Cross talk between cardiac myocytes and fibroblasts: from multiscale investigative approaches to mechanisms and functional consequences

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Zhang P, Su J, Mende U. Cross talk between cardiac myocytes and fibroblasts: from multiscale investigative approaches to mechanisms and functional consequences. Am J Physiol Heart Circ Physiol 303: H1385–H1396, 2012. First published October 12, 2012; doi:10.1152/ajpheart.01167.2011.—The heart is comprised of a syncytium of cardiac myocytes (CM) and surrounding nonmyocytes, the majority of which are cardiac fibroblasts (CF). CM and CF are highly interspersed in the myocardium with one CM being surrounded by one or more CF. Bidirectional cross talk between CM and CF plays important roles in determining cardiac mechanical and electrical function in both normal and diseased hearts. Genetically engineered animal models and in vitro studies have provided evidence that CM and CF can regulate each other’s function. Their cross talk contributes to structural and electrical remodeling in both atria and ventricles and appears to be involved in the pathogenesis of various heart diseases that lead to heart failure and arrhythmia disorders. Mechanisms of CM-CF cross talk, which are not yet fully understood, include release of paracrine factors, direct cell-cell interactions via gap junctions and potentially adherens junctions and nanotubes, and cell interactions with the extracellular matrix. In this article, we provide an overview of the existing multiscale experimental and computational approaches for the investigation of cross talk between CM and CF and review recent progress in our understanding of the functional consequences and underlying mechanisms. Targeting cross talk between CM and CF could potentially be used therapeutically for the modulation of the cardiac remodeling response in the diseased heart and may lead to new strategies for the treatment of heart failure or rhythm disturbances.

Cardiac myocytes; fibroblasts; cardiac remodeling; cross talk; intercellular communication

Introduction

Cardiac myocytes (CM) and cardiac fibroblasts (CF) are the two main cell types in the myocardium. The proportion of each cell type varies among species and changes during maturation and in disease. Generally, CM make up <50% and CF between 40 and 60% of the total cell population in the heart (2, 8, 84, 155). Fibroblasts are generally defined as cells of mesenchymal origin that produce a variety of extracellular matrix proteins (ECM); CF are flat, spindle-shaped cells with multiple processes and are the only cell type in the heart lacking a basement membrane (123).

Importantly, CM and CF are spatially intermingled in the myocardium, with virtually every CM bordering one or more CF (64). Bidirectional cross talk between CM and CF can be mediated by paracrine signals, direct cell-cell interactions, and indirect interaction via ECM (Fig. 1). Both cell types and their cross talk are important determinants of structural, mechanical, and electrical characteristics in the healthy and remodeled myocardium.

Cardiac remodeling is a common feature in many cardiovascular diseases (including hypertension, coronary artery disease, valvular defects, genetic disorders, arrhythmia disorders) that is defined as alterations in the size, shape, and function of the myocardium in response to changes in mechanical, chemical, and/or electrical signals (126). It can be divided into structural (hypertrophy and fibrosis) and electrical remodeling. In response to stress, CM become hypertrophic and can change their electrical properties, whereas CF convert into “activated” myofibroblasts (MyoFb), proliferate and enhance ECM deposition, which leads to fibrosis. Hypertrophy is a risk factor for cardiovascular morbidity and mortality that can lead to heart failure, predispose the heart to arrhythmias, and cause sudden cardiac death (50). Cardiac fibrosis affects CM metabolism and performance and ultimately ventricular function, because it can 1) impair diastolic function and later on also systolic function, 2) impair electrical coupling by separating CM with ECM proteins, and 3) reduce the capillary density and increase the oxygen diffusion distance that can lead to CM hypoxia (76). Fibrosis has long been viewed as a disease modifier secondary
to CM injury that affects the progression, severity, and outcome of myocardial remodeling and failure. Recent evidence suggests that it may also be a primary event in cardiac remodeling (127, 131) that impairs contractile function (76) and electrical coupling (29). Changes in the operation of ion channels and transporters that promote the occurrence of arrhythmias are part of the electrical remodeling response [reviewed in Nattel et al. (87)].

Cross talk between CM and CF plays an important role in cardiac remodeling in both ventricles and atria. Animal models with cell type-specific gene targeting strongly support the notion of CM-to-CF cross talk in vivo. However, the intricate interspersion of CM and CF makes investigations of CM-CF cross talk in the myocardium in vivo difficult, so that cell culture models and computational models have been mainly used to investigate the functional interactions between the two cell types in a direct and controllable manner.

In this review, we first provide an overview of the existing multiscale experimental and computational approaches for investigations of cross talk between CM and CF (Fig. 2) and summarize their respective characteristics, benefits, and limitations. We then review recent advances in our understanding of paracrine intercellular communication and direct cell-cell interactions and their functional consequences. Detailed information on ECM interactions as another means for CM and CF cross talk can be found in other recent reviews (13, 28, 60, 123), including the role of matricellular proteins, which unlike structural matrix proteins do not play a primary role in tissue architecture but are induced following tissue injury and modulate cell-cell and cell-ECM interactions (40). Finally, we highlight important questions in the field that warrant further investigation and outline potential avenues to address them and possible therapeutic implications. This review focuses on cross talk between CM and CF in the postnatal and adult heart. The importance of nonmyocytes (including CF) for CM differentiation and embryonic cardiac development was recently reviewed elsewhere (132).

**Approaches to Investigate Cross Talk Between CM and CF**

**Experimental models.** CELLULAR MODELS. In conventional two-dimensional (2-D) coculture, CM and CF randomly attach to the culture surface with varying degrees of homo- and heterotypic cell-cell interactions. In general, this configuration limits the type of investigations that can be performed in a cell type-specific manner. The intercellular conductance between single adjacent cell pairs of neonatal rat ventricular CM and CF has been measured using a double whole cell patch-clamp...
cell-ECM interactions are expected to dominate in scaffold-based cultures, whereas cell-cell interactions likely dominate in the absence of a scaffold when cells reestablish mutual contacts and specific environments (including ECM production) that allow them to express a more tissue-like phenotype. We recently developed a scaffold-free cardiac culture comprised of neonatal rat heart cells that can easily and inexpensively create many microtissues (>800 per 6 well), which mimic the cellular distribution and functional behavior of CM and CF in cardiac tissue (31). Importantly, these microtissues were amenable to cell type-selective gene transfer (31), paving the way for future investigations of CM and CF behavior and their interactions in a 3-D environment. Importantly, when compared with 2-D models, cell-cell interactions are enhanced in 3-D models, balancing the source-sink effect on action potential and conduction.

Both the developmental stage and the activation state of the cells used for investigation must be considered for the experimental design and interpretation since they are important determinants of cellular function. Most studies to date have used CM from neonatal rats, because they can be cultured in a confluent manner and recapitulate morphological and gene expression changes associated with hypertrophy in vivo (121), and they are widely used to investigate CM excitation and conduction (139). CM from neonatal mouse hearts (30) and CM derived from human inducible pluripotent stem cells (70) have also been used. Adult ventricular CM are terminally differentiated and more closely resemble CM in the intact heart tissue with respect to contractile protein composition, signaling properties and function, but they cannot be cultured in a confluent manner. In contrast, CF from animals of all developmental stages can be used in cocultures, but they may have functional differences depending on the age of the animal. For example, embryonic CF promote hyperplasia, whereas adult CF promote hypertrophy in embryonic CM (55). The cellular activation state is also particularly important for CF, because CF markedly change their morphology and functional properties when they convert into “activated” (i.e., contractile and hypersecretory) MyoFb in response to stress (102). CF activation can be experimentally induced in culture (e.g., by TGFβ1 treatment). Alternatively, fibrotic hearts can be used as a source for activated CF [e.g., (142)], because they retain their activated phenotype in culture throughout early passaging (37, 124). Importantly, CF have a propensity to turn into MyoFb in culture (107, 157), an effect that depends on the rigidity of the substrate and time in culture and must be taken into account in the design of cell culture models. It is also important to bear in mind that continuous electrical stimulation as well as mechanical stretch of both CM and CF during culture alters their morphology and electrical properties (5, 118, 145) and can impact cross talk via paracrine factors (137, 140).

All cell culture models share the requirement that CM and CF must first be released from the myocardial tissue context by serial digestion steps and are often enriched by purification steps before the cells are seeded in different configurations to generate 2-D or 3-D cell cultures. Advantages are minimal “contamination” by other cell types (e.g., endothelial cells, vascular smooth muscle cells, inflammatory cells), the ability to investigate drug and other effects in the absence of neurohumoral regulation, and flexibility in using specified numbers of CM and CF. However, the cells have been exposed to...
isolation conditions and lack their physiological tissue environment.

MYOCARDIAL TISSUE SLICES. Myocardial tissue slices have been developed as an intermediate between cell culture and organ studies (27, 46) that can be used as multicellular models for electrophysiological and pharmacological studies (14, 19). They hold promise for future investigations of CM-CF cross talk (see Conclusions and Future Outlook), but so far most studies have been conducted in cell culture models.

WHOLE HEART. Animal models with cell type-selective gene manipulation provide evidence for cross talk between CM and CF in vivo. Models with no obvious effect of CM-restricted gene targeting on CM but marked effects on fibrosis development strongly support the notion of CM-to-CF cross talk in vivo. Examples include mice with CM-restricted overexpression of connective tissue growth factor (CTGF) that had no effect on hypertrophy development but markedly enhanced fibrosis in response to pressure overload (153). Similarly, CM-restricted inhibition of G12/13 signaling suppressed pressure overload-induced fibrosis without affecting hypertrophy or systolic function (89). Gene manipulation in only a subset of targeted CM that have different effects on the neighboring CF provide the most direct evidence for CM-to-CF cross talk. For example, transgenic mice with mosaic, CM-specific overexpression of the α-subunit of the heterotrimeric G protein Gs developed fibrosis that was not randomly distributed but most prominent in areas with greatest density of Gs-expressing hypertrophic CM (143). In chimeric mice with Gs-coupled angiotensin II (ANG II) type 1A receptor-deficient and -intact CM, the local proliferative response of CF to ANG II was determined by the neighboring CM (77).

Conversely, CF-to-CM cross talk has been implicated in a study showing that reexpression of the hypertrophic marker gene β-myosin heavy chain occurred predominately in CM clusters within and around foci of fibrosis (94). Ideally, CF-to-CM cross talk in vivo should be investigated using CF-specific gene targeting. However, in contrast to CM-specific promoters (such as the α-myosin heavy chain promoter and myosin light chain promoter), which have long been used to selectively alter gene expression in CM (105), CF-specific promoters are not available at present. Instead, investigators have been using promoter fragments that drive gene expression only in nonmyocytes (primarily fibroblasts). For example, promoters for fibroblast-specific protein 1 and peroxisome have been used to target fibroblasts in the mouse heart, though they only become fully activated in response to injury and hemo-dynamic stress [e.g., myocardial infarct (103) or pressure overload (127)]. A study in which a transcription factor (Kruppel-like factor 5) was deleted primarily in murine fibroblasts upon peroxisome promoter-driven Cre expression after transverse aortic constriction strongly suggests CF-to-CM cross talk, because the phenotype entailed not only a reduction in fibrosis but also in CM hypertrophy, whereas CM-restricted deletion of Kruppel-like factor 5 did not affect either remodeling response to pressure overload (127).

Cross talk between the two cell types in the atrial myocardium in vivo has also been suggested by animal models, although much less is known about its mechanisms and functional significance to date. Atrial fibrosis in mouse models with CM-specific transgene expression in both atria and ventricles [e.g., Hirose et al. (51)] could be the result of atrial CM-to-CF cross talk and/or atrial stretch induced by ventricular dysfunction. However, studies in which atrial enlargement and fibrosis were observed in the absence of ventricular dysfunction (85, 150) suggest that CM-to-CF cross talk participates in the pathogenesis of atrial remodeling.

Computational models. Computational models integrate functional and structural information across subcellular, cellular, tissue, and organ levels on multiple scales, both temporally and spatially. Iterative interactions between experimentation and modeling lead to continuously refined computational models and new hypotheses for experimental investigations.

IONIC AND CELLULAR LEVEL. At the ionic and cellular level, integration of experimental and biophysical data have been used to investigate the electrical interactions between CM and CF. CF play important roles in both triggered activity and conduction blocks, depending on the degree and numbers of coupling between two cell types. Importantly, various coupling configurations can be evaluated individually in computational models. For example, mechanistic insights in the electrophysiological role of CF and their interaction with CM have been obtained by varying resting membrane potential, membrane capacitance, coupling strength, CM-to-CF ratio, cell distribution, and geometries. Simulations have been mainly performed to examine electrophysiological interactions of one or more CF with a single CM, one-dimensional cable-like CM strands and 2-D CM monolayers; models with virtual cells have been developed based on the electrophysiological characteristics from both ventricular (57, 73, 112) and atrial cells (74, 75). A hybrid biological computational approach and dynamic patch-clamp technique was recently used to develop a new model that coupled a real ventricular CM with virtual CF (88).

REALISTIC CARDIAC STRUCTURE. Computational modeling of realistic cardiac structure can simulate the propagation of a wave of transmembrane potential on user-defined tissue and whole heart geometries based on high-resolution magnetic resonance imaging and/or histology images, which enable inclusion of details of fiber orientations, laminar structures, fibrosis, and vascular structures. These models have been developed at the tissue and whole heart level for both ventricles and atria [reviewed in Dossel et al. (34), Plank et al. (99), Roberts et al. (106), and Trayanova (135)]. For example, information from ventricular microstructure measurements and intramural electrical mapping were used to demonstrate that the laminar arrangement of CM in pig ventricles influences electrical behavior (52). The same group recently introduced the first image-based model of the surface geometry and myofiber orientations in sheep atria and modeled the spread of electrical activation on this model (160).

These modeling approaches have been used to investigate how structural heterogeneities at both macro- and microlevels can influence arrhythmogenesis. For example, a magnetic resonance imaging-based, 3-D computational model of chronically infarcted rabbit ventricles suggests that MyoFb can either promote or prevent ventricular arrhythmias depending on their density (80) (see Gap junctions). Whole heart models also allow investigation of the coupling between electrical and mechanical events, including studies on mechanoelectrical feedback (65, 136).

The complex interrelations among models, simulations, and experiments and the importance of understanding what each model represents, as well as validation challenges and strate-
Mechanism and Functional Consequences of Cross Talk Between CM and CF

Paracrine intercellular communication. Intercellular communication via paracrine factors is well known to play an essential role in CM-CF cross talk and in the regulation of both CM and CF function. Both CM and CF can secrete many different chemokines, cytokines, growth factors, and other soluble factors. Many of them, such as TGFβ, IL family members, TNFα, VEGF, FGF2, ANG II, and endothelin-1, have been extensively studied, and information regarding their expression pattern/regulation as well as mechanisms of action has been reviewed elsewhere (9, 15, 60, 76, 92, 101). Here, we will focus on recent findings that provide novel mechanistic insights and/or point toward new paracrine signaling systems participating in the cross talk between CM and CF.

CTGF, which is induced by TGFβ and expressed in both CM and CF, is a mediator of TGFβ-induced CF proliferation, migration, and ECM deposition (23). Paracrine release of CTGF from CM and subsequent intercellular communication with CF has been implicated in cardiac fibrosis development (156): it was shown in neonatal CM that knockdown of Rad, a small G protein that suppresses both basal and TGFβ-induced CTGF expression, leads to elevated CTGF expression in CM. Conditioned medium from Rad knockdown CM markedly increased ECM production by CF, an effect that can be completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolished by adding a CTGF-neutralizing antibody into the medium. In the same study, Rad knockout in mice in vivo completely abolishe

Another mouse model showed that increased CTGF from serum response factor-null CM exerts paracrine control on CF proliferation, which could be counteracted by normalization of CTGF expression in CM (134).

Among the members of the IL family members, IL-33 is a more recently discovered, mechanically activated member of the IL-1 family that is predominantly synthesized by CF. IL-33, among other cytokines, activate CTGF and procollagen secretion of ANG II and reactive oxygen species, which promote the secretion of IL-6, as shown by enhanced levels in conditioned medium from CM-CF cocultures compared with CM or CF only cultures (12, 117). These findings indicate that communication between the two cell types is required for the secretion of some paracrine factors. Several additional IL family members (i.e., IL-4, IL-5, IL-9, IL-10, IL-12p70, IL-17, and IL-18) are secreted from CF (69); their potential roles in cross-regulating CM remain to be investigated.

A new role for VEGF [reviewed in Ottaviano and Yee (92)] as a paracrine factor between CM and CF is emerging. Recent studies showed that interaction of S100B (Ca2+ binding protein) with the receptor for advanced glycation end products (RAGE) induces VEGF secretion from injured CM, which in turn acts in a paracrine manner to induce MyoFb proliferation (138), suggesting that VEGF might contribute to fibrosis development in diseased heart. Placental growth factor (PGF), another member of the VEGF superfamily, was shown to regulate cardiac adaptation and hypertrophy through paracrine communication (1): the proposed mechanism involves PGF release from CM, which activates CF and endothelial cells (but not CM) with subsequent CM proliferation and capillary growth, respectively. The authors propose that PGF-induced CM hypertrophy involves a mechanism that is at least in part based on paracrine factors that are secondarily released from CF upon PGF-induced activation.

In addition to structural remodeling, paracrine factors also can affect the expression and function of ion channels and gap junctions in CM (32, 43, 81, 128). For example, paracrine factors released from CF can alter impulse conduction and ion channel expression of CM, as shown by a marked slowing of conducting velocity, increase of action potential duration (APD), and resting membrane potential in neonatal rat CM exposed to CF-conditioned medium (97). These changes were associated with downregulation in the expression of ion channels in CM, but no changes in gap junction expression or function. This study provides evidence that CF can cross-regulate CM electrical properties not only via direct cell-cell interactions (see below) but also via paracrine intercellular communication.

A requirement for a collaborative regulatory effect from paracrine factors and ECM proteins is illustrated in Ieda et al. (55): fibronectin, collagen, and heparin-binding, EGF-like growth factor were identified as embryonic CF-specific paracrine signals that jointly promoted CM proliferation via a mechanism that required β1-integrin on CM. Another important and new concept in paracrine signaling is epicardial paracrine conditioning of the subendocardial environment, which was suggested by a study demonstrating that the adult mouse epicardium modulated myocardial injury by secreting paracrine factors that promoted angiogenesis (161).

Most studies to date have used ventricular cells, but paracrine intercellular communication has also been demonstrated between atrial CM and CF. For example, conditioned medium from rapidly paced atrial CM was shown to alter canine atrial CF function (reduced proliferation, increased protein synthesis and collagen formation, phenotypic change to MyoFb), which could be attenuated in part by ANG II type 1 receptor blockade (18), suggesting that paced atrial CM regulate atrial CF via ANG II. Pacing of HL-1 atrial CM-induced collagen expression by CF via a mechanism that involves paracrine CM secretion of ANG II and reactive oxygen species, which activate CTGF and procollagen α1 in cocultured mouse atrial CF via TGFβ1 (137). Atria are generally more vulnerable to fibrosis than ventricles (47, 85, 150), which may be due to an enhanced response to multiple growth factors in atrial compared with ventricular CF (17).

In contrast to the conventional exocytosis of paracrine factors, a pannexin-based mechanism appears to also exist in the heart. Pannexins [reviewed in D’Hondt et al. (25), Shestopalov and Panchin (120), and Sosinsky et al. (122)] were initially proposed to form gap junction-like structures based on their sequence homology and similarity in membrane topologies with innexins and connexins, which are invertebrate and vertebrate gap junctional proteins, respectively. However, in contrast to connexins, pannexins were found to readily form channels that are functional in single membranes (and only
under rare and still poorly understood conditions intercellular channels in appositional membranes). Importantly, they can facilitate paracrine intercellular communication by releasing ATP and other small signaling molecules from the cytoplasm to the extracellular space. To date, little is known about pannexins in the heart and their role under physiological and pathophysiological conditions. One study provided evidence for a pannexin-mediated paracrine mechanism of CM-to-CF cross talk: G_{12/13}-mediated release of ATP and UTP from CM via pannexins that leads to activation of purinergic P2Y subtype 6 receptors on CF and subsequent induction of profibrotic genes (89).

**Cell-cell interactions. GAP junctions.** The best characterized means of direct cell-cell interactions in the myocardium is connexin-mediated gap junctional communication between CM, which connects the cytoplasm of both cells, enables intercellular exchange of small regulatory molecules and metabolites, and is essential for impulse propagation [reviewed in Jansen et al. (58)]. Each gap junction is made up of two hemichannels (connexons), of which one is provided by each cell. Each connexon is made up of six connexin molecules. Of the over 20 connexin (Cx) isoforms, Cx43, Cx45, and Cx40 predominate in the heart with differences in regional expression between the chambers. Gap junctional coupling between CF also exists and is mediated by Cx40, Cx43, and Cx45 (21, 82, 158). A novel physiological function of Cx43 in the activation of neonatal CF has been reported (3). While the extent at which CM and CF coupling occurs in myocardial tissue in vivo and its functional significance are still subject of debate, adjacent CM and CF clearly form functional gap junctions in vitro [reviewed in Camelliti et al. (20) and Vasquez et al. (141)]. Rohr and colleagues (41) showed electrical coupling of distant CM (up to 300 μm) that are interconnected by CF, using micropatterned 2-D culture of neonatal rat ventricular cells. A recent study (4) demonstrated that the arrhythmogeneity of a monolayer of cocultured neonatal rat ventricular CM and CF is mediated by Cx43 expressed in CF. Here, we will briefly summarize key findings that are supported by both experimental and computational studies with a focus on the modulation of action potential characteristics.

CM are not electrically excitable, but their plasma membrane contains voltage-dependent and other ion channels (24, 71, 141). Without coupling to CM, CF act as pure passive electrical insulators (151). When coupled to CM, they can act as current sources and/or sinks and thereby modulate action potential characteristics and conduction velocity in CM. CF coupling affects CM depolarization. Since the resting membrane potential of CF is less negative than that of CM, CF coupling elevates the resting membrane potential of coupled CM and modulates their excitation threshold. When the coupling is weak, the increased resting membrane potential of coupled CM leads to a decrease of the CM excitation threshold (57, 82), which can be sufficient to induce spontaneous ectopic activity (44, 83). However, when the coupling is strong, the resting membrane potential of coupled CM is progressively elevated, which in turn can increase the CM excitation threshold by leaving CM sodium channels in a less excitable state (57, 82). It has been reported in a 2-D computational model that the increase in CM excitation threshold by CF coupling prolongs the CM refractory period and thereby makes reentry more inducible by premature stimulation (151). Similarly, CF coupling (at intermediate densities) was shown to exacerbate arrhythmia propagation in a high-resolution, MRI-based, 3-D model of the infarcted rabbit ventricles (80). The same study also suggested that, at high CF densities, the resting depolarization of CM is sufficient to suppress the propensity to reentry, despite other proarhythogenic ion channel remodeling, and therefore may have antiarrhythmic effects (80). These findings highlight the complexity of the effects of CM-CF coupling that are determined by CM-CF coupling conditions (such as cell density and ratios, coupling strength, and activation state), spatial distribution, and tissue structures and warrant further investigations.

In contrast to the resting state, during action potential upstroke—when the CM membrane potential is higher than that of CF-CF act as a current sink, which slows down CM activation, and can decrease maximum upstroke velocity and the peak amplitude of the CM action potential. These effects are more pronounced with increased numbers of coupled CF and/or the coupling strength (56, 57, 73). CF coupling can also affect CM repolarization, which is strongly dependent on the CF resting membrane potential (56, 73, 74). Because the effects of CF on CM APD depend on the morphology of the action potential during repolarization, species-specific differences exist. For example, in human ventricular CM (with a much longer plateau phase than rodent CM), repolarization is accelerated and APD is shortened (73), whereas in mouse ventricular CM (with a triangular shape of the action potential and very short duration), APD is prolonged at similar coupling conductance (57). For atrial CM, the effects of CF coupling are more complicated (56, 74), because atrial CM have a different action potential shape, smaller cell capacitance, and diminished repolarization reserve compared with ventricular CM.

Associated with changes in action potential, conduction velocity can be affected by CM-CF coupling as well: consistent in experimental and computational studies, conduction velocity generally increases initially and then decreases as CF density and/or coupling strength increase (56, 57, 82, 112, 113). Conversely, with the use of neonatal rat cells, reduced CM-CF coupling by silencing Cx43 in CF increased conduction velocity (162). However, the same study also reported that conduction velocity can be increased by overexpression of Cx43 in CF. The authors interpreted this phenomenon as a potential switch of CF from current sink to short-range current transmitter upon markedly enhanced CM-CF coupling (162).

CM-CF coupling has also been shown to alter intercellular calcium cycling alternans, which could play an additional role in arrhythmogenesis in fibrotic heart tissue as well (152).

Electrophysiological effects of CM on coupled CF have also been examined but are much less understood. A computational study, in which a human ventricular CM action potential was coupled to up to four adult rat CF, revealed hyperpolarization of the CF resting membrane potential; each CM action potential was shown to drive electrotonic depolarization and repolarization in coupled CF (73). The authors speculate that this may modulate Ca^{2+} homeostasis and excitation-secretion coupling in CF and thereby may regulate the release of procollagen and other humoral factors.

Ventricular remodeling of the diseased heart, regardless of the etiology, is generally characterized by changes in the expression and distribution of connexins in both human and animal models [reviewed in Jansen et al. (58)]. A reduced
content of Cx43 gap junctions in CM has been described in most models and can be associated with a more heterogeneous distribution and lateralization. In contrast, CF isolated from infarcted rodent hearts, which display features of activated MyoFb, show enhanced Cx43 protein expression as well as enhanced intercellular coupling among themselves (159) and with CM (142). MyoFb have a 5–10-fold higher membrane capacitance than CF (24), leading to enhanced effects on CM electrophysiology (73, 142).

Importantly, it is still controversial whether or not gap junctional CM-CF coupling in the intact myocardium occurs at a level that is functional and impacts cardiac conduction in vivo (10, 11, 20, 63, 90), in part because of the challenges in obtaining the necessary evidence in vivo (20, 63). Thus, despite ample morphological and functional evidence for gap junctional coupling between CM and CF in vitro, further investigations are required to determine whether the effects observed may be limited to the cell culture environment or exist in the native myocardium.

ADHERENS JUNCTIONS. Mechanical coupling through adherens junctions and the cadherin/catenin complex at their core is another mechanism for intercellular communication. Cadherins are transmembrane receptors that bind together adjacent cells, link intracellularly to actin and intermediate filaments via catenins, and facilitate bidirectional transmission of cytoskeletal tension between cells (48, 78). Cadherins respond to intercellular mechanical load by actively remodeling the cytoskeleton to strengthen the adherens junction. Adherens junctions between CM play a well-known role in coordinating myofibrillogenesis and developing and maintaining a functional cardiac phenotype (78). In fibroblasts, adherens junction formation accompanies the transformation into MyoFb and is essential for the synchronization of periodic calcium oscillations between physically contacting MyoFb (39, 129). Cadherin staining has also been detected between cocultured CM and MyoFb (96, 129, 130). Tung and colleagues (129) showed that TGFβ-activated MyoFb can exert tonic contractile forces on CM and slow electric propagation as a result of increased mechanosensitive channel activation. Thus three potential mechanisms could be involved in mechanoelectrical regulation of CM by CF: 1) activation of mechanosensitive channels in response to mechanical stress that is imposed on CM by CF presumably via adherens junctions containing N-cadherin (129, 130), 2) mechanical deformation-induced change in CF electrophysiology that affects CM via electrotonic coupling (61, 62), and 3) stretch-induced release of paracrine factors from CF [reviewed in Porter and Turner (101)] that may affect CM ion channel expression and gap junctions (see *Paracrine intercellular communication*). However, if and to what extent the mechanisms contribute to mechanoelectrical feedback in the intact myocardium remain to be investigated.

The regulation of cell-to-cell adherens junctions and gap junctions can be closely related in some settings [reviewed in Saffitz and Kleber (115)]. Destabilization of gap junctions has been reported when adherens junctions are compromised because of loss or mutations in cadherins/catenins (72, 114). In contrast, the organization of adherens junctions and desmosomes was shown to be independent of gap junctions (45).

MEMBRANE NANOTUBES. While gap and adherens junctions provide means of communication for CM and CF that are adjacent to each other, membrane nanotubes (or tunneling nanotubes) represent a more recently discovered mechanism of cell-cell communication that facilitates intercellular communication over longer distances by enabling trafficking of organelles, plasma membrane components, and cytoplasmic molecules between cells, either inside or along their surface [reviewed in Davis and Sowinski (26) and Gerdes and Carvalho (42)]. Membrane nanotubes have been found in several cell types, including between CM and endothelial progenitor cells or mesenchymal stem cells (66, 100). Membrane continuity and connectivity via nanotubes were recently reported for cultured neonatal rat ventricular CM and CF (49), indicating for the first time that this mode of intercellular communication may play a role in CM and CF cross talk. The authors demonstrated long, thin membrane nanotubular structures containing actin and microtubules, through which intercellular organelle transfer (vehicles and mitochondria) and calcium signal propagation occurred from CM to CF and vice versa. It has been shown (albeit for noncardiac mammalian cell types) that connection via nanotubes can lead to bidirectional spread of electrical signals over distances of 10–70 μm (147). Connexins appear to be involved, since punctuate Cx43 immunoreactivity was frequently detected at one end of nanotube and electrical coupling was inhibited by a gap junction blocker and absent in cell types lacking gap junctions (147).

Much more work is needed to fully understand the functional role of this novel biological interaction between cultured CM and CF in vitro as well as to prove the existence of membrane nanotubes that connect CM and CF in myocardial tissue and to characterize their functionality and physiological role in vivo. Nevertheless, based on the few existing studies in this emerging field, one can speculate that cell-cell communication via nanotubes may play a significant role in cross talk between CM and CF via exchange of organelles and cytoplasmic proteins (26) and in long distance electric coupling between the two cell types (146). Nanotubes enable not only heterotypic CM-CF connections; homotypic CM-CM and CF-CF connections have been detected as well in vitro but have not yet been further characterized (49). Both types of interactions must be taken into account when interpreting results from studies with mixed cell populations both in vitro and in vivo.

**Conclusions and Future Outlook**

This review highlights the main concepts and most recent advances in our understanding of cross talk between the two major cell types in the myocardium and its important role in regulating cardiac function by modulating CM excitability, conductivity, and contraction and CF proliferation, migration, and ECM production. Many signaling pathways and electrophysiological and mechanical interactions are involved in regulating normal cardiac function and participate in the remodeling responses to stress. Insights into the molecular networks that integrate and control the function of CM and CF and their cross talk will be required for a comprehensive understanding of the regulatory events and their functional consequences that determine cardiac function in the normal and diseased heart.

Key conceptual and mechanistic questions that await further investigation are as follows: 1) How do multiple paracrine factors work together and have coordinated effects? 2) How are paracrine effects integrated with cell-cell or cell-ECM medi-
ated cross-regulatory effects? 3) Do other mechanisms of intercellular communication exist that can regulate CM-CF cross talk? 4) What are the effects of other cell types (such as stem cells, endothelial cells or inflammatory cells) on CM and CF function and their cross talk [see Brutsaert (16) and Tirziu et al. (133)]? 5) What is the relative contribution of biochemical, electrical, and mechanical communication to CM-CF cross talk under normal conditions in vitro and in the normal heart? 6) How are the different means of communication affected by developmental and disease-related structural and electrical remodeling?

Experimental and computational models need to be expanded and advanced. With regard to cell sources and their origin, CM and/or CF that originate from atria and ventricles, from different species, and from different developmental stages are needed to advance our understanding of species- and chamber-specific and developmental mechanisms. CM and CF from diseased hearts and/or those with genetic gene targeting are needed to investigate activation state-dependent and disease-specific mechanisms. Importantly, the cell population generally labeled as fibroblasts is not homogenous with regard to origin and functional properties (123), which along with their marked phenotypic changes upon activation in response to stress, needs to be carefully considered. The use of CM that are genetically reprogrammed from CF will be important to incorporate in experimental CM-CF coculture models as well. Proof-of-principle studies have shown that CF can be reprogrammed into CM-like cells not only in culture but also in their native tissue environment in vivo [reviewed in Srivastava and Ieda (125)]. The investigation of cross talk between CF-derived CM and CF is important, since CF reprogramming into CM is pursued as a potential therapeutic strategy for cardiac regeneration. Similarly, CM that are derived from human induced pluripotent stem cells should be investigated because of their importance and potential in disease modeling and regeneration (86).

Experimentally, technologies that can advance the existing experimental models are continuously evolving. First, a novel laser-guided cell micropatterning system enables selection and patterning of individual cells (including adult CM) on a substrate with high precision (98), which can facilitate cross-talk studies in adult CM but is limited to small numbers of cells. Second, diffusion tensor magnetic resonance imaging-based cell micropatterning technology has been developed that enables the fabrication of monolayers enriched in neonatal rat ventricular CM to replicate realistic cross-sectional ventricular tissue structure (6, 7). This approach has been used to vary the relative numbers of CM and CF plated (7), which could be used to model interstitial and diffuse fibrosis (29). Directing the two cell types to specific areas will be needed to model patchy and compact areas of fibrosis (29). Third, a valuable ex vivo model that maintains individual cells in their in situ environment are cardiac tissue slices, which partially preserve electrotonic and paracrine interactions and are amenable to targeted gene transfer (36). Importantly, functionally viable tissue slices can be successfully obtained from human endocardial biopsies (14, 19). Fourth, high-resolution scanning confocal microscopy can enable 3-D reconstruction of gap junction distribution in the myocardium and their quantitative analysis (68). Important insights are to be gained into the complex arrangement of connexins in myocardial tissue not only with respect to connexin remodeling and lateralization in CM but also for the investigation of gap junctional CM-CF coupling. Fifth, laser-capture microdissection, which fully preserves the molecular integrity for subsequent quantitative analyses (35, 67), can be used to harvest histologically enriched CM and CF populations from micropatterned 2-D cultures and cardiac tissue slices. Finally, comprehensive proteomic and functional analyses of paracrine factors are needed to better understand the signaling networks that mediate paracrine communication.

With regard to computational models for the investigation of CM-CF cross talk, incorporation of comprehensive data on ion channel expression and their electrophysiological properties in CF (154) from normal and diseased hearts, including human CF (71), will help to further refine the existing models. Incorporation of coexistence of CF with different activation states into the simulations will also provide information more closely related to the circumstance in diseased heart. Second, recent advances in high-resolution cardiac imaging including computer tomography and magnetic resonance imaging open up the possibility of performing computational and experimental studies in the same animal and of ultimately creating patient-specific heart models that may influence clinical practice (135). Finally, computational models also have significant potential for pharmacological research including drug screening (33, 106). So far little is known about the contribution of CM and CF cross talk on the effectiveness as well as side effects of drugs.

In conclusion, while many questions are still unanswered and await further investigation, targeting cross talk between CM and CF is believed to have significant potential as another promising avenue for the treatment of heart failure and rhythm disturbances. The design of new therapeutic strategies to mitigate and prevent cardiac remodeling have so far focused mainly on CM, but CF have increasingly attracted attention as direct therapeutic targets in recent years (15, 108). Understanding the mechanisms and functional consequences of their cross talk will aid and enhance therapeutic development.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

P.Z. and U.M. conception and design of research; P.Z., J.S., and U.M. prepared figures; P.Z., J.S., and U.M. drafted manuscript; P.Z., J.S., and U.M. edited and revised manuscript; P.Z., J.S., and U.M. approved final version of manuscript.

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Review

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