Many considerations make heart failure an appealing clinical candidate for gene therapy. Heart failure is an increasingly common clinical problem that is only partly mitigated by our current pharmacological therapy. In contrast to the decreasing mortality from coronary disease, heart failure deaths more than doubled from 1979 to 1995. In part, this may reflect the aging demographics of the US population, because the rates of new and recurrent heart failure events increase substantially with age. As with cancer, the poor prognosis of specific subsets of heart failure patients heightens interest in novel therapeutic approaches. This interest is further intensified, because the elderly population in which heart failure is most common has generally been excluded from consideration for cardiac transplantation. Moreover, in contrast to younger patients with more favorable prognoses, waiting years for vector improvements may not be a realistic option for the heart failure patient, and the fact that treatment with current vectors may preclude future administration because of overlapping immune responses may have little practical consequence. Although vector delivery is not a trivial problem, the well-circumscribed geography of the heart makes it an attractive target that benefits from a wealth of clinical interventional experience. A variety of catheter or...
surgical approaches to in vivo cardiac gene transfer show promise in animal and clinical studies. Although achieving “lifelong” transgene expression may be more feasible in this population than in infants with inborn errors of metabolism, there may also be clinical settings in which transgene expression would be required only during a period of defined risk, such as remodeling after myocardial infarction. Importantly, a variety of animal models of heart failure exist that reasonably reflect the human condition and have previously paved the way for clinical therapies.2,3 Moreover, a sophisticated array of clinical tools are currently available to evaluate objectively the functional consequences of any intervention. Thus, potential gene-based therapies for heart failure could be validated in realistic animal models and, when appropriate, rigorously analyzed in clinical trials for effects on both physiological and clinical endpoints.

Clinical success in any application of gene therapy will require 3 essential elements appropriate to the specific setting. First, a vector or packaging system is necessary for the genetic material that will be delivered. To a large extent, features of the vector determine the range of host cells that can be transduced, as well as the efficiency, level, and duration of transgene expression. Of note, only a few of the currently available vectors achieve efficient, high-level transgene expression in postmitotic cells, such as cardiomyocytes. These include recombinant adenoviruses (see below), adeno-associated viruses,4 and possibly lentivirus.5 The interested reader is referred to a more detailed discussion of available vector systems.4 Secondly, the vector must be delivered to the affected tissues. This poses a particularly formidable barrier in conditions with an extensively distributed phenotype and may be more achievable in conditions localized to one organ, such as the heart. Finally, an appropriate gene to be expressed in a particular clinical setting must be identified. Over the past decade, there have been substantial advances in all 3 of these areas that provide the basis for a renewed but cautious optimism that gene therapy may prove clinically useful in specific settings. However, it is important to acknowledge that the field of gene therapy has not yet proven its clinical value in any context. Moreover, this laudable long-term goal is likely to be achieved only through a logical progression of rigorous basic and clinical investigation. In this review, we will first highlight recent progress toward achieving effective, global cardiac gene transfer in vivo and then outline some of the molecular pathways being considered for gene therapy in heart failure.

**Cardiac Gene Delivery**

The feasibility of in vivo cardiac gene transfer by viral vectors has been consistently demonstrated over the last few years.7–9 Recombinant adenoviruses have been the most common vectors used in these initial studies largely because of their flexible packaging constraints and their ability to transduce nonreplicating cells. However, the robust immune response these vectors evoke suggests that clinical applications will likely require other vectors or further refined adenoviral systems. A number of mechanical approaches have been used to achieve cardiac gene transfer, as shown in Figure 1. Intracoronary catheter delivery of an adenovirus encoding β-galactosidase achieved transduction of ≈30% of the myocytes in the distribution of the coronary artery.10 Direct injection of adenovirus into the ventricular wall using an epicardial approach has also been shown to induce significant expression of reporter constructs; however, the expression was focal, and the injections within the myocardium caused needle damage.9–11 Intramyocardial delivery of adenovirus using an intraventricular approach with retroinfusion of coronary veins has also been used in larger animals yielding regional areas of transduction.12 In rodents, injection of an adenovirus carrying β-galactosidase into the pericardial sac transduced only the pericardial cell layers.13 The addition of collagenase and hyaluronidase together with the adenovirus led to a larger diffusion of the transgene activity within the ventricle.13 Effective therapy in heart failure will likely require a gene delivery method capable of globally transducing the myocardium. Using intracoronary perfusion in explanted hearts, Donahue et al14 reported highly effective gene transfer to the heart and identified critical parameters influencing the efficiency of adenoviral gene transfer.14 These included (1) the use of crystalloid solution as opposed to whole blood, (2) high coronary flow rate, (3) exposure time, (4) virus concentration, and (5) temperature. More recently, Donahue et al14 found that decreasing perfusate Ca2+ concentration, or pretreating with serotonin or bradykinin, significantly decreased the exposure time necessary to achieve widespread infection.15 To achieve diffuse cardiac gene transfer in vivo, we recently developed a catheter-based technique in rodents.16 In this approach, a catheter is inserted in the left ventricular apex and advanced beyond the aortic valve. A high-concentration adenoviral preparation is then injected through the catheter (Figure 1E) while the aorta and pulmonary artery are cross-clamped distal to the catheter tip for a period of 10 to 40 seconds. This method achieves grossly homogeneous transduction of cardiac myocytes throughout the left and right ventricles of the heart.16 More importantly, this technique can produce dramatic, transgene-specific physiological effects on ventricular function in vivo.16 The success of this approach likely reflects in vivo optimization of the parameters previously shown to be important for ex vivo gene transfer,14 as well as high-perfusion pressure that presumably allows the opening of capillaries and optimizes the myocardial area of virus exposure. Other investigators have confirmed the effectiveness of similar approaches in other animal models. Recently, Maurice et al17 used this technique to express β3 receptors in rabbit hearts; however, they only clamped the aorta (Figure 1D) and achieved predominantly epicardial transgene expression. By cross-clamping both the pulmonary artery and the aorta, the left ventricular end-diastolic pressure does not increase, because blood return to the left ventricle is minimal.16 This allows perfusion of the virus at relatively low downstream pressure, and the endocardium can be efficiently infected. Correlates of this method in humans have not yet been established. However, it is noteworthy that aortic occlusion during aortic valvuloplasty is well tolerated in generally ill patients for periods of time comparable with those required for gene transfer in animal models.18,19 Optimizing conditions for gene transfer in large animals and eventually humans will require substantial further investigation.

**Gene Targets**

Cardiac myocytes isolated from failing hearts are characterized by a number of abnormalities that affect excitation-contraction coupling. These include changes at the level of the sarcotubula, sarcoplasmic reticulum (SR), myofilaments, and mitochondria, all of which contribute to depressed contractile function and reserve. Identifying the mechanisms by which these changes contribute to the observed pathology is frequently confounded by simultaneous alterations in multiple signaling pathways in the
complex milieu of the failing heart. Targeting genes to the heart through somatic gene transfer allows us to assess the efficacy of highly specific interventions in models of heart failure. Recent work using gene transfer in animal models has helped identify potential molecular targets for therapy in heart failure.

**Calcium Handling**

In the heart, $[\text{Ca}^{2+}]$ is tightly regulated at several levels. The SR plays an important role in orchestrating the movement of calcium during each contraction and relaxation. As shown in Figure 2, excitation leads to the opening of voltage-gated L-type $\text{Ca}^{2+}$ channels, allowing the entry of a small amount of $\text{Ca}^{2+}$ into the cell. Through coupling of the L-type $\text{Ca}^{2+}$ channel and the SR release channels (ryanodine receptors), a larger amount of $\text{Ca}^{2+}$ is released, activating the myofilaments and leading to contraction. During relaxation, $\text{Ca}^{2+}$ is reaccumulated in the SR by the SR $\text{Ca}^{2+}$-ATPase pump (SERCA2a) and extruded extracellularly by the sarcolemmal Na/Ca exchanger. The contribution of each of these mechanisms toward lowering cytosolic $\text{Ca}^{2+}$ varies with species. In humans, $\approx75\%$ of the $\text{Ca}^{2+}$ is removed by SERCA2a and $\approx25\%$ by the Na/Ca exchanger. The $\text{Ca}^{2+}$ pumping activity of SERCA2a is influenced by phospholamban. In the unphosphorylated state, phospholamban inhibits the $\text{Ca}^{2+}$-ATPase, whereas phosphorylation of phospholamban by cAMP-dependent protein kinase and by $\text{Ca}^{2+}$-calmodulin–dependent protein kinase reverses this inhibition. Studies in cardiac muscle strips, trabeculae, or single cardiomyocytes from failing hearts show reduced systolic force, elevated diastolic force, and slowed relaxation, as well as prolonged $\text{Ca}^{2+}$ transient with elevated end-diastolic $[\text{Ca}^{2+}]$. A decrease in SR $\text{Ca}^{2+}$ ATPase activity and $\text{Ca}^{2+}$ uptake appears responsible for abnormal $\text{Ca}^{2+}$ homeostasis not only in animal models but also in human heart failure. Associated with a defective $\text{Ca}^{2+}$ uptake, there is a decrease in the relative ratio of SERCA2a/phospholamban in these failing hearts. Using transgenic and gene transfer approaches, increasing levels of phospholamban relative to SERCA2a in isolated cardiac myocytes significantly altered intracellular $\text{Ca}^{2+}$ handling by prolonging the relaxation phase of the $\text{Ca}^{2+}$ transient, decreasing $\text{Ca}^{2+}$ release, and increasing resting $\text{Ca}^{2+}$. These results support the hypothesis that an abnormal ratio of phospholamban to SERCA2a contributes significantly to abnormalities in $\text{Ca}^{2+}$ handling and contraction observed in failing ventricular myocardium, but leave unanswered the questions about the benefit that would be derived from restoring this ratio through gene transfer. In neonatal rat myocytes in vitro, overexpression of SERCA2a largely “rescued” the phenotype cre-
ated by increasing the phospholamban-to-SERCA2a ratio. More importantly, in human cardiomyocytes isolated from the left ventricles of patients with end-stage heart failure, gene transfer of SERCA2a resulted in an increase in both protein expression and pump activity and induced a faster contraction velocity and enhanced relaxation velocity, restoring these parameters to levels observed in nonfailing hearts. Furthermore, diastolic Ca\(^{2+}\) was decreased in failing human cardiomyocytes overexpressing SERCA2a, whereas systolic Ca\(^{2+}\) was increased and the frequency response was normalized. These in vitro models may not reflect the behavior of intact hearts. However, in an animal model of pressure-overload hypertrophy in transition to failure, in which SERCA2a protein levels and activity are decreased and severe contractile dysfunction is present, overexpression of SERCA2a by gene transfer in vivo (using the technique described above) restored both systolic and diastolic function to normal levels. Overexpression of SERCA2a decreased left ventricular size and restored the slope of the end-systolic pressure–dimension relationship, a load-independent parameter of contractility, to control levels. These recent studies provide strong evidence that overexpression of SERCA2a to rescue disturbed Ca\(^{2+}\) cycling and myocardial function of the failing heart is indeed possible and suggest the feasibility of cardiac gene transfer in failing hearts as a therapeutic modality. The effective SERCA2a/phospholamban ratio can also be normalized by decreasing or inhibiting phospholamban. Overexpression of an antisense phospholamban construct or a dominant-negative mutant of phospholamban has recently been shown to enhance SERCA2 activity. This is consistent with the observation that genetic ablation of phospholamban prevents the functional abnormalities otherwise seen in a mouse model of dilated cardiomyopathy. Of note, increased SR Ca\(^{2+}\) ATPase activity, however, achieved decreases in intracellular diastolic Ca\(^{2+}\) by increasing uptake into the SR and enhancing Ca\(^{2+}\) release. Thus, in addition to the contractile benefits of SERCA2a expression, diastolic Ca\(^{2+}\) is decreased, which may help prevent activation of signaling molecules, including calcineurin and stress-activated protein kinases (SAPKs) capable of inducing myocyte hypertrophy and cell death.

**β-Adrenergic Signaling**

Other pathways in excitation-contraction coupling also provide targets for intervention in heart failure. β-Adrenergic signaling defects, including downregulation of myocardial β-adrenergic receptors (β-AR), β-AR uncoupling, and upregulation of the β-AR kinase (β ARK1), are central features of human and animal heart failure. In isolated ventricular myocytes from a model of heart failure in the rabbit, adenoviral gene transfer of the human β2-AR or an inhibitor of β ARK1 led to the restoration of β-AR signaling and an increase in cytosolic cAMP levels. This study, along with the finding that overexpression of an inhibitor of β ARK1 prevents the development of cardiomyopathy in a murine model of heart failure, emphasizes the importance of β-adrenergic signaling defects in the pathogenesis of heart failure and raises the possibility that targeting this system may restore function in failing cardiomyocytes. However, stimulation of the β-adrenergic system induces an increase in intracellular cAMP that, when sustained, can be cardiotoxic and arrhythmogenic. It is possible that this mechanism may

![Figure 2](https://example.com/image2.png)
underlie the clinical observation that inotropic interventions that increase cellular cAMP increase mortality in chronic heart failure.47 In fact, a recent study found that in mice overexpressing β2-adrenergic receptors, development of heart failure was exacerbated when these mice were subjected to aortic stenosis.48 Moreover, the transgenic mice had more severe left ventricular dysfunction and higher incidence of premature deaths.54 Nevertheless, the critical role of the β-adrenergic pathway suggests further investigation of this pathway as a target for intervention despite the cautionary clinical and experimental experience of direct β-agonism.

Apoptosis
In response to specific stimuli, cells can activate intrinsic suicide pathways and undergo programmed cell death or apoptosis. Morphological and biochemical markers of apoptosis have been identified in a wide variety of cardiac conditions, including experimental50–51 and human heart failure,52–54 suggesting that these pathways may contribute to cardiomyocyte loss and cardiac dysfunction in heart failure. Cardiac-specific deletion of the signaling receptor subunit, gp130, leads to massive cardiac apoptosis and accelerated dilated cardiomyopathy after aortic banding,55 suggesting a functional role of apoptosis in heart failure that may represent an additional target for therapeutic intervention.56 In cardiomyocytes, manipulating a number of conserved pathways through somatic gene transfer can block apoptosis in response to a variety of stimuli. Overexpression of Bcl-2 through adenoviral gene transfer blocks p53-induced apoptosis in ventricular cardiomyocytes.57 This observation is consistent with the powerful protective effect of antiapoptotic Bcl family members in a variety of cell systems. In addition, a number of “viability factors” have been identified that can play an important role in modulating apoptosis. These include growth factors, such as insulin-like growth factor-I (IGF-I), which blocks apoptosis in many settings, including models of cardiac ischemia-reperfusion injury.58 The ability of IGF-I to block apoptosis is often dependent on activation of phosphatidylinositol (PI) 3-kinase59 and, in some systems, its downstream target, Akt.60 Adenoviral gene transfer of activated forms of PI 3-kinase and Akt can block hypoxia-induced cardiomyocyte apoptosis in vitro.61 There is also some evidence that the SAPKs (especially p38α) may be involved in cardiac apoptosis. Stimulation of p38α in cardiac myocytes induced apoptosis, which was abrogated by gene transfer of a dominant-negative p38α mutant.62 The ability to block cardiomyocyte apoptosis through somatic gene transfer with such vectors should allow us to examine the functional significance of specific pathways and apoptosis in general in animal models of heart failure to determine whether these pathways hold promise as targets for clinical intervention.63

Future Directions
Heart failure represents a growing clinical challenge in need of novel therapeutic approaches. Improvements in vector technology; cardiac gene delivery; and, most importantly, our understanding of the molecular pathogenesis of heart failure, prompt careful consideration of gene therapy for heart failure at this time. Several interventions, particularly those enhancing sarcoplasmic calcium transport, show therapeutic promise in animal models of heart failure and in myopathic cardiomyocytes derived from patients. In this effort, somatic gene transfer provides an important tool to help understand the relative contribution of specific pathways and validate molecular targets for therapeutic intervention, whether pharmacological or genetic. Nevertheless, bridging the gap between these basic investigative studies and clinical gene therapy remains a formidable and not insurmountable task. Early proof of concept experiments in rodents will need to be extended to large-animal models with clinical-grade vectors and delivery systems to assess both efficacy and safety. On the basis of a foundation of rigorous science and a growing understanding of heart failure pathogenesis, there is reason for cautious optimism for the future.

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