expression using either viral or cellular promoters, (2) potential stage-specific effects of transgene expression are investigated, (3) the relationship between transgene abundance and phenotype is examined, and (4) transgene expression is reversibly controlled. We discuss each of these paradigms in greater detail below.

Toxic Gene Products
Increasing numbers of investigators are generating transgenic mice with dysregulated expression of wild-type or mutationally engineered gene products in the heart, often using the well-characterized mouse α-MHC promoter.4 As many as 30 new models were presented at a recent meeting of cardiovascular scientists.5 Although occasionally the goal is to examine the function of a previously uncharacterized gene product, more often the investigator wishes to understand the consequences of dysregulated expression of a protein with known activity. In many instances, the goal is to generate a mouse model of a clinically relevant human disease syndrome, where alterations in expression of the gene in question are hypothesized to contribute to the pathophysiology. Not infrequently, such efforts result in early lethal phenotypes, such as the intense inflammatory myocarditis observed with overexpression of tumor necrosis factor-α, the massive atrial dilation and perinatal mortality associated with overexpression of the type 1a angiotensin II receptor, or the disruption of normal looping morphogenesis and embryonic lethality observed with constitutively activated transforming growth factor-β receptor mutants.6-8 From a purely pragmatic point of view, early lethality precludes the establishment of transgenic lineages, necessitating the de novo generation of transgenic founders for any additional experimentation. More
important, these outcomes prevent examination of the consequences of dysregulated gene expression in the context of the adult organism, where most forms of acquired cardiovascular disease are manifest.

**Stage-Specific Transgene Expression**

In most mammalian tissues, profound developmental alterations in gene expression are observed; this phenomenon has been especially well explored in the heart, where the transition from the so-called “fetal” to “adult” program of transcriptional activity has been extensively characterized.\(^5\)–\(^12\) Inasmuch as the cellular environment is undergoing profound ontogenic modulation, the effects of ectopically expressed genes are likely to vary as a function of developmental stage. For example, dysregulated expression of a mutationally activated retinoic acid receptor in the heart under the control of regulatory elements from the α-MHC isoform is without obvious phenotype, whereas earlier activation in the ventricular myocardium using β-MHC regulatory sequences leads to profound effects on cardiac structure and function.\(^13\) Recently, when a conditional strategy was used (described more fully below), it was shown that induction of a mutationally activated protein kinase C β-isoform in the neonatal heart leads to sudden death, whereas activation beginning in adult mice is better tolerated, with modest concentric ventricular hypertrophy.\(^14\) Finally, phenotypes observed in late stages, such as the abnormal multinucleation and sustained DNA synthesis observed in adult cardiac myocytes overexpressing cyclin D1, may be predicated on the perinatal expression observed with the α-MHC promoter.\(^15\) Together, these studies highlight the concept that susceptibility to the phenotypic effects of various gene products may be stage specific and underscore the need for tools to more fully define the precise windows of vulnerability.

**Manipulation of Transgene Abundance**

Gene dosage may profoundly influence phenotype. Whereas transgene expression may not necessarily correlate with copy number, phenotypic severity frequently does reflect abundance of the transgene product. For example, overexpression of transcription factors may result in transcriptional squelching and unanticipated effects on gene expression.\(^16\) Similarly, the abundance of mutationally activated or dominantly negative polypeptides may potently influence phenotype. Consider the incorporation of a mutant dominant-negative polypeptide into a multimeric structure,\(^17\) such as a potassium channel formed by the association of four α-subunits.\(^18\) If all channels with at least one mutant subunit are nonfunctional, the current (\(I\)) may be described by the following equation: \(I = (1 - f_{\text{mut}})^3 \times I_w\), where \(f_{\text{mut}}\) is the fraction of mutant subunits, and \(I_w\) is the wild-type current.\(^18\) The relative abundance of the mutant protein reduces function (ie, current) dramatically over an extremely narrow window. Thus, quantitative control of the magnitude of mutant protein expression renders the extent of ionic flux amenable to precise regulation and greatly enhances the power of the experimental paradigm. These observations suggest that animal models in which phenotypic severity is predicted to vary in a quantitative manner with respect to the abundance of the transgene might be ideally suited for study using regulated gene expression systems.

**Reversibility**

A major impetus for transgenic and gene-targeting strategies is the development of suitable mouse models of human disease states. Whereas gene disruption and gene replacement strategies are appropriate to create null or gain-of-function mutants, neither is ideal to model “acquired” diseases, ie, those with polygenic etiologies and environmental influences. Gene-targeting strategies (with the exception of those using recombinase-based techniques, considered below) create germline mutations, and the potential to initiate a cascade of secondary responses during the earliest stages of development is substantial. These responses, even if phenotypically silent in the unstressed organism, may considerably alter behavior to additional challenges in the adult mouse. Although this information, in itself, may be of interest, rendering a transgene silent during development and inducing its expression in the adult provide a potentially more suitable environment in which its effects may be examined, an environment not complicated by potential developmental perturbations. Moreover, in contrast to the permanent modification of gene expression achieved with traditional gene-targeting strategies, conditional transgenic approaches allow one to reversibly manipulate the expression of a transgene. Thus, one may examine not only the process of disease progression but also the adaptive and potentially reparative mechanisms that are used during disease regression. For example, conditional overexpression of a G\(_\text{q}\)-coupled heptahelical receptor has recently been reported to result in a syndrome resembling human congestive heart failure. Repression of transgene expression, even in the later stages of the syndrome, leads to a dramatic reversal of the illness.\(^19\) Conceptually, it is easy to imagine that such reversibility will facilitate dissection of the manifold interactions that give rise to complex syndromes such as congestive heart failure.

**Conditional Gene Expression In Vivo**

An ever-expanding repertoire of systems to conditionally regulate gene expression has been described; these systems are highly effective in vitro. Several of these regulatory schemes have been successfully adapted for in vivo use, and details of these paradigms have recently been reviewed.\(^2\) Inducible promoters, such as the glucocorticoid-responsive mouse mammary tumor virus long-terminal repeat, the interferon-inducible Mx1 promoter, and the heavy-metal inducible metallothionein promoter are experimentally straightforward, and relatively robust inductions of heterologous sequences may be achieved.\(^20\)–\(^22\) Nonetheless, pragmatic difficulties and

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**Selected Abbreviations and Acronyms**

- CMV = cytomegalovirus
- FRB = FKBP12-rapamycin–binding domain of FRAP
- HSV = herpes simplex virus
- MHC = myosin heavy chain
- rtTA = reverse tTA
- tetO = tet operator
- tTA = tetracycline-controlled transactivator

\(f_{\text{mut}}\) is the fraction of mutant subunits, and \(I_w\) is the wild-type current.
includes a strong transactivation domain. Expression of the cognate DNA binding site. Third, the chimeric protein negatively) of the transactivator to interact productively with its second component is a ligand-binding domain, occupancy of which efficiently regulates the ability (either positively or negatively) of the transactivator to interact productively with its cognate DNA binding site. Only in binary transgenic mice (+/+) is the target gene transactivated, in a TCN-repressible manner.

inherent theoretical limitations render use of these systems unsuitable for the majority of investigators, accounting for their failure to be more widely used. Nonrepressible baseline transcription, ie, leakiness, is a persistent technical limitation with most of these systems. For others, toxicity or pleiotropic effects of the inducing agents are problematic. More important, for almost all of the earliest systems, there is no inherent mechanism for restricted cell-type–specific expression. Although the abundance of transgene expression may be regulatable, the spatial profile is not amenable to experimental control, and tissue specificity simply reflects the intrinsic activity of the inducible promoter used. Thus, in contrast to traditional gene-targeting experiments using tissue-specific promoters, here one gains temporal control but relinquishes the arguably more important spatial regulation.

Ligand-Regulated Binary Systems

In theory, the emergence of ligand-regulated binary transgenic systems has addressed the dual problems of spatial specificity and temporal regulation.23–26 These systems require two genetic elements typically integrated independently into separate transgenic mouse strains, as schematized in Figure 1. The first element, the transactivator, uses tissue-specific regulatory elements, such as the α-MHC promoter (MHCα Pro) to drive expression of the chimeric tTA. The chimeric transactivator includes the DNA-binding domain (B) and ligand-binding domain (L) from the tet repressor and the transactivation domain (A) from HSV virion protein 16. The target gene includes an array of tetO sequences fused to a human CMV minimal promoter (CMVm), upstream from the gene of interest. Transgenic lines harboring each of the two constructs are generated and mated to produce progeny with each of four genotypes. Only in binary transgenic mice (+/+) is the target gene transactivated, in a TCN-repressible manner.

Figure 1. Binary strategy for tetracycline (TCN)-regulated transgene expression. The tissue-specific transactivator uses cell-type–specific regulatory elements such as the α-MHC promoter (MHCα Pro) to drive expression of the chimeric tTA. The chimeric transactivator includes the DNA-binding domain (B) and ligand-binding domain (L) from the tet repressor and the transactivation domain (A) from HSV virion protein 16. The target gene includes an array of tetO sequences fused to a human CMV minimal promoter (CMVm), upstream from the gene of interest. Transgenic lines harboring each of the two constructs are generated and mated to produce progeny with each of four genotypes. Only in binary transgenic mice (+/+) is the target gene transactivated, in a TCN-repressible manner.

transactivator in a tissue-specific manner imparts spatial control on the system, whereas ligand administration regulates the efficiency and hence the magnitude of transcription. The second element in this binary system is the target gene, consisting of a transcriptionally quiescent minimal promoter flanked by multimerized copies of the site-specific DNA binding site. By crossbreeding, compound heterozygous progeny that harbor both elements of the regulatory scheme are generated, allowing ligand-dependent and tissue-specific target gene transactivation.

To date, most experimental work has been carried out with variants of two chimeric transactivators, based on either the lac or tet repressors of Escherichia coli.22,27 Taking advantage of the modular nature of most transcription factors, the DNA- and ligand-binding domains from these two proteins have been retained, whereas their transcriptional repression domains have been replaced with strong trans-activation regions from heterologous proteins, such as HSV virion protein 16. The resulting chimeric proteins, such as the recently described tTA, are thus converted from normal repressors to ligand-regulated transactivators. The tetracycline system has some theoretical and pragmatic advantages compared with lac or tet repressors of Escherichia coli.22,27 Taking advantage of the modular nature of most transcription factors, the DNA- and ligand-binding domains from these two proteins have been retained, whereas their transcriptional repression domains have been replaced with strong trans-activation regions from heterologous proteins, such as HSV virion protein 16. The resulting chimeric proteins, such as the recently described tTA, are thus converted from normal repressors to ligand-regulated transactivators. The tetracycline system has some theoretical and pragmatic advantages compared with lac or tet repressors of Escherichia coli.22,27

On the basis of these considerations, several laboratories, including our own, have examined the ability of tTA to conditionally transactivate appropriately designed target genes in the cardiovascular system and elsewhere. Using regulatory elements from the α-MHC gene to impart cardiac myocyte-restricted expression, we initially examined the ability of mice harboring an α-MHC–tTA transgene to

Figure 2. Tetracycline-regulated transgene expression in the heart. The tTA was expressed with regulatory elements from the cardiac-specific α-MHC gene. Binary α-MHC–tTA/tetlacZ′ mice harboring the cardiac-specific transactivator and lacZ reporter transgenes were maintained either in the presence (A) or absence (B) of tetracycline in the drinking water. Hearts from adult mice were stained for β-galactosidase activity. Expression of lacZ is heterogeneous but fully repressed by antibiotic treatment.
successfully transactivate coinherited luciferase or nuclear-localized β-galactosidase transgenes. These studies demonstrated the efficacy of the binary system but also underscored the potential for tTA-independent transcriptional leakiness, as well as the existence of heterogeneity of expression on a cell-by-cell basis (Figure 2). Both of these undesirable features may primarily reflect the integration site of the target gene rather than the behavior of the transactivator line, per se, since independent lines harboring identically constructed target transgenes show substantially different degrees of background expression and also vary in the extent of spatial heterogeneity. Importantly, for the investigator, for most target genes we have examined, transgenic lines can be identified with virtually no background expression and complete repression by exogenous tetracycline analogues. To date, when this same strain of cardiac-specific transactivator has been used, successful transactivation of a number of different target strains has been achieved, resulting in stage-dependent and/or reversible phenotypes and including proteins as diverse as a mutationally activated form of protein kinase Cβ, an engineered variant of a Gα-coupled heptahelical transmembrane receptor and the diphtheria toxin A gene. This last example is of particular interest with regard to the issue of毒性 and tTA-independent transcriptional leakiness. Transgenic founders harboring the tetracycline-responsive diphtheria toxin A target gene were recovered with rather low efficiency, presumably reflecting toxic effects from transcriptional leakiness in most embryos injected with the construct and embryonic lethality. Nonetheless, lines could be identified that appeared healthy and had normal viability, including one that was appropriately transactivated by tTA in a tetracycline-dependent manner. Thus, founders that harbor constructs with even the most toxic of gene products may be successfully generated with this approach.

Initial studies examining the feasibility of conditional transgene expression elsewhere in the cardiovascular system have recently been reported. With a strategy similar to that described above, tTA has been expressed using transcriptional regulatory elements from the SM22α promoter. In embryos, tetracycline-repressible transactivation was observed in the heart, developing vascular tree, and somitic mesoderm, similar to the known pattern of endogenous SM22α expression at this stage of development (Figure 3A). In adult mice, reporter gene expression is restricted to smooth muscle cells of the arterial tree, as illustrated in

Figure 3. Tetracycline-regulated transgene expression in vascular smooth muscle cells. A, tTA was expressed with regulatory elements from the vascular smooth muscle-specific SM22α gene. Binary SM22α-tTA/tet-lacZ embryos maintained either in the presence (left) or absence (right) of doxycycline were whole-mount-stained for β-galactosidase activity. Antibiotic-repressible staining is evident in the heart, the developing vascular tree, and the somitic mesoderm. B, Aortas from adult binary SM22α-tTA/tetlacZ mice exposed (top aorta) or not exposed (bottom aorta) to doxycycline treatment were processed for lacZ staining. Significant inhibition of tTA-dependent lacZ staining was observed in antibiotic-treated animals.
Figure 3B. It is likely that the ability to conditionally express target genes within distinct lineages of the cardiovascular system will be expanded, limited only by the specificity of regulatory elements used to drive transcription of tTA and other chimeric transcription factors.

Outside the cardiovascular system, several laboratories have also reported success with tetracycline-regulated transgenic models. In two instances, genetic crosses with mice harboring a tetracycline-responsive simian virus 40 T-antigen target gene have resulted in conditional cellular transformation. When an insulin promoter was used to restrict expression of tTA to pancreatic islet beta cells, binary offspring developed infrequent islet cell tumors, from which cell lines were established with tetracycline-repressible proliferation. 33 A similar phenomenon was observed in submandibular glands of binary mice harboring the T-antigen target transgene and tTA under transcriptional control of the mouse mammary tumor virus long-terminal repeat. 33 In a particularly interesting result, conditional expression of a mutationally activated form of calcium-calmodulin–dependent kinase II in the forebrain resulted in reversible defects in memory formation. 35

Modified Tetracycline-Controlled Transactivators
Several variants of tTA have recently been generated in an effort to expand the utility of the regulatory system, particularly with respect to in vivo use. In a screen of random mutants within the repressor domain, one isolate was identified that rendered the protein paradoxically a transcriptional activator in the presence of tetracycline analogues, in contrast to the repression observed with tTA itself. 36 Since target gene induction with tTA requires metabolism and clearance of tetracycline, especially from bone, 37 de novo transcription follows with some temporal delay. Thus, the so-called rT TA has some theoretical advantages for studies when very rapid induction of target genes is desirable. 38 Although the rT TA approach has worked well in tissue culture applications, the experience to date in transgenics is limited to the original report describing successful transactivation of a reporter gene using a CMV-rT TA construct. Finally, T TA truncation mutants with graded transactivation potential have been described. 39 These variants, which have truncated VP16 transactivation domains, may minimize interactions with endogenous cellular transcription factors and reduce the likelihood of potentially toxic transcriptional squelching. The utility of these modifications for in vivo use has yet to be examined.

Hormone-Regulated Transgene Expression
Similar ligand-dependent strategies using hormone-responsive transcriptional activators to regulate target transgenes have been described. For example, a chimeric transactivator consisting of a mutated progesterone receptor ligand–binding domain, the yeast GAL4 DNA–binding domain, and the HSV VP16 transactivation domain has been shown to transactivate appropriate target genes only in the presence of mifepristone (RU486), a progesterone antagonist, at concentrations substantially lower than those that block the normal biological effects of progesterone. 40 By use of this approach, expression of human growth hormone was conditionally expressed in mouse liver, resulting in ligand-inducible growth enhancement. 41 The Drosophila ecdysone receptor, which normally heterodimerizes with ultraspiracle (a homologue of the mammalian retinoid X receptor) to mediate the biological activity of the steroid hormone 20-OH ecdysone, has been similarly modified, also resulting in hormone-dependent transactivation of appropriately engineered response genes. 42 Although the single study in transgenic mice suggests that ecdysone-regulated transgene expression compares quite favorably with tetracycline-controlled systems, to date, no reports of regulated expression of functional genes have appeared, 43 and additional experience will be required to directly compare these two approaches. Moreover, the potential exists for inappropriate sequestration of endogenous retinoid X receptor by the targeted ecdysone receptor and unanticipated phenotypic consequences.

Dimeric Ligands
To some extent, transcription factors have structurally distinct DNA-binding and activation domains, and site-specific transactivation may be reconstituted by close physical approximation of these two independent domains. This association may be facilitated by fusion of each domain to heterologous proteins that directly interact, as in the yeast two-hybrid system, 44 or by means of a ternary complex with a bridging dimeric ligand, as illustrated in Figure 4. Several such chemical inducers of dimerization, including synthetic compounds and natural products such as FK506 (which mediates the interactions of FKBP12 with calcineurin) and rapamycin (which mediates the interaction of FKBP12 with FRB, the FKBP12-rapamycin–binding domain of FRAP), have been examined for their ability to pharmacologically control gene expression. 45,46 For example, coexpression of chimeric constructs consisting of a site-specific DNA-binding domain fused to FKBP12 and the carboxy-terminal transactivation domain of nuclear factor-κB linked to FRB will effectively transactivate engineered target reporter genes in a rapamycin-dependent manner, 47 as schematized in Figure 4. Since rapamycin itself is growth inhibitory and immunosuppressive, nontoxic derivatives have been synthesized and compensatory mutations in FRB have been identified that still allow for ternary complex formation and transcriptional
activation. Although this approach has not yet been demonstrated in vivo, the potential utility of small dimeric ligands for regulation of transcription in intact animals is considerable.

Future Directions

Conditional Recombination

Substantial impetus for the development of temporally regulated gene expression systems has come from the results of recent gene-targeting experiments in the mouse, where loss-of-function mutations have highlighted the potential multiplicity of functions of individual gene products. For example, endothelin-1 is a 21–amino acid peptide originally characterized as a potent vasoconstrictor. Remarkably, homozygous mice with targeted disruption of the endothelin-1 locus display a range of cardiovascular malformations in pharyngeal arch–derived tissues and organs. Similarly, the connexin43 gene encodes a gap junction channel critical for electrotonic coupling and impulse propagation in the heart, yet mice lacking connexin43 develop right ventricular outflow tract obstruction, perhaps reflecting a defect in the migration of neural crest–derived cells during cardiac morphogenesis. However, although these loss-of-function mutations uncover unanticipated functions for various gene products, these early lethal outcomes confound studies of gene function during later stages of development. Moreover, since the heart is the earliest organ to develop, there may be a bias ascribing embryonic lethality to defective cardiogenesis even for genes with critical functions in a variety of tissues. Therefore, for genes that are widely expressed or with multiple time-dependent functions, strategies for circumscribed gene disruption, restricted in terms of lineage or the timing of inactivation, are required.

Already, substantial progress has been made with lineage-specific knockouts. Several groups have demonstrated the ability of Cre recombinase, expressed under the control of tissue-specific promoters, to catalyze site-specific recombination of appropriately engineered luxP-containing genomic target sites. The timing of tissue-specific recombination is subject to the same limitations of all conventional transgenic experiments; i.e., the onset of recombination is determined by the properties of the specific regulatory sequences controlling the recombinase. Since few promoters are active only in the mature organism, until very recently, it has been difficult to even envision techniques to specifically inactivate genes in the adult mouse. To impart temporal control on recombinase activity, several novel strategies have been explored, including delivery of Cre recombinase by viral vectors, where anatomically restricted recombination has been achieved using either replication-deficient adenoviral or HSV vectors. An alternative strategy has been to fuse Cre recombinase with modified ligand-binding domains of human steroid receptors, resulting in chimeric proteins with activities dependent on administration of synthetic hormone analogues. Although Cre recombinase has also been conditionally expressed using the tetracycline-regulated system, the efficiency of site-specific recombination varied substantially from tissue to tissue. Thus, although the relative efficiency and utility of any of these approaches remains to be determined, the ability to control precisely both the timing and location of genomic recombination promises to greatly extend the power of traditional gene-targeting strategies.

Knockins and Conditional Gene Expression

To achieve precise lineage-specific expression of heterologous genes, strategies using homologous recombination have recently been described whereby endogenous protein coding regions are replaced with foreign sequences, thereby ensuring that virtually all potential regulatory elements, even those quite distant from site of recombination, remain intact. Within the cardiovascular system, this so-called “knockin” strategy has been used to place Cre recombinase under the transcriptional control of the myosin light chain-2v locus, resulting in cardiac-restricted recombination of appropriate luxP-flanked genomic target sites. A similar knockin approach could be used to express chimeric transcription factors, such as tTA, under the transcriptional control of an endogenous locus. Inasmuch as the intracellular concentration of tTA and the corresponding likelihood for undesirable transcriptional squelching will vary as a function of the endogenous locus selected, the previously described tTA mutants with graded squelching may prove useful in this setting.

Finally, it is also conceivable that homologous recombination could be used to replace upstream transcriptional regulatory elements of a gene of interest with arrays of tetO sequences, thereby rendering expression of the modified locus dependent on the appropriate transcriptional activator and its ligand. This approach would allow one to conditionally regulate endogenous gene expression at the transcriptional level, a strategy that has significant theoretical benefits, including the creation of reversible null phenotypes that do not depend on dominant-negative approaches. Such a strategy has recently been described in vitro, where enhancer elements of the pancreatic elastase I gene were replaced with a tetO element, resulting in gene expression that was induced by tTA and repressible by tetracycline, while retaining cell-type specificity.

The ability to manipulate gene expression in the intact organism with precise control is becoming less of a theoretical concept and more of an experimental reality. The marriage of conditional gene expression systems with other existing and emerging gene-targeting technologies should provide a wealth of opportunity to study gene function, physiology, and pathophysiology in vivo. These advances in mouse molecular genetics are paralleled by equally remarkable triumphs in the ability to measure functional parameters in the intact mouse. Echocardiography and magnetic resonance imaging, hemodynamics, angiography, and electrophysiology of the mouse heart have all been described in recent years. If we are wise enough to exploit these new tools in a rational manner, we should expect to learn a great deal more about the molecular determinants of function and dysfunction in the intact organism.
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References
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