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Cell-Cell Connection to Cardiac Disease

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Abstract

Intercalated disks (ICDs) are highly organized cell-cell adhesion structures, which connect cardiomyocytes to one another. They are composed of three major complexes: desmosomes, fascia adherens, and gap junctions. Desmosomes and fascia adherens junction are necessary for mechanically coupling and reinforcing cardiomyocytes, whereas gap junctions are essential for rapid electrical transmission between cells. Because human genetics and mouse models have revealed that mutations and/or deficiencies in various ICD components can lead to cardiomyopathies and arrhythmias, considerable attention has focused on the biologic function of the ICD. This review will discuss recent scientific developments related to the ICD and focus on its role in regulating cardiac muscle structure, signaling, and disease.

Intercalated Disks: Structure and Components

Intercalated disks (ICDs) are highly organized components of cardiac muscle, which maintain structural integrity and synchronized contraction of cardiac tissue. The ICD is categorized into three major junctional complexes: desmosomes, fascia adherens junctions, and gap junctions (Figures 1 and 2). Desmosomes are major cell adhesion junctions that anchor cell membranes to the intermediate filament network, particularly in tissues undergoing constant physical stress, such as skin and heart (Jamora and Fuchs 2002). Desmosomal proteins include (a) tissue-specific desmosomal cadherins (desmogleins and desmocollins)—transmembrane proteins that form Ca^{2+} -dependent heterophilic cell-cell adhesive interactions, (b) armadillo proteins (plakoglobin (γ -catenin) and plakophilins)—cytoplasmic cadherin binding partners that signal and regulate cadherin adhesive activity, and (c) plakins (desmoplakins) that link to intermediate filaments such as desmin (Capetanaki 2000, Jamora and Fuchs 2002). Fascia adherens junctions anchor cells firmly by linking the cell membrane to the actin cytoskeleton. Components of this complex include (a) cadherins (N-cadherin)—transmembrane proteins responsible for Ca^{2+} -dependent homophilic cell-cell adhesion, (b) catenins (α -, β -, and γ -catenin (plakoglobin))—cytoplasmic proteins that bind to cadherins and regulate their adhesive activity, and (c) other catenin-related proteins including vinculin and α -actinin, which link the ICD to the cytoskeleton (Jamora and Fuchs 2002, Yamada et al. 2005).

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In contrast to these mechanical linkages, gap junctions form dynamic inter-cellular aqueous pores or channels from connexins (Severs et al. 2008). Six connexin molecules interact with one another to form two half-channels (hexameric hemichannels or connexons) across an intercellular space. These channels enable electrical and metabolic coupling between two adjoining cells by enabling small molecules (<1000 Da) to shuttle from the cytoplasm of one cell to another. The pivotal role of connexins in the myocardium has been comprehensively reviewed elsewhere (Noorman et al. 2009, Severs et al. 2008), and therefore, only a limited discussion of connexin 43 within the context of desmosomal and fascia adherens junction proteins will be included in this review.

A Role for Desmosomal-Associated Proteins in Arrhythmogenic Right Ventricular Cardiomyopathy

Human genetic studies have identified mutations in almost every known component of the desmosomal complex in patients harboring the human disease arrhythmogenic right ventricular cardiomyopathy (ARVC) (Table 1). Despite the genetic evidence, there are a limited number of studies, which have directly assessed the role of desmosomal proteins in ARVC. With this in mind, we will now discuss the role of plakoglobin, desmoplakin, plakophilin-2, desmoglein-2, and desmocollin-2 in ARVC (Tables 1 and 2).

Plakoglobin

Plakoglobin (~82-kD protein) is an ICD component found in both fascia adherens junctions and desmosomes (Figure 2). A 2-bp deletion (Pk2157del2) in *plakoglobin* causes a C-terminal truncation and was identified in patients with the recessively inherited cardiocutaneous form of ARVC, Naxos disease (McKoy et al. 2000). The truncated *plakoglobin* of Naxos disease is thought to disrupt plakoglobin's abilities to interact with other desmosomal proteins within the ICD and therefore alters normal desmosome structure. With this, Cx43 protein is also lost from the ICD, which likely leads to abnormal electrical coupling of myocytes and an increase in cardiac arrhythmias associated with this disease (Kaplan et al. 2004b).

As is obvious by its name, ARVC had been historically characterized by right ventricular dysfunction. More recent studies have shown that left ventricular dysfunction also occurs (Sen-Chowdhry et al. 2004, Thiene et al. 2007). Typically, fibrotic or fatty replacement of the ventricle is seen and ventricular arrhythmias leading to sudden cardiac death occurs. After its initial discovery, it was recognized that *plakoglobin* mutations play a causal role in both dominant and recessive forms of ARVC (Asimaki et al. 2007, Protonotarios et al. 2001). The dominant *plakoglobin* mutation (S39_K40insS) is thought to cause ARVC by altering turnover kinetics of plakoglobin (Asimaki et al. 2007). A mutant of human *desmoplakin* in its plakoglobin binding domain (S299R) also causes a dominant form of ARVC (Rampazzo et al. 2002).

Heterozygous *plakoglobin* deficient mice are viable but display postnatal cardiac abnormalities associated with ARVC, including abnormal RV size and function as well as spontaneous and exercise-induced ventricular arrhythmias (Kirchhof et al. 2006). These results suggest a direct role for *plakoglobin* in provoking ARVC; however, it remains to be determined whether ARVC can also be recapitulated in animal models by the many dominant and recessive *plakoglobin* mutations identified in ARVC patients.

Desmoplakin

Desmoplakin (~250-kD (I, large isoform) and ~210-kD (II, small isoform)) is a central component of the desmosomal complex (Figure 2). It appears to link the tissue-specific

desmosomal cadherins to the cytoskeletal intermediate filament network, i.e., desmin in cardiac muscle, through the intermediary armadillo proteins plakoglobin and plakophilin (Sonnenberg and Liem 2007). Mouse genetic studies have identified a role for *desmoplakin* in ARVC (Garcia-Gras et al. 2006, Yang et al. 2006). Cardiac-myocyte– specific ablation of the *desmoplakin* gene, with the use of the α -myosin heavy chain (MHC) Cre mouse, resulted in significant embryonic lethality between E10 and E12, when embryos displayed poorly formed hearts with no chamber specification and unorganized cardiac myocytes (Garcia-Gras et al. 2006). Knockout mice surviving this embryonic period predominantly died within the first 2 weeks after birth; however, heterozygous *desmoplakin* knockout mice were viable and displayed some features associated with ARVC. Although the mechanisms remain to be determined, these studies suggested that *desmoplakin* deficiency results in mislocalization of plakoglobin from the ICD with decreased canonical Wnt/ β -catenin signaling that promoted adipogenic/fibrogenic gene expression.

A variety of *desmoplakin* mutations in man have been linked to ARVC. For instance, R2834H affects the binding of desmoplakin to intermediate filaments as well as other junctional proteins such as plakoglobin, β -catenin, desmin, and plakophilin-2 (Yang et al. 2006). Cardiac-myocyte– specific transgenic mice generated to recapitulate this mutation also exhibited features of ARVC. These studies clearly point to an important role for *desmoplakin* in ARVC; however, the early lethality of some of these mouse models has precluded the ability to assess the molecular mechanisms underlying the postnatal onset of the human disease.

Other *desmoplakin* mutations that have varied modes of inheritance have been shown to produce a variety of ARVC phenotypes (Rampazzo et al. 2002). For example, two recessive mutations in *desmoplakin* (Gly2375Arg and 7901delG) both disrupt desmoplakin-intermediate filament interactions but result in slightly different clinical phenotypes (Alcalai et al. 2003, Kaplan et al. 2004a, Norgett et al. 2000). Although both result in patients with woolly hair and keratoderma, the Gly2375Arg mutation results in more typical ARVC (Alcalai et al. 2003), whereas the 7901delG mutation identified in Ecuadorian individuals predominantly involved the left ventricle (LV) and has been termed *Carvajal syndrome* (Kaplan et al. 2004a, Norgett et al. 2000). Several other recessive mutations in *desmoplakin* have also been linked to either fully expressive Carvajal syndrome or to a more restricted syndrome with only heart failure (Turkay et al. 2006, Uzumcu et al. 2006). Interestingly, Carvajal syndrome patients do not exhibit typical fibrofatty replacement of the myocardium, but rather, with reduced expression of desmosomal proteins (plakoglobin, desmin) and connexin 43, show contractile and electrical abnormalities (Kaplan et al. 2004a).

Clinical heterogeneity has also been observed in other dominant *desmoplakin* mutations (e.g., three missense and one with abnormal splicing) (Bauce et al. 2005). For example, the *desmoplakin* mutation 2034insA causes production of a truncated protein with disrupted desmoplakin-intermediate filament binding and results in a predominantly LV phenotype (Norman et al. 2005). The heterogeneity of ARVC is seen in a recent study that has identified additional mutations associated with ARVC (Yang et al. 2006). These affect either binding of desmoplakin to linker proteins and, therefore, alter its localization in desmosomes (V30M, Q90R), to increase decay of a nonsense mRNA (W233X) or alter binding of desmoplakin to intermediate filaments (R283H).

Plakophilin-2

Plakophilin (~97-kD protein) is a cytoplasmic desmosomal cadherin-binding partner, similar to plakoglobin, which is involved in regulating cadherin adhesive activity and signaling (Figure 2). Although not as well characterized as some of the other desmosomal proteins associated with ARVC, mutations in *plakophilin-2* have also been detected in ARVC

patients and associated with abnormalities in connexin 43 expression (Antoniades et al. 2006, Fidler et al. 2008, Kannankeril et al. 2006, Lahtinen et al. 2008). It remains to be determined whether ARVC can be recapitulated in animal models by any of the *plakophilin-2* mutations.

Desmocollin-2/Desmoglein-2

The tissue-specific desmosomal cadherins, desmogleins (~122-kD protein) and desmocollins (~100-kD protein), are transmembrane proteins that form Ca^{2+} -dependent heterophilic cell-cell adhesive interactions (Figure 2). Mutations that cause either premature truncation or mislocalization of *desmocollin-2* have been recently identified in autosomal dominant ARVC (Beffagna et al. 2007, Heuser et al. 2006, Syrris et al. 2006). A truncation mutant of *desmocollin-2* has also been associated with recessive ARVC with mild palmoplantar keratoderma and woolly hair (Simpson et al. 2008). In addition, mutations in *desmoglein-2* have also been identified as causal to ARVC (Pilichou et al. 2006, Posch et al. 2008, Syrris et al. 2007). These mutations can have both variable penetrance and clinical heterogeneity. These studies clearly implicate a role for a range of desmosomal proteins in human ARVC.

A Role for Proteins Associated With the Fascia Adherens Junction in Cardiac Disease and Arrhythmias

Extensive studies in animal models have also begun to show that components of the fascia adherens junctions play important roles in all aspects of cardiac muscle biology, including cardiac development, disease, and arrhythmias (Tables 1 and 2). This portion of the review will focus on N-cadherin, α -E-catenin, β -catenin, and vinculin because genetically manipulated mice (i.e., transgenic and gene knockout models) have been generated to address their role in vivo.

N-cadherin

N-cadherin (~88-kD protein) is an essential component of the fascia adherens complex and a member of the classical cadherin family (Figure 2). As such it is a single-pass transmembrane glycoprotein that mediates calcium-dependent cell-cell adhesion primarily through homophilic interactions (Vlemminckx and Kemler 1999). In the adult murine myocardium, cardiac-specific overexpression of either *N-cadherin* or *E-cadherin* caused a dilated cardiomyopathy (DCM) phenotype, cardiac calcification, and intracardiac thrombus (Li et al. 2006). The *N-cadherin* mice have a less severe phenotype than the *E-cadherin* ones, but neither transgenic line showed alterations in ICD structure though both had increased cardiac expression of α - and β -catenin, down-regulation of Cx43, and redistribution of vinculin.

In contrast, recent studies used an approach where *N-cadherin* was inducibly deleted from cardiac myocytes after the mice had reached adulthood (Li et al. 2006). These mice showed absence of identifiable ICDs with loss of components within the fascia adherens junction and desmosomes, resulting in a modest DCM. They also displayed a significant decrease in Cx40 and Cx43 expression, with evidence of conduction slowing, inducible, and spontaneous ventricular arrhythmias, which lead to sudden death. Although a role for *N-cadherin* in humans remains to be determined, the above studies as well as ones performed in *N-cadherin* heterozygous null mice, suggest that mutation or reduced expression of N-cadherin might predispose patients to rhythm disturbances (Li et al. 2008). Although there is no direct evidence for a role of N-cadherin–based adhesion signaling in cardiomyocytes, classical cadherin engagement (transinteracting cadherin) has been linked to the induction of Rac1 activation via the Rap1-PI3K-Vav2 pathway in nonmuscle cells (Fukuyama et al. 2006, Noren et al. 2001). This may also be of importance in muscle because some evidence

suggests a critical role for Rac1 activation in cardiomyocyte cell alignment after mechanical stress (Yamane et al. 2007).

α -Catenin

α -Catenins (~100–102-kD protein) are key cytoplasmic molecules, which are thought to indispensably link the cytoplasmic domain of cadherin to the actin cytoskeleton (Rimm et al. 1995). To do so, α -catenin binds to β - or γ -catenin through their N-terminal domains, and to actin, directly through its C-terminus. It also binds indirectly to other actin-binding proteins, such as vinculin and α -actinin (Figure 2). Among the three α -catenin subtypes, α -E-catenin is the most widely studied (Hirano et al. 1992, Nagafuchi et al. 1991). It is ubiquitously expressed in all tissues, with particularly high amounts in epithelial cells and cardiac myocytes (Ehler et al. 2001, Janssens et al. 2001). α -E-catenin is highly expressed in the adherens junction of the cardiac ICD and perturbations in its expression are also associated with DCM (Ehler et al. 2001). Cardiac-myocyte-specific ablation of the α -E-catenin gene in mice resulted in a progressive DCM and unique defects in the right ventricle, which included ICD ultrastructural defects and complete loss of vinculin from the ICD (Sheikh et al. 2006). These mice were also predisposed to ventricular free wall rupture after myocardial infarction. A reduction in expression or alteration in localization of α -E-catenin has also been associated with postmyocardial infarction ventricular rupture in man (Van den Borne et al. 2008). Interestingly, the expression and distribution of other fascia adherens components such as β -catenin, γ -catenin, and N-cadherin were not altered in the rupture prone regions of these same patients. The cytoskeletal linker protein α -T-catenin, maps to a region of human chromosome 10 associated with DCM (Janssens et al. 2003). However, no mutations in α -T-catenin have been identified in patients with DCM so far. These in vivo studies highlight the importance of α -E-catenin in the myocardial cadherin/catenin/vinculin complex and the necessity to continue to study its role in human cardiac injury and cardiomyopathy.

β -Catenin

β -Catenin (~88-kD protein) has multi-functional capabilities depending on its intracellular localization (Nelson and Nusse 2004). In cardiac muscle, it is localized at the fascia adherens junction where it is part of the N-cadherin–actin complex (Figure 2). It can also be localized in the cytoplasm and nucleus and act as a transcriptional activator. Cytoplasmic β -catenin is soluble and is involved in the Wnt signaling pathway. In the absence of Wnt signaling, β -catenin expression is low in cells because glycogen synthase kinase- β phosphorylates β -catenin and targets it for destruction by the ubiquitin–proteasome pathway. Alternatively, when Wnt signaling is activated, glycogen synthase kinase-3 β is inactivated, thereby, leading to stabilization of β -catenin, accumulation of it in the cytosol and subsequent translocation of it to the nucleus, where it results in the transcription of Wnt-responsive genes. The role of β -catenin in adult myocyte adhesion remains unknown because up-regulation of plakoglobin was shown to compensate for the loss of β -catenin in the adult myocardium of various cardiac-myocyte-specific β -catenin deficient models (Chen et al. 2006, Zhou et al. 2007). Plakoglobin has structural and functional similarities to β -catenin and can also interact and interfere with β -catenin signaling (Rudiger et al. 1998). Targeted deletion of β -catenin in the cardiac myocyte, however, did result in a blunted hypertrophic response to pathologic stress-induced growth, which was attributed to the altered transcriptional activating activity of β -catenin (Chen et al. 2006).

Increased as well as decreased expression of the fascia adherens component, β -catenin have both been identified in various cases of cardiomyopathies (Mahmoodzadeh et al. 2006, Masuelli et al. 2003). β -Catenin expression accumulates in the ICDs of patients exhibiting hypertrophic cardiomyopathies (HCM) (Masuelli et al. 2003). In contrast, in human end-stage heart failure, the expression of β -catenin and one of its associated partners, estrogen

receptor- α , is lost from the ICD (Mahmoodzadeh et al. 2006), suggesting that some ICD proteins may have unique functions in different types of myopathies or at least in different stages of an evolving cardiac disease.

Vinculin

Vinculin (~117-kD protein) and its muscle-enriched splice variant, *metavinculin* (~124-kD protein), are ubiquitously expressed membrane-associated proteins that link cell matrix adhesions, cell-to-cell contacts (fascia adherens junctions), and costameres (subsarcolemmal adhesion plaques) to the actin cytoskeleton (Belkin et al. 1988, Rudiger et al. 1998) (Figure 2). To determine a role for *vinculin* in the adult myocardium, both heterozygous conventional and cardiac-myocyte-specific *vinculin* knockout mice have been generated and characterized (Zemljic-Harpf et al. 2004, Zemljic-Harpf et al. 2007). Heterozygous conventional *vinculin* deficient mice displayed cardiac dysfunction and increased mortality after acute hemodynamic stress imposed by transverse aortic constriction, which was preceded by ultrastructural defects in the ICD when the mice were phenotypically and physiologically normal (Zemljic-Harpf et al. 2004). Cardiac-myocyte-specific *vinculin* knockout mice display cardiac adherens junction/ICD abnormalities accompanied by reduced cardiac myocyte N-cadherin and β 1D integrin expression as well as mislocalization of Cx43, leading to sudden death caused by ventricular tachycardia before 3 months of age (Zemljic-Harpf et al. 2007). Mice surviving this period displayed a DCM phenotype, leading to death by 6 months of age. These studies demonstrate that *vinculin* expression is necessary for preservation of ICD ultra-structure as well as both cardiac contractile and electrical function.

Mutations and deficiencies in *vinculin* and *metavinculin* have also been associated with DCM and HCM (Maeda et al. 1997, Olson et al. 2002, Vasile et al. 2006a, 2006b, 2006c). One study reported that *metavinculin* deficiency was caused by a defect in alternative mRNA splicing in a patient with DCM (Maeda et al. 1997). A more comprehensive study of 350 unrelated patients with DCM detected three *metavinculin* mutations (Arg975Trp; Leu954del; Ala934Val) (Olson et al. 2002). In vitro studies performed on the *metavinculin* mutant proteins showed that they affected actin filament organization. These studies suggested that the interactions between metavinculin and actin could alter force transmission within the cardiomyocyte sarcomere and between cardiac myocytes. It is interesting to note that the *vinculin* mutation, Arg975Trp, has been found in patients exhibiting not only DCM but also obstructive HCM, suggesting that modifier genes and/or environmental stressors could be critical in the phenotypic plasticity displayed by some vinculin mutations (Vasile et al. 2006c). Recent evidence has also identified additional *vinculin* and *metavinculin* mutations (Ala934Val, Pro943Ala, Leu277Met) in patients exhibiting obstructive HCM (Vasile et al. 2006a, 2006b, 2006c). These mutations may cause alterations in the secondary structure or helical organization of the protein or perhaps lead to a specific reduction in the expression of vinculin/metavinculin at the ICD but not at the Z disk.

Novel Fascia Adherens Junction Proteins in the Cardiac ICD and Their Role in the Heart

Over the last few years, a growing number of novel fascia adherens junction proteins have been identified and shown to play an important role in cardiac disease and arrhythmias (Table 2). These newly identified proteins are associated with the fascia adherens junction through their interactions with β -catenin and/or N-cadherin (Figure 2) and include such genes as muscle-specific mouse Xin α (*mXina*) (Choi et al. 2007, Gustafson-Wagner et al. 2007, Lin et al. 2005), lysosomal integral membrane protein 2 (*LIMP-2*) (Schroen et al.

2007), and the coxsackievirus and adenovirus receptor (*CAR*) (Lim et al. 2008, Lisewski et al. 2008).

Muscle-specific mouse *Xin* α

Muscle-specific mouse *Xin* α (~155 kD) is a mouse striated muscle-specific protein, which directly associates with β -catenin and actin filaments in vitro (Choi et al. 2007, Lin et al. 2005). It colocalizes with N-cadherin and β -catenin throughout mouse embryogenesis and into adulthood (Lin et al. 2005). These results suggested a role for *mXina* in cell-cell adhesion and specifically in modulating the Wnt/ β -catenin/N-cadherin-mediated signaling pathway and organizing actin filament assembly (Lin et al. 2005). The role of *mXina* in early cardiac development remains unknown because the conventional knockout mice develop normally as *mXin β* expression appeared to compensate for the loss of *mXina* (Gustafson-Wagner et al. 2007). However, the conventional *mXina* knockout mice did exhibit significant postnatal defects in ICD ultrastructure, myofilament assembly as well as the abnormal expression of a number of fascia adherens and desmosomal proteins (p120 catenin, β -catenin, N-cadherin, desmoplakin, Cx43) leading to cardiac hypertrophy, cardiomyopathy, and conduction defects. The role of *Xin* remains to be determined in human cardiomyopathies and arrhythmias.

Lysosomal integral membrane protein 2

Lysosomal integral membrane protein 2 (*LIMP-2*) is a highly glycosylated protein (~54 kD) that decorates the luminal surface of lysosomal membranes and plays a role in lysosomal biogenesis (Eskelinen 2006). Recent studies have evaluated the role of *LIMP-2* in mouse and man (Schroen et al. 2007). This work showed *LIMP-2* is a novel component of the cardiac ICD based on its interactions with N-cadherin and its ability to modulate interactions between phosphorylated β -catenin and N-cadherin in cardiomyocytes. Conventional *LIMP-2* knockout mice were viable, demonstrating that *LIMP-2* is dispensable for mouse embryogenesis. However, postnatally *LIMP-2* knockout mice failed to mount a hypertrophic response after transverse aortic constriction and displayed ICD abnormalities with N-cadherin mislocalization and development of a DCM after angiotensin II treatment. Interestingly, this study also showed *LIMP-2* was increased in hearts of patients with clinically severe pressure loading. Thus, *LIMP-2* appears important in the hemodynamically challenged heart.

Coxsackievirus and adenovirus receptor

Coxsackievirus and adenovirus receptor (*CAR*) is a transmembrane protein (~40 kD) that functions as an intercellular adhesion molecule and the common receptor for the viruses for which it was named (Lim et al. 2008). *Coxsackievirus and adenovirus receptor* has recently been identified as a novel component of the ICD based on its localization within cardiomyocytes and its interactions with the fascia adherens protein, β -catenin, and the gap junction proteins, Cx45 as well as zona occludens 1 (ZO-1) (Lim et al. 2008). It should be noted that ZO-1 can also directly interact with connexins independent of *CAR* (Hunter and Gourdie 2008). One group showed that cardiac-myocyte-specific *CAR* knockout mice developed normally but exhibited postnatal abnormalities including atrioventricular (AV) block associated with loss of Cx45, β -catenin, and ZO-1 localization at the ICD, as well as ICD ultrastructural abnormalities leading to DCM (Lim et al. 2008). A second laboratory independently generated these same mice and similarly identified effects of *CAR* on AV conduction and Cx45 expression; however, defects in sinus node function, AV morphology, and Cx43 expression were also observed (Lisewski et al. 2008). These results demonstrate a clear role for *CAR* in cardiac development, disease, and arrhythmias. Although *CAR* has been implicated in human myocarditis and DCM (Bowles et al. 1986), these animal studies invite future studies investigating unique functions of *CAR* in the human heart.

Cross-Talk Between the Desmosome, Fascia Adherens, and Gap Junctional Proteins

A significant amount of crosstalk has been shown to exist among the various junctional proteins within the complexes of the ICD. This occurs both because of the close spatial proximity of the junctional complexes to one another and also because the complexes contain common binding partners. Elegant studies with the use of immunoelectron microscopy have demonstrated that desmosomal components can also be localized in the fascia adherens junction, whereas fascia adherens and gap junction components can also be localized in the desmosomes. These data suggest that the ICD components could be thought of as a common specialized region termed *area composita* (Borrmann et al. 2006, Franke et al. 2006). *Plakoglobin* is an example of an ICD-associated protein, which is found both within the fascia adherens junction and desmosomes. Deficiencies in *plakoglobin* resulted in cardiac rupture of the embryonic heart, whereas heterozygous loss/mutations in *plakoglobin* in the adult myocardium are associated with ARVC (Bierkamp et al. 1996, Kirchhof et al. 2006). These defects have primarily been observed in mice with deficiencies in desmosomal components (Gallicano et al. 2001) (Table 1) and further suggest that defects in *plakoglobin* expression or function dominantly cause problems related to the desmosomal as opposed to fascia adherens junction complex. Recent studies where *N-cadherin* was specifically reduced in the adult mouse myocyte have also suggested a hierarchical relationship among junctional proteins/complexes (Li et al. 2006). These mice displayed a loss in all components of the fascia adherens junction and desmosomes (Li et al. 2006, Li et al. 2008), suggesting that desmosomal stability is dependent on the fascia adherens junction protein, N-cadherin. Although fascia adherens and desmosomal proteins are essential to maintain muscle integrity, their loss frequently leads to gap junction instability, suggesting a certain level of cross-talk between mechanical and electrical junctional components. In contrast, when Cx43 was conditionally ablated in the myocyte, fascia adherens, and desmosome structure was not affected indicating that gap junctions are not necessary for the establishment of these other cellular adhesion junctions at the ICD (Gutstein et al. 2003). These studies are further corroborated by ones that suggest that mechanical junctions are established before gap junctions in the developing myocyte (Noorman et al. 2009). Further studies will be required to directly assess how this cross-talk occurs among the various complexes of the ICD and how this relationship relates to the variety of cardiac pathologic conditions observed in man.

Key Questions/Areas for Future Research

Although a number of studies have sought to uncover the biological role of ICD components in the heart with the use of mouse models and human genetics, several key questions remain to be answered. For example, we must determine the molecular mechanisms, which are responsible for the clinical heterogeneity of distinct mutations in ICD proteins. Studies aimed at comprehensively dissecting the functional role of specific mutations with the use of mouse knock-in strategies, coupled with protein biochemistry and cell biology techniques, could be invaluable. These studies could also be pivotal in understanding why there are distinct clinical phenotypes associated with alterations in different complexes (e.g., *N-cadherin* and *α -E-catenin* deficiencies associated with DCM and desmosomal deficiencies associated with ARVC). This may be especially important given recent evidence of the “area composita” which proposes overlapping roles of cardiac cell-junction components within the ICD. Another key question is to determine whether ICD proteins have only a structural role or if they also function in signal transduction. There is some evidence for *β -catenin* and *plakoglobin* functioning as signal transducers, but additional studies aimed at identifying and functionally characterizing novel cardiac ICD interacting proteins may give further insights into this prospect. This research may also be important in elucidating the

cross-talk that occurs between the various ICD components in both basal and cardiac disease states.

Conclusions

The discovery of mutations in genes encoding ICD proteins in patients with cardiomyopathy and arrhythmias has driven research investigations to uncovering the function of the ICD and its associated proteins. Mouse models have played a pivotal role in uncovering the role of certain ICD components in cardiac disease. However, the importance of additional, still to be discovered, ICD proteins in these processes is highly likely and will be of value in furthering our understanding of ICD function. Study of the molecular mechanisms underlying the diseases linked to these ICD components is necessary so that (a) unique therapeutic targets for ICD-associated myopathies might be identified and (b) to identify patients that may have distinct genetic susceptibilities to particular inherited forms of cardiomyopathy. The results of these studies could have profound effects on future characterization of disease prognosis, as well as clinical decisions involving treatment.

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Figure 1. Transmission electron micrograph of the major junctional components within the ICD of an adult mouse heart. Fa indicates fascia adherens junction; Ds, desmosome; and Gj, gap junction. White bar represents 500 nm.

Figure 2.
Schemata of the major complexes and components found in the cardiac ICD.

Table 1

Summary of ICD-associated proteins linked to human cardiac disease, pathologic conditions, and arrhythmias

ICD-associated junctional complexes	ICD component	Human cardiomyopathies/pathologic conditions/arrhythmias	Reference(s)
Fascia adherens junction	α -E catenin	Post-MI ventricular rupture	Van den Borne et al. 2008
	α -T-catenin	DCM	Janssens et al. 2003
	Vinculin/metavinculin	DCM, HCM	Maeda et al. 1997, Olson et al. 2002, Vasile et al. 2006a, 2006b, 2006c
	β -catenin	HCM, Heart failure	Mahmoodzadeh et al. 2006, Masuelli et al. 2003
	CAR	Myocarditis, DCM	Bowles et al. 1986
	Plakoglobin	ARVC, Naxos disease	Asimaki et al. 2007, McKoy et al. 2000, Protonotarios et al. 2001
Desmosomes	Plakoglobin	ARVC, Naxos disease	Asimaki et al. 2007, McKoy et al. 2000, Protonotarios et al. 2001
	Desmoplakin	ARVC, Carvajal syndrome, heart failure, left ventricle dominant form of ARVC	Alcalai et al. 2003, Baucé et al. 2005, Kaplan et al. 2004a, Norgett et al. 2000, Norman et al. 2005, Rampazzo et al. 2002, Turkay et al. 2006, Uzumcu et al. 2006, Yang et al. 2006
	Plakophilin-2	ARVC	Antoniades et al. 2006, Fidler et al. 2008, Kannankeril et al. 2006, Lahtinen et al. 2008
	Desmocollin-2	ARVC	Beffagna et al. 2007, Heuser et al. 2006, Simpson et al. 2008, Syrris et al. 2006
	Desmoglein-2	ARVC	Pilichou et al. 2006, Posch et al. 2008, Syrris et al. 2007

MI indicates myocardial infarction.

Table 2

Summary of mouse models generated to determine the role of mechanical components of ICD (fascia adherens junction and desmosomes) in postnatal myocardium and cardiac disease

ICD-associated junctional complexes	ICD component	Mouse models and cardiac phenotypes	Reference(s)
Fascia adherens junction	N-cadherin	CS-overexpression: DCM (mild), cardiac calcification, thrombosis	Li et al. 2006
		CS-I-KO: DCM (modest), ventricular arrhythmias, sudden cardiac death	Li et al. 2006, Li et al. 2008
	α -E-catenin	CS-KO: progressive DCM, RV defects, Predisposed to MI-induced ventricular rupture	Sheikh et al. 2006
		CS-I-KO: predisposed to MI-induced ventricular rupture	Van den Borne et al. 2008
	β -Catenin	CS-KO: no basal phenotype but blunted response to hypertrophy following TAC	Chen et al. 2006
		CS-I-KO: no basal phenotype due to compensation by plakoglobin	Zhou et al. 2007
	Vinculin	Heterozygous KO: predisposed to postnatal sudden death after TAC	Zemljic-Harpf et al. 2004
		CS-KO: postnatal sudden death via ventricular tachycardia, DCM	Zemljic-Harpf et al. 2007
	mXin α	Conventional KO: postnatal cardiac hypertrophy, cardiomyopathy, and conduction defects	Gustafson-Wagner et al. 2007
	LIMP-2	Conventional KO: viable but blunted response to hypertrophy after TAC, DCM after angiotensin II treatment	Schroen et al. 2007
Desmosomes	CAR ^a	CS-KO: viable but exhibit postnatal AV conduction defects and DCM	Lim et al. 2008, Lisewski et al. 2008
	Plakoglobin ^a	Heterozygous KO: viable but displayed postnatal cardiac abnormalities associated with ARVC, including exacerbation in ventricular arrhythmias after exercise	Kirchhof et al. 2006
	Desmoplakin	CS (α -MHC-Cre)-KO: lethality at E10–E12 with defects in chamber specification and myocyte organization; embryonic survivors died at 2 weeks after birth	Garcia-Gras et al. 2006
		CS (α -MHC-Cre) heterozygous KO: features associated with ARVC	Garcia-Gras et al. 2006
		CS-R283H mutation overexpression: features associated with ARVC	Yang et al. 2006

KO indicates knockout; CS, cardiac-specific; CS-I, cardiac-specific-inducible; MI, myocardial infarction; TAC, transverse aortic constriction.

^aIt should be noted that CAR is also present within gap junctions and that plakoglobin is also found at the fascia adherens junction.