This Review is part of a thematic series on Gene Expression in Hypertrophy and Stress, which includes the following articles:

Gene Expression in Fibroblasts and Fibrosis: Involvement in Cardiac Hypertrophy
Molecular Mechanisms of Csx/Nkx2-5 Involvement in Cardiac Hypertrophy
Ras, Akt, and Mechanotransduction in the Cardiac Myocyte
G Protein–Coupled Signaling and Gene Expression
Genetic Models and Mechanisms of Transcription in Cardiac Hypertrophy
Calcineurin Pathway

Ryozo Nagai, Guest Editor

Gene Expression in Fibroblasts and Fibrosis
Involvement in Cardiac Hypertrophy
Ichiro Manabe, Takayuki Shindo, Ryozo Nagai

Abstract—Structural remodeling of the ventricular wall is a key determinant of clinical outcome in heart disease. Such remodeling involves the production and destruction of extracellular matrix proteins, cell proliferation and migration, and apoptotic and necrotic cell death. Cardiac fibroblasts are crucially involved in these processes, producing growth factors and cytokines that act as autocrine and paracrine factors, as well as extracellular matrix proteins and proteinases. Recent studies have shown that the interactions between cardiac fibroblasts and cardiomyocytes are essential for the progression of cardiac remodeling. This review addresses the functional role played by cardiac fibroblasts and the molecular mechanisms that govern their activity during cardiac hypertrophy and remodeling. A particular focus is the recent progress toward our understanding of the transcriptional regulatory mechanisms involved. (Circ Res. 2002;91:1103-1113.)

Key Words: transcription ■ fibrosis ■ myofibroblast ■ cardiac remodeling

Regardless of the origin, injury to the heart evokes a diverse and complex array of cellular responses involving both cardiomyocytes and nonmuscle cells that initiate and sustain a process of structural remodeling of the myocardium.1 The importance of the structural remodeling has been addressed in randomized clinical trials.2 For example, angiotensin-converting enzyme (ACE) inhibitors, which may delay and, in some cases, reverse cardiac remodeling, have proven to have a beneficial effect on morbidity and mortality in heart failure. Thus, remodeling is now generally accepted as a key determinant of the clinical course of heart failure.2

Cardiac remodeling is manifested clinically as changes in the size, shape, and function of the heart.2 Histopathologically, it is characterized by a structural rearrangement of components of the normal chamber wall that involves cardiac myocyte hypertrophy, cardiac fibroblast proliferation, fibrosis, and cell death.3 Fibrosis, which is a disproportionate accumulation of fibrillar collagen, is an integral feature of the remodeling characteristic of the failing heart. Accumulation of type I collagen, the main fibrillar collagen found in cardiac fibrosis, stiffens the ventricles and impedes both contraction and relaxation.4 Fibrosis can also impair the electrical coupling of cardiomyocytes by separating myocytes with extracellular matrix (ECM) proteins.3 Furthermore, fibrosis results in reduced capillary density and an increased oxygen diffusion distance that can lead to hypoxia of myocytes.5 Thus, fibrosis profoundly affects myocyte metabolism and performance and ultimately ventricular function.6

In the myocardium, ECM proteins are mainly produced by fibroblasts that also produce matrix metalloproteinases
(MMPs), growth factors, and cytokines, all of which are involved in the maintenance of myocardial structure, and in diseased hearts play pivotal roles in remodeling. In this review, we present an overview of the mechanism that controls the functions of cardiac fibroblasts in cardiac remodeling with a special emphasis on transcriptional regulation of gene expression.

**Cardiac Interstitium and ECM Regulation**

The cardiac interstitium, ie, the space between the cardiomyocytes, contains fibroblasts, blood vessels, lymphatic vessels, adrenergic nerve endings, and ECM. The myocardial ECM is made up of a fibrillar collagen network, a basement membrane, proteoglycans, and glycosaminoglycans and contains a diverse array of bioactive signaling molecules. The major ECM proteins are type I and III collagens, although type IV, V, and VI collagens, as well as elastin are also present. The fibrillar collagen network ensures the structural integrity of the adjoining myocytes, provides the means by which myocyte shortening is translated into ventricular pump function, and is essential for maintaining alignment of the myofibrils within the myocytes through a collagen-integrin-cytoskeletal myofibril relation.

The disproportionate increase in synthesis and/or inhibition of degradation of ECM proteins would result in fibrosis. Fibrosis has been classified into two groups: reparative and reactive fibrosis. Reparative (replacement) fibrosis or scarring accompanies myocyte death. Reactive fibrosis appears as “interstitial” or “perivascular” fibrosis and does not directly associate with myocyte death. In interstitial fibrosis, fibroblast collagen appears in intermuscular spaces. Perivascular fibrosis refers to the accumulation of collagen within the adventitia of intramyocardial coronary arteries and arterioles. “Focal” and “diffuse” are also used to describe the distribution of fibrosis. Although there are a number of apparent differences between reparative and reactive fibrosis (eg, cells involved and the time course of fibrotic change), many factors likely work in common to control fibroblast function (discussed subsequently).

Collagens are degraded by a family of MMPs capable of enzymatically digesting a wide variety of ECM proteins. The activity of MMPs is controlled at the transcriptional level as well as through activation and inhibition by other proteins including tissue inhibitors of MMPs (TIMPs). Cardiac fibroblasts produce both ECM proteins and MMPs, thus playing a central role in the regulation of ECM.

The expressions of both ECMs and MMPs change dynamically during the developmental process of heart failure. For instance, loss of collagen and enhanced MMP activity begin within minutes of the onset of myocardial ischemia. This rapid initial loss of collagen is followed by a rapid and progressive increase in collagen and fibronectin gene expression. MMPs seem to be involved in several aspects of infarct healing processes, including early ECM degradation, cell migration of inflammatory cells and fibroblasts, angiogenesis, remodeling of newly synthesized connective tissue, and the regulation of growth factor activities. Progressive activation of MMPs has also been demonstrated to accompany the progression of left ventricular (LV) dilation and dysfunc-

tion. Moreover, increased MMP activity was found in the hearts of patients with ischemic and dilated cardiomyopathy.

Recent studies using genetic and pharmacological manipulation of collagens and MMPs have further demonstrated that the balance between matrix synthesis and degradation plays an essential role in the maintenance of the integrity of the myocardium. Disruption of this balance would lead to cardiac remodeling and dysfunction. Cardiac-specific overexpression of MMP-1 (interstitial collagenase) resulted in structural changes in ECM and marked deterioration of systolic and diastolic function. Disruption of MMP inhibitor control by TIMP-1 gene knockout resulted in LV dilation. MMP-9 (gelatinase B) gene knockout mice showed attenuation of LV enlargement and collagen accumulation after myocardial infarction. MMP inhibitor treatment of pigs with chronic rapid pacing and spontaneously hypertensive heart failure rats attenuated the degree of LV dilation and improved LV function.

ECM remodeling in the processes of the progression and repair of myocardial infarction has also been investigated using mouse models. For instance, urokinase-type plasminogen activator (u-PA) deficiency protected against rupture at the acute phase. However, the u-PA knockout mice showed impaired scar formation. Reduced leukocyte infiltration and angiogenesis were observed in the infarcted region. In a separate study, the lack of wound healing and infiltration of inflammatory cells were observed in plasminogen knockout mice. Therefore, although suppression of ECM degradation may inhibit acute myocardial rupture, it could impede the normal healing processes later. Collectively, these studies have clearly demonstrated the importance of dynamic regulation of the synthesis and degradation of ECM in the repair of acute cardiac damage and subsequent development of cardiac remodeling.

**Cardiac Fibroblasts and Myofibroblasts**

In the normal heart, two thirds of the cell population is composed of nonmuscle cells, the majority of which are fibroblasts. External stress causes fibroblasts to change their phenotype. These altered cells are referred to as myofibroblasts because they express several smooth muscle (SM) markers, including SM α-actin, SM22α, SMemb/nonmuscle myosin heavy chain-B, and tropomyosin. However, more stringent SM markers (eg, SM myosin heavy chains) are not expressed in myofibroblasts.

With the exception of heart valve leaflets, myofibroblasts are not found in normal cardiac tissue. Upon injury, myofibroblasts appear in the myocardium and are generally believed to arise from resident interstitial and/or adventitial fibroblasts. Their origin is not yet completely clear; however, they might also originate from progenitor stem cells. Given that recent reports suggest that stem cells in local tissues and bone marrow may be involved in various diseases, the differentiation of cardiac myofibroblasts from stem cells cannot be ruled out. In any case, a growing body of evidence indicates that by producing growth factors, cytokines, chemokines, ECM proteins, and proteases, myofibroblasts play a pivotal role in inflammation, tissue repair, fibrosis, and organogenesis.
As summarized in the Table, a number of genetically engineered mouse models have been reported to have cardiac fibrosis. Of note, cardiac fibrosis developed in the myocardium of both hypertrophy and dilated cardiomyopathy models. Therefore, the mechanisms of fibrosis induction in these animals appear to be divergent. In some cases, the alterations in target gene function per se may have induced fibrosis. In others, the deterioration of myocardial function due to the genetic manipulation may have secondarily caused fibrosis.

In mice with cardiac-specific overexpression of Fas and tumor necrosis factor-α (TNF-α), inflammatory cells likely activated fibroblasts. These studies indicate that even if the dysfunction is initially confined within nonfibroblasts (eg, cardiomyocytes), disturbances in myocardial function would lead to fibroblast activation. Humoral factors, ECM proteins, and adhesion molecules mediate the interaction between cardiac fibroblasts and other cell types.

**Autocrine and Parocrine Factors Controlling Cardiac Fibroblasts**

Humoral factors that affect the phenotype and function of cardiac fibroblasts include angiotensin II (Ang II), basic fibroblast growth factor (bFGF/FGF-2), transforming growth factor-β (TGF-β), catecholamines, and insulin-like growth factor-1 (IGF-1). Among these factors, Ang II appears to be one of the most important regulating cardiac fibrosis and remodeling, although other factors are also important, as clearly demonstrated in recent studies in which pressure overload caused cardiac hypertrophy and fibrosis in type 1a Ang II (AT1a) receptor knockout mice. Evidence suggests that Ang II synthesis and degradation in the heart is compartmentalized and mediated by mechanisms different than those in intravascular spaces. Furthermore, most components of the renin-angiotensin-aldosterone (RAA) system are present in the heart and are produced locally, although the extent of local production of the RAA system components and how they are produced still remains somewhat controversial. Regardless of the exact mechanisms, the RAA system appears to be upregulated by mechanical stretch and other insults to the myocardium within which Ang II acts as an autocrine/paracrine factor critically affecting the progression of hypertrophy and remodeling.

Recent studies have also demonstrated the local production of aldosterone in the heart and the expression of mineralocorticoid receptors in cardiac cells, suggesting a paracrine function for aldosterone in the heart. This is particularly interesting, because a clinical study, Randomized Aldactone Evaluation Study (RALES), showed that treatment of patients...
with spironolactone, an antagonist of the aldosterone receptor (mineralocorticoid receptor), improved both morbidity and mortality in heart failure patients. Subsequently, this beneficial effect of spironolactone was shown to be at least partly a result of the reduced ECM turnover.

Ang II acts via receptors that are members of the G protein–coupled receptor (GPCR) superfamily. There are two types of Ang II receptors: type 1 (AT₁) and type 2 (AT₂). Most of the known physiological effects of Ang II are mediated through the AT₁ receptor. Binding of Ang II to the AT₁ receptor leads to activation of well-defined G protein–linked pathways, such as activation of phospholipase C (PLC), which causes the release of Ca²⁺ and subsequent activation of calmodulin kinase and protein kinase C (PKC). In addition to these classical pathways, AT₁ receptors can activate tyrosine kinases, such as Jak2, Tyk2, c-Src, and Pyk2, and can transactivate cell surface receptors possessing an intrinsic tyrosine kinase domain (RTKs), in particular the EGF receptor. This effect is at least partly mediated by heparin-binding EGF-like growth factor (HB-EGF). Importantly, Ang II may activate different signaling pathways in cardiac fibroblasts than in cardiomyocytes. Zou et al recently reported that in cardiac fibroblasts, Ang II activated extracellular signal-regulated kinases (ERKs) through a pathway including the Gₛ subunit of Gₛ and tyrosine kinases (eg, Src, Ras, and Raf), whereas Gₛ and PKC are important in cardiomyocytes. These differences in signal activation may lead to differential activation of genes in these two cell types.

In certain cases, the AT₂ receptor has been shown to counterregulate the AT₁ receptor effects. AT₂ receptor stimulation suppresses the growth of cardiomyocytes and cardiac fibroblasts. AT₂ receptor expression is upregulated in human failing hearts mainly in cardiac fibroblasts. Therefore, it has been suggested that selective block of the AT₁ signal might have an additional beneficial effect in heart failure via AT₂ receptors. However, previous studies have also provided conflicting results regarding the role of the AT₂ receptor. AT₂-deficient mice showed reduced cardiac fibrosis in pressure overload and chronic Ang II infusion. Therefore, the role of AT₂ may depend on other signals, including that via AT₁.

In vitro studies of cultured cardiac fibroblasts have shown that Ang II directly stimulates fibroblast proliferation, collagen synthesis, and the expression of ECM proteins (collagen, fibronectin, and laminin) via AT₁ receptors. Ang II also regulates cardiac fibroblast function indirectly through induction of TGF-β, endothelin-1 (ET-1), IL-6, and osteopontin, which function in autocrine and paracrine manners. Recent studies suggest that this indirect effect may be the major mechanism by which Ang II controls cardiac fibroblasts. This mechanism has been directly addressed in recent studies using knockout mice. TGF-β–deficient mice subjected to chronic subpressor doses of Ang II showed no significant LV hypertrophy or fibrosis. In another study, an Ang II–dependent renovascular hypertension model was applied to FGF-2– (bFGF) deficient mice. In these mice, compensatory hypertrophy and fibrosis were abolished, at least partly because of the deficiency in the paracrine function of cardiac fibroblasts. That is, FGF-2–/– cardiac fibroblasts had a defective capacity for releasing growth factors, including FGF-2, to induce hypertrophic responses in cardiomyocytes. These studies clearly demonstrate the importance of cell-cell communication in cardiac remodeling.

Cell-Cell Communication in Cardiac Remodeling

As discussed above, acute injury to the myocardium activates the RAA system. For example, mechanical stretch of cardiomyocytes induces the release of Ang II, which in turn stimulates the release of multiple growth factors and cytokines from cardiac fibroblasts that affect both the fibroblasts themselves and other cell types, including cardiomyocytes (Figure). Although the paracrine factors released by cardiac fibroblasts are required for induction of hypertrophic responses in cardiomyocytes, the paracrine activities of cardiomyocytes are also crucially important for regulating the function of cardiac fibroblasts. For example, Matsusaka et al generated chimeric mice that expressed both AT₁ receptor–intact and AT₁ receptor–null cells. Infusion of Ang II caused mild cardiac hypertrophy and fibrosis in the chimeric mice, and importantly most of the proliferating fibroblasts were found to be surrounding cardiomyocytes carrying the intact AT₁ gene. Fibroblasts adjacent to AT₁-null myocytes showed a lesser degree of cell proliferation. Consistent with these findings, chimeric mice carrying cells overexpressing Gₛ protein and wild-type cells showed clustered fibrosis and hypertrophy in loci predominantly containing myocytes overexpressing Gₛ again indicating the importance of local communication between fibroblasts and myocytes, presumably via a paracrine mechanism. Finally, cardiac fibrosis is observed in several transgenic mouse lines specifically overexpressing genes in cardiomyocytes (Table).

Other cell types, including endothelial cells, pericytes/smooth muscle cells (SMCs), and immune cells are also involved in the cell-cell communication mediating the pathogenesis of cardiac remodeling. The myocardium contains a dense network of blood vessels, and all of the muscle fibers are situated in close proximity to the vessels. As evident in the pathogenesis of atherosclerosis and other chronic inflammatory conditions, endothelial cells act as a mediator of inflammatory responses by promoting the infiltration of inflammatory cells and interacting with the surrounding SMCs and pericytes. It is therefore likely that cells comprising the blood vessels and blood cells are also involved in the pathogenesis of cardiac remodeling.

The role of inflammatory cells has been extensively studied in myocardial infarction. In reperfused myocardial infarction, inflammatory cells are involved in both myocardial injury and healing. Ischemic myocardial injury activates the complement cascade, production of reactive oxygen species, and cytokine cascade triggered by mast cell–derived mediators, all of which directly affect the function and fate of cardiac cells as well as recruit inflammatory cells. The major cell type first recruited to the reperfused area is the neutrophil. Subsequently, monocytes and lymphocytes infiltrate the infarcted myocardium in the first few hours of reperfusion. Histamine and TNF-α released from mast cells...
induce IL-6 in myocytes and mononuclear cells. IL-6, in turn, induces ICAM-1 expression in myocytes, leading to ligand-specific adhesion of neutrophils that injure myocytes. During the healing phase, the infarcted tissue is repaired and remodeled by degradation and production of matrix, angiogenesis, and cell apoptosis and proliferation. Macrophages and mast cells accumulate in the healing scar and secrete a variety of growth factors and cytokines, inducing fibroblast proliferation. Fibroblasts become myofibroblasts that produce ECM proteins and growth factors. In the healing phase, angiogenesis is also induced by a variety of angiogenic factors, such as VEGF, bFGF, and IL-8. As such, the complex processes of tissue repair and remodeling in myocardial infarction are mediated by a network of molecules, such as cytokines and growth factors. In the network, these factors interact synergistically and antagonistically with each other and have pleiotropic effects depending on cellular, spatial, and temporal variables. Therefore, cytokines and other inflammatory mediators that play an injurious role in the early stages of the inflammatory response may also be necessary as regulators of cardiac repair.

Taken together, the networks of various molecules and cells control the complex processes of tissue injury, repair, and remodeling in cardiac diseases. The functional role of each player in the networks is highly dependent on environmental variables. Therefore, it would be important to address the function of each molecule in the context of networks for understanding of the molecular mechanisms of cardiac remodeling as well as the development of novel therapeutics.

Transcriptional Control of Cardiac Remodeling

Injury to the myocardium evokes multiple intracellular signaling pathways that ultimately lead to changes in gene expression, which are largely conducted by transcription factors and cofactors. The first group of transcription factors activated by external stress (e.g., growth factors, cytokines, hypoxia, and mechanical stretch) includes products of immediate early response genes, such as AP-1, NF-κB, Egr-1, and Stat3. Activation of these transcription factors leads to sequential transcriptional control events that coordinately change gene expression and control the function of cells responding to environmental cues. In the next section, we present an overview of the transcription factors activated at the immediate early stage and later stages. After this overview, we discuss specific gene regulation by the cooperative interaction between transcription factors.

Immediate Early Response Genes

Activating Protein-1 (AP-1)

AP-1 is activated by diverse environmental cues and is involved in numerous cellular functions. It often functions...
primarily to control cell proliferation and apoptosis. Ap-1 proteins are homodimers and heterodimers composed of basic region-leucine zipper (bZIP) proteins, which include Jun, Fos, and Jun dimerization partners and activating transcription factors. External stimuli can differentially activate stimulus- and cell type-specific sets of AP-1 proteins. It is likely that the different AP-1 dimers execute specific cellular programs.

In cardiac fibroblasts, mechanical stretch, Ang II, and hypoxia have all been shown to activate AP-1. Ang II directly induces c-fos, c-jun, and JunB expression in cardiac fibroblasts. Potential AP-1 binding sites have been identified in the transcriptional regulatory regions of numerous genes, including transcription factors, ECM proteins, MMPs, cell adhesion molecules, growth factors, cytokines, and cyclins. In cardiac fibroblasts, the potential target genes identified so far include collagen, fibronectin, ICAM, and VCAM. It is very likely that AP-1 also controls genes involved in the cell cycle and apoptosis in cardiac fibroblasts.

**Nuclear Factor-κB (NF-κB)**

NF-κB is a redox-sensitive transcription factor that controls a number of genes involved in inflammation and apoptosis; it mediates cell survival or apoptosis depending on the cell type and environment. NF-κB consists of homodimers or heterodimers of NF-κB/Rel family proteins. In unstimulated cells, NF-κB is bound by an inhibitory IκB family protein and is retained within the cytoplasm, where it can be activated by a wide range of stimuli, including reactive oxygen species (ROS), hypoxia, hyperoxia, cytokines, growth factors, bacterial and viral products such as lipopolysaccharide (LPS), and UV radiation. Cell stimulation leads to degradation of IκB. Free NF-κB translocates into the nucleus and works as a transcription factor.

NF-κB target genes include proinflammatory cytokines, chemokines, leukocyte adhesion molecules, MMPs, NO synthase, and antiapoptotic factors. In cultured cardiac fibroblasts, NF-κB has been shown to be activated by hypoxia, TNF-α, and IL-1β. and its potential target genes include ICAM, VCAM, and monocyte chemoattractant protein-1 (MCP-1). It may also induce expression of angiotensinogen, AT1 receptor, and IL-6.

**Early Growth Response Factor-1 (Egr-1)**

Egr-1 is a zinc-finger transcription factor that is rapidly and transiently activated by growth factors and other injurious stimuli. In cardiac fibroblasts, Egr-1 is induced by Ang II, EGF, and IGF-1. Once expressed, Egr-1 has been shown to control a battery of genes important for cell growth and inflammatory responses. In cardiac fibroblasts, Egr-1 has been shown to induce PDGF-A and KLFS. It is also known to control PDGF-B, FGF-2, TNF-α, IL-2, tissue factor, plasminogen activator, macrophage colony stimulating factor (M-CSF), apolipoprotein A1, ICAM-1, and NF-κB in other cells.

**Signal Transducers and Activators of Transcription (Stat)**

Janus kinase (Jak) and Stat proteins are found mainly coupled with cytokine receptors. However, recent studies have shown Jak/Stat to be involved in other receptors, including receptor tyrosine kinases (eg, PDGF receptor) and GPCRs (eg, AT1 receptor). Upon ligand binding, Jaks phosphorylate Stat proteins, resulting in their rapid translocation to the nucleus, where they function as specific transcription factors.

There are seven Stat proteins, many of which can be activated in cardiac cells. Stat3 is unique among Stats in that it appears to be involved in multiple signaling pathways and biological activities. Stat1 and Stat3 is activated by cytokines that signal through gp130 (eg, IL-6, LIF, and CT-1). The importance of the gp130/Stat3 pathway in heart disease was clearly demonstrated by recent studies with transgenic and knockout mice. Continuous activation of gp130 signaling resulted in cardiac hypertrophy, whereas cardiac-specific ablation of gp130 caused massive apoptosis of cardiomyocytes and dilated cardiomyopathy. Activation of Stat3 and other Stats in the heart has been documented in acute infarction, ischemic preconditioning, and pressure overload. In both cultured cardiac myocytes and fibroblasts, the Jak/Stat pathway is activated by Ang II. On the other hand, Stat3 activates transcription of angiotensinogen via direct binding to its promoter, suggesting a close interaction between Ang II and the Jak/Stat pathways. Ang II also induces expression of IL-6, CT-1, and LIF in cardiac fibroblasts, which in turn activate Jak/Stat in adjacent cardiomyocytes. In ischemia preconditioning of the myocardium in vivo, Stat1 and Stat3 expression is reduced.

**Transcription Factors Activated at the Later Stages**

**Smad**

TGF-β participates in the regulation of cell proliferation, differentiation and migration, apoptosis, and ECM production. Moreover, TGF-β plays a central role in the development of fibrosis in a variety of chronic inflammatory conditions. For instance, it stimulates fibroblast chemotaxis and the production of collagen and fibronectin while inhibiting collagen degradation. It can also induce expression of SM-α-actin in fibroblasts and is thus considered to be one of the factors responsible for myofibroblast formation.

In the heart, TGF-β is induced by pressure overload, infarction, and Ang II infusion, and this upregulation persists for at least 4 to 8 weeks, during which concomitant expression of fibronectin and collagen is observed. Cardiac specific overexpression of TGF-β resulted in cardiac hypertrophy and fibrosis. Neutralization of TGF-β by anti-TGF-β antibody resulted in reduced fibrosis in pressure-overloaded rats. Furthermore, TGF-β knockout mice showed no hypertrophic responses to subpressor doses of Ang II. TGF-β can activate multiple signaling pathways, including mitogen-activated protein kinase (MAPK) pathways, but the Smad pathway is thought to be the predominant one. After ligand activation, activated TGF-β receptor phosphorlates R-Smads (Smad2 and Smad3), which in turn associate with a Co-Smad (Smad4) and are translocated into the nucleus where they act as transcription factors.
Transcriptional control by Smads involves interactions with other transcription factors. For instance, analysis of COL1A2 demonstrated that responsiveness to TGF-β is governed by the overlapping potential cis-elements for Smad, AP-1, and NF-κB. Association with coactivators and corepressors is also critically important for the function of Smads. Indeed, Smads can either activate or repress target genes depending on the binding partners.

Changes in Smad expression have been associated with heart diseases, for instance, the levels of Smad2 and Smad4 are upregulated in scar tissue after myocardial infarction. This effect is attenuated by the AT1 receptor blocker losartan, suggesting the presence of crosstalk between Ang II and Smad signaling. Conversely, Smad7, an I-Smad that inhibits phosphorylation of R-Smads, is downregulated in scar tissues after myocardial infarction. Interestingly, Smad7 expression is induced by the proinflammatory cytokines interferon-γ (IFN-γ) and TNF-α via NF-κB and Stat in at least some cell types, suggesting a crosstalk between signaling pathways important for chronic inflammation.

Kruppel-Like Factor (KLF)
Kruppel-like factors are a family of transcription factors that contain three Kruppel-like C2H2-type zinc finger domains and have diverse functions in normal development and disease. Several KLFs are crucially involved in the function of cells of mesenchymal origin: KLF2/LKLF is required for the investment of SMCs in the vascular wall; KLF5/BTEB2/IKLF is required for the investment of SMCs in the function of cells of mesenchymal origin; KLF2/LKLF is required for the investment of SMCs in the vascular wall; KLF5/BTEB2/IKLF is required for the investment of SMCs in the function of cells of mesenchymal origin; KLF10/TIEG1 and KLF11/TIEG2 are both induced by KLF5 in the activation of SMCs and fibroblasts. Furthermore, KLF2/LKLF binds to the promoter of c-fos, a marker of phenotypic modulation of SMCs, is downregulated in mature cells, and is reexpressed in phenotypically modulated cells present in neointimal lesions in rats and in atherosclerotic lesions in human coronary arteries. We recently identified KLF5 as a transcription factor that binds to the promoter of Smemb, a marker of phenotypic modulation of SMCs. KLF5 is abundantly expressed in embryonic vascular SMCs, is downregulated in mature cells, and is reexpressed in phenotypically modulated cells present in neointimal lesions in rats and in atherosclerotic lesions in human coronary arteries. We also noted that KLF5 was expressed in activated fibroblasts (myofibroblasts).

We generated lines of KLF5 knockout mice. Homozygous mice died in utero at a very early stage of embryonic development. KLF5−/− mice are viable and apparently normal. The cardiovascular system showed relatively minor abnormalities. However, KLF5−/− mice demonstrated attenuated responses to cardiovascular injuries. Vascular injury resulted in much less neointimal formation and reduced reactions in the adventitia of KLF5−/− mice. KLF5−/− mice also showed reduced cardiac hypertrophy and fibrosis by Ang II infusion, indicating its important role in cardiac fibroblasts. These results clearly demonstrate an important role played by KLF5 in the activation of SMCs and fibroblasts. Furthermore, KLF5 is induced by Ang II via the MEK pathway. Once expressed, KLF5 directly controls expression of PDGF-A and TGF-β. As such, KLF5 appears to be a key element linking external stress and cardiovascular remodeling.

Nuclear Receptor (NR)
Nuclear receptors are a group of ligand-inducible transcription factors that include receptors for steroid hormones (glucocorticoids and estrogens), nonsteroidal ligands such as retinoids, and various lipid metabolites. A number of NRs are expressed in the cardiovascular system, and their respective functions in cardiovascular disease have been extensively studied.

In addition to activation and repression via direct binding to target sites, NRs can affect the activity of other classes of transcription factors. For example, ligand-coupled glucocorticoid receptors can inhibit the transcriptional activities of AP-1 and NF-κB without directly binding to the DNA. Such transrepression is considered to be the major mechanism by which glucocorticoids inhibit inflammatory gene expression. Transrepression is not limited to glucocorticoid receptors; however, the activity of KLF5 is transrepressed by retinoic acid receptors, and those of AP-1, STAT, and NF-κB are transrepressed by peroxisome proliferator-activated receptors (PPAR-γ). Thus, NRs appear to be critically involved in chronic inflammatory responses by modifying the functions of other transcription factors.

Context-Dependent Combinatorial Transcriptional Regulation in Cardiac Remodeling
Injury to the myocardium activates a number of transcription factors reviewed so far. These transcription factors, particularly immediate early factors, are activated in many cell types and induce expression of genes generally required for stress responses. Still, activation of these transcription factors also leads to the coordinate control of genes specifically required for the function of a given cell type. Thus, a key question is how do these transcription factors control specific sets of genes in a specific cell type in a context-dependent manner? An interesting feature of the promoters of stress-responsive genes is that they often contain binding sites for multiple immediate early factors. For example, the c-fos promoter is controlled by both AP-1 and Stat, IL-8 by AP-1 and NF-κB, and angiotensinogen by NF-κB and Stat. Although AP-1 per se may not have specificity for a particular target gene, interactions at composite regulatory elements likely produce protein complexes with a high degree of sequence and regulatory selectivity. Recent studies have demonstrated that combinatorial transcriptional control of such genes as IFN-β and TNF-α involves formation of higher-order nucleoprotein complexes called enhanceosomes. Enhanceosomes have important features that enable transcription factors to control context-dependent transcription. First, the binding of all required transcription factors is necessary for enhanceosome activation. Second, the activity of enhanceosomes is not determined by the simple linear sum of that of individual transcription factors, but rather by the highly synergistic interaction between transcription factors and cofactors involved. As a result, the activation of enhanceosomes requires inputs from multiple signaling pathways, each of which leads to activation of a different set of transcription factors. For instance, stimulation that activates an individual subset of transcription factors does not turn on IFN-β; activation of all relevant signaling pathways is required before IFN-β is transcribed. Although enhanceosomes have been formally analyzed for only a limited number of genes,
they are almost certainly responsible for transcriptional control of many others.

Another important feature of the transcriptional control mechanism that has been revealed recently is that gene transcription is controlled by multiple cis-regulatory modules, each executing one of the functions of the entire regulatory program. One example involving fibroblasts is the control expression of type I collagen. Type I collagen is synthesized by multiple cell types, including fibroblasts, osteoblasts, and odontoblasts. Analysis in transgenic mice showed that type I collagen genes were controlled by multiple regulatory modules that were spread over large genomic regions (more than several kilobases) and differentially required in the different types of cells. In other words, different regions (more than several kilobases) and differentially regulated expression of the gene in different cells, associated with cardiac hypertrophy, myocardial infarction, and other diseases. This modularity of the transcriptional regulatory system likely enables the system to respond to divergent environmental cues.

A view on transcriptional regulation in vivo emerging from these studies is that genes are controlled by the network rather than by individual transcription factors. The network of factors participating in the transcriptional control is linked with the network of signaling molecules. In this way, cells control gene expression in response to changing environmental cues. Analysis of these networks likely requires new technologies that enable us to determine pathways linking factors and to evaluate the network experimentally and computationally. In this regard, global mRNA and protein expression analysis and various bioinformatics techniques will become increasingly important.

Microarray analysis has identified transcriptional changes associated with cardiac hypertrophy, myocardial infarction, cardiomyopathy, and heart failure in human and animal models. Genome wide expression analysis has also been applied to map the effects of upregulation or downregulation of genes in mice. Redfern et al. generated a conditional expression of a G-coupled receptor Rho1 mouse model, in which Rho1 expression can be regulated by drug administration in a cardiomyocyte-specific manner. In this study, the changes in gene expression caused by Rho1 expression were mapped to the G protein signaling cascade and other functionally grouped molecules. This analysis revealed a complex feedback regulation of each G protein signaling pathway and interactions between the pathways. A number of genes involved in fibrosis were also reported to be modulated in the study. When linked with other databases, such as those of promoter structures and protein interactions, expression profiling would potentially provide us valuable data with which to analyze the network of signaling and transcriptional regulation.

We did not discuss the involvement of chromatin structure in transcriptional regulation in this review, although clearly chromatin remodeling profoundly affects gene transcription. For instance, chromatin remodeling is essential for transcription of SM α-actin. Chromatin remodeling is also crucially important for the activation of enhanceosomes.

Analysis of the complex interaction between protein, DNA, and chromatin is clearly important for elucidation of the molecular mechanism that regulates dynamic gene expression during the processes of cardiac remodeling and heart failure. It would also provide us novel therapeutic targets for heart diseases. For instance, if the interaction but not the expression of transcription factors is specific to control a gene, it might be possible to develop a drug targeting the interaction.

**Conclusions**

Cardiac fibroblasts, along with cardiomyocytes, play an essential role in the progression of cardiac remodeling. Injuries to the heart evoke multiple signaling pathways in cardiac cells that lead to coordinate and sequential gene regulation. Initial transcriptional events lead to the activation of cardiac fibroblasts. Activated fibroblasts (myofibroblasts) produce ECM proteins and proteinases, as well as autocrine/paracrine factors, and mediate tissue remodeling processes. Recent studies have demonstrated that specific transcriptional control is governed by the complex interaction between factors participating in transcriptional control. The activity at transcriptional regulatory regions (eg, enhancers and promoters) is not a collection of activities of individual transcription factors, but instead emerges as a network of synergistic interactions between cis-elements within the region and their cognate transcription factors. Signaling molecules also form networks. Therefore, to fully understand the mechanism of cardiac remodeling, we will need to investigate the molecular networks that control the activities of cardiac fibroblasts and cardiomyocytes.

**Acknowledgments**

This work was supported in part by a Grant-in-Aid for Millennium Projects from the Ministry of Health, Labor and Welfare, Japan, a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to R.N.), and grants from the Takeda Medical Foundation and the Sankyo Foundation of Life Science (to I.M.).

**References**


30. Manabe et al Cardiac Fibroblasts in Remodeling


74. Stephanou A, Brar B, Scarrabelli TM, Jonassen AK, Yellon DM, Marber MS, Knight RA, Latchman DS. Ischemia-induced STAT-1...


Gene Expression in Fibroblasts and Fibrosis: Involvement in Cardiac Hypertrophy
Ichiro Manabe, Takayuki Shindo and Ryozo Nagai

Circ Res. 2002;91:1103-1113
doi: 10.1161/01.RES.0000046452.67724.B8
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/91/12/1103

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2002/12/02/91.12.1103.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/
Reference for Table 1


Progressive atrioventricular conduction defects and heart failure in mice expressing a mutant Csx/Nkx2.5 homeoprotein. *J Clin Invest.*

2001;280:H1782-1792.


1999;276:H2148-2158.


