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N-cadherin/Catenin Complex as a Master Regulator of Intercalated Disc Function

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Abstract

Intercellular adhesive junctions are essential for maintaining the physical integrity of tissues; this is particularly true for the heart that is under constant mechanical load. The correct functionality of the heart is dependent on the electrical and mechanical coordination of its constituent cardiomyocytes. The intercalated disc (ID) structure located at the termini of the rod shaped adult cardiomyocyte contains various junctional proteins responsible for the integration of structural information and cell-cell communication. According to the classical description, the ID consists of three distinct junctional complexes: adherens junction (AJ), desmosome (Des), and gap junction (GJ) that work together to mediate mechanical and electrical coupling of cardiomyocytes. However, recent morphological and molecular studies indicate that AJ and Des components are capable of mixing together resulting in a 'hybrid adhering junction' or 'area composita'. This review summarizes recent progress in understanding the *in vivo* function(s) of AJ components in cardiac homeostasis and disease.

Keywords

N-cadherin; catenin; adherens junction; desmosome; arrhythmogenic cardiomyopathy

INTRODUCTION

The coordinated contraction of the heart is dependent on the proper mechanical and electrical coupling of cardiomyocytes. To achieve this goal cardiomyocytes are connected end-to-end by a specialized structure called the intercalated disc (ID) that serves as an organizing center for various cell surface proteins including junctional complexes critical for cell-cell attachment and cell-cell communication (Figure 1). The ID contains three different intercellular junctions: adherens junction (AJ), desmosome (Des), and gap junction (GJ) (Forbes and Sperelakis, 1985). AJ and Des provide mechanical attachment between the myocytes by anchoring the actin cytoskeleton and intermediate filaments, respectively, at the ID. GJs are plaques of multiple intercellular channels that connect the cytoplasm of adjacent

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Conflict of interest

None

cells. A major role of GJs in the myocardium is to enable the rapid and coordinated electrical excitation, a prerequisite for normal rhythmic cardiac function. The expression, distribution, and/or function of many of the junctional components are often perturbed in heart disease (Severs et al., 2008, Saffitz, 2009), however their specific contribution to the disease phenotype is less well understood.

The AJ consists of classical cadherins, including E-, P- and N-cadherin, that mediate calcium-dependent cell-cell adhesion. The classical cadherins are single pass transmembrane proteins with five extracellular 'cadherin domains', a transmembrane domain, and a cytoplasmic domain (Meng and Takeichi, 2009). Through their homophilic binding and adhesive specificities, cadherins play a critical role in embryonic development and the maintenance of normal tissue architecture in the adult. Cadherin adhesive activity is dependent on cytosolic proteins called catenins that link the cadherin cytoplasmic domain to the actin cytoskeleton. N-cadherin is the sole cadherin expressed in heart muscle. In contrast, different catenin subtypes with overlapping and distinct functions are available for N-cadherin/catenin complex formation. Catenins can be divided into two families: the armadillo (Arm) domain containing catenins that bind directly to the cadherin (β-catenin, γ-catenin, and p120ctn) and the vinculin-homology domain containing catenins (αE-catenin and αT-catenin). β-catenin and γ-catenin (also known as plakoglobin) bind to the carboxy-terminus in a mutually exclusive manner whereas p120ctn binds to the juxtamembrane region of cadherin. The function of p120ctn has not been studied in the heart and will not be discussed further in this review. α-catenin which binds to the cadherin-β-catenin or cadherin-plakoglobin complex, mediates linkage to the actin cytoskeleton.

Desmosomes are present in tissues under constant mechanical stress such as skin and heart, and thus reinforce intercellular adhesion in those tissues (Green et al., 2010). The molecular structure of Des is relatively similar to AJ, however these adhesive junctions are anchored to intermediate filaments (i.e. desmin). The desmosomal cadherins, desmoglein-2 (DSG2) and desmocollin-2 (DSC2), serve as the transmembrane adhesion receptors in the Des. The Arm proteins plakoglobin (PG) and plakophilin-2 (PKP-2) bind directly to the cytoplasmic tail of DSG2 and DSC2, which in turn binds to the plakin protein desmoplakin (DP). DP links the desmomosal cadherins to desmin.

The importance of these adhesion molecules in the heart is highlighted by the fact that human mutations in genes encoding desmosomal proteins cause arrhythmogenic cardiomyopathy (AC, also known as arrhythmogenic right ventricular cardiomyopathy or ARVC) a hereditary heart muscle disease that causes sudden cardiac death (SCD) in young people and athletes (Basso et al., 2012). The pathological features of AC consist of progressive loss of cardiomyocytes, myocardial degeneration, and compensatory replacement with fibro-fatty tissue. AC is considered a disease of the desmosome since almost half of the patients carry a mutation in one of the five genes encoding desmosomal proteins expressed in the heart. A hallmark of AC is incomplete penetrance and variable expressivity of the disease phenotype making it difficult for clinicians to advise patients of their risk of SCD. Adding further to the genetic complexity AC patients were recently identified with more than one mutation in the same or different desmosomal gene,

suggesting that AC might require multiple genetic hits in the cell adhesion complex to elicit a cardiac phenotype (den Haan et al., 2009, Bauce et al., 2010, Xu et al., 2010).

Until recently it was thought that AJ and Des represent distinct junctional complexes of the ID. The idea of a “hybrid junction” as part of the normal heart structure was first suggested in 2006 (Franke et al., 2006). In this study, the authors revealed the presence of DP and PKP2 in “desmosome-like” structures as well as “adherens junction-like” structures by immunoelectron microscopy. These hybrid junctions that they called “area composita”, comprise the majority of intercellular junctions in the heart (Franke et al., 2006, Borrmann et al., 2006) (Figure 2). This novel junctional complex is only found in the heart of higher vertebrates raising the intriguing possibility that this reinforced adhesive junction evolved to withstand the increased mechanical load of the four-chamber mammalian heart (Pieperhoff and Franke, 2007, Pieperhoff and Franke, 2008). The concept of a mixed junctional complex containing both AJ and Des components has important ramifications for understanding the molecular mechanisms underlying ID remodeling observed in heart disease. The area composita will be discussed later in the context of specific interactions between the AJ and Des components, αT-catenin and PKP-2.

Gap junction channels connect the cytoplasmic compartments of adjacent cells and allow the intercellular flow of ions. In the heart, GJ are critical for the efficient propagation of the action potential throughout the myocardium (Severs et al., 2008). An individual channel is created by stable, noncovalent interactions of two hemichannels, referred to as connexons. Each connexon is composed of six connexin (Cx) proteins. In the mammalian heart, gap junction channels are primarily composed of three different Cx proteins: Cx43, Cx40 and Cx45. Cx43 is the main constituent of cardiac GJ and in rodents is expressed in all atrial and ventricular myocytes. Cx40 is expressed in atrial myocytes, while Cx45 is expressed in the conduction system. In cardiomyopathies, Cx43 is often down-regulated or mislocalized to the lateral membranes instead of being restricted to the ID (Kostin et al., 2003). Experimental animal models demonstrated that altered Cx expression results in slow, heterogeneous conduction and conduction block critical for reentrant arrhythmias (Remo et al., 2012).

In this review, we will focus on AJ components and their essential roles in the organization and function of the cardiac ID. As the ID can only be fully appreciated in the context of the working myocardium, we will focus our discussion on animal models that have enhanced our understanding of the functional interdependence of the different intercellular junctions in the heart. Finally, the new concept of the ‘area composita’ will be discussed and how it may explain the increased risk of arrhythmia in AC patients.

N-cadherin function in the heart

N-cadherin-mediated adhesion plays a critical role in both cardiac morphogenesis and in the adult working myocardium. The importance of N-cadherin in mammalian development was appreciated after germline deletion of N-cadherin in mice (Radice et al., 1997). N-cadherin knockout embryos display multiple embryonic abnormalities that include severe cardiovascular defects. The loss of cardiomyocyte adhesion in the N-cadherin mutant

embryos caused malformation of the primitive heart tube and early embryonic lethality. Interestingly, cardiac-specific expression of the epithelial cadherin, E-cadherin, was able to rescue the adhesion defect and restore cardiac looping in the N-cadherin-null embryos demonstrating that these two cadherins are functionally interchangeable during early cardiac development (Luo et al., 2001). In addition to the requirement of N-cadherin in the nascent myocardium, this protein also plays essential roles in other cardiac cell lineages including cardiac neural crest, epicardial, and endocardial cells (Luo et al., 2006, Luo et al., 2005).

Initially, during early cardiac development, the junctional components are distributed all along the cell borders of the polygonal shaped embryonic cardiomyocyte (Hirschy et al., 2006). During the later fetal and early postnatal period the myocyte elongates, myofibrils align, and maturation results in a significantly larger rod shaped cardiomyocyte. During this morphological progression the AJ, Des, and GJ components eventually become restricted to the polarized ends of the cell to form the mature ID (Angst et al., 1997). Pathological remodeling of the ID has been observed in diseased human myocardium (Schaper et al., 1991, Tomaselli et al., 1994) and animal models (Matsushita et al., 1999, Wang and Gerdes, 1999) demonstrating that a heightened risk of arrhythmia and sudden death is associated with abnormal cell-cell coupling.

Mouse models have allowed a detailed characterization of the consequences of interfering with mechanical junctions in the heart (Table I). To overcome the requirement for N-cadherin during embryogenesis, an inducible cardiac-specific N-cadherin (N-cad) conditional knockout (CKO) model was used to investigate N-cadherin function in the adult heart (Kostetskii et al., 2005). Induced deletion of the *N-cadherin* (*Cdh2*) gene in the working myocardium results in complete disassembly of the ID as determined by transmission electron microscopy. This study demonstrated the paramount importance of N-cadherin for ID structural integrity. The loss of desmosomes after N-cadherin depletion illustrates the functional hierarchy of these adhesive junctions in the heart (Figure 3). In contrast, mutations in genes encoding desmosomal proteins have minimal, if any effect, on N-cadherin expression in the heart (Kwon et al., 2013, Tavora et al., 2013, Oxford et al., 2007, Cerrone et al., 2012).

It is remarkable that N-cad CKO animals survive even for a limited time without any recognizable ID. The mutant heart retains the ability to contract, albeit weakly, without these mechanical junctions. It is possible that other adhesion systems compensate for loss of the normal end-to-end connection between the myocytes. For instance, increased β1-integrin at the sarcolemma of the N-cadherin-deficient cardiomyocytes suggests enhanced cell-ECM interactions that may provide additional structural stability in the absence of AJ and Des (Kostetskii et al., 2005).

The N-cad CKO mice exhibit sudden cardiac death (SCD) beginning about 5 weeks after deleting the gene. Electrocardiographic recordings from transmitters implanted into freely mobile animals showed that SCD in N-cad CKO mice was the result of spontaneous ventricular fibrillation in the absence of any clinical signs of heart failure (Kostetskii et al., 2005). The investigation into the mechanism(s) by which N-cadherin depletion leads to an arrhythmogenic substrate and SCD is complicated by the fact that multiple proteins involved

in electrical activity in the heart are affected by disassembly of the ID. Evidence from diseased human myocardium and animal models indicate that alterations in electrical coupling via gap junctions are a critical determinant in the development of the arrhythmogenic substrate (Remo et al., 2012). Both the number and size of Cx43- and Cx40-containing GJ plaques are reduced in the ventricular and atrial myocardium of the N-cad CKO, respectively (Li et al., 2005). The carboxy-terminus of Cx43 is phosphorylated at specific serine residues when it forms an active gap junction complex at the cell surface. In contrast, it is dephosphorylated during trafficking/endocytosis in the cytoplasm. Researchers have taken advantage of a series of antibodies generated by the Lampe lab that recognize the phosphorylation status of Cx43 (Sosinsky et al., 2007). N-cad CKO hearts exhibit an increase in dephosphorylated Cx43 consistent with less functional gap junctions at the sarcolemma. Consistent with this idea, optical mapping studies revealed slowing of conduction velocity in the N-cad CKO hearts (Li et al., 2005). Of note, the reduction in epicardial conduction velocity is similar to that observed in the Cx43 CKO mice which also die from spontaneous lethal arrhythmias (Gutstein et al., 2001). Based on these studies, gap junction remodeling is considered a major determinant of the arrhythmogenesis observed in the N-cad CKO animals.

N-cadherin regulation of gap junction communication

Previous studies demonstrated that N-cadherin-mediated cell-cell contacts are a prerequisite for the aggregation of gap junction channels at the sarcolemma (Hertig et al., 1996, Luo and Radice, 2003). More recent evidence indicates N-cadherin regulates connexon assembly, trafficking and functional gap junction cellular coupling. It is becoming better appreciated that N-cadherin maintains gap junction homeostasis by multiple mechanisms. AJ stabilize the plasma membrane of adjacent cardiomyocytes, which then helps to form an environment at the ID that protects the GJ from mechanical stress. Interestingly, in response to pulsatile stretch neonatal rat ventricular cardiomyocytes markedly upregulate N-cadherin and Cx43 which increases propagation velocity (Zhuang et al., 2000). N-cadherin/catenin complex also organizes the underlying cytoskeleton and associated scaffold proteins thus facilitating the aggregation of connexons into large arrays of gap junctions. The actin-binding scaffold protein, zonula occludens-1 (ZO-1), interacts with Cx43 via a PDZ-binding motif found in the large carboxy terminus (Giepmans and Moolenaar, 1998). ZO-1 has been shown to regulate Cx43 plaque size and distribution (Hunter et al., 2005). N-cadherin-deficient hearts exhibit a loss of ZO-1 from the myocyte termini providing a possible explanation for the reduced Cx43 plaque size in the mutant hearts (Li et al., 2008). Recent data indicate that vinculin, another actin-binding scaffold protein, and ZO-1 together regulate GJ in the heart (Zemljic-Harpf et al., 2014). Lastly, efficient delivery of connexons to the cell-cell border is dependent on tethering of microtubule plus-ends via N-cadherin and β-catenin at AJ (Shaw et al., 2007). The plus-end binding protein EB1 binds to Cx43 and facilitates its transport along the microtubules to the AJ. In the mouse heart, it was shown experimentally that the EB1/Cx43/AJ association can be disrupted by ischemia-reperfusion injury or oxidative stress induced by treatment with hydrogen peroxide (Smyth et al., 2010). Interestingly, N-cadherin association with Cx43 and EB1 is disrupted by stress whereas N-cadherin-β-catenin interaction at the membrane remains intact.

In addition to gap junctions, ion channels are also dependent on proper arrangement of the cytoskeleton. Remodeling of the actin cytoskeleton including downregulation of the actin-binding scaffold protein cortactin is likely responsible for decreased Kv1.5 function in the N-cad CKO model (Cheng et al., 2011). Reduction of the voltage-gated potassium channel Kv1.5 at the sarcolemma likely causes electrical heterogeneity thus facilitating the development of arrhythmia in the N-cad CKO mice. Further studies will be necessary to determine if trafficking of Kv1.5 to the ID is dependent on N-cadherin-mediated cytoskeletal organization.

N-cad CKO mice exhibit a severe cardiac phenotype and SCD. In comparison, N-cadherin heterozygous null mice expressing half the normal level of N-cadherin display normal ID structure, cardiac pathology, and cardiac function (Li et al., 2008). Further examination revealed a reduction in the size of the large GJ plaques and an increase of the dephosphorylated or less active isoform of Cx43 in the N-cad −/+ mice. The gap junction remodeling was accompanied by an increased susceptibility to induced arrhythmias in the N-cad −/+ compared to control littermates. Interestingly, the susceptibility to arrhythmias was similar in N-cad −/+ compared to Cx43 −/+ mice. The generation of N-cad −/+; Cx43 −/+ compound mutants showed a further decrease in gap junction plaque size and increased susceptibility to inducible arrhythmias in comparison to the single mutant mice demonstrating that these two genes function synergistically to regulate gap junction communication in the heart.

To date, no human *CDH2* mutations have been identified in people suffering from heart disease. However, recent evidence suggests that N-cadherin and Cx43 expression may be generally affected in heart failure patients. Based on optical mapping data, end-stage non-ischemic human hearts (n=10) exhibit slower transmural activation compared with non-failing hearts (Glukhov et al., 2012). The conduction slowing was associated with decreased total Cx43, phosphorylated Cx43 isoform, as well as reduced co-localization with N-cadherin.

Functional overlap and distinct roles for β-catenin and plakoglobin

β-catenin and plakoglobin (PG) are members of the armadillo (arm) protein family (Zhurinsky et al., 2000). The central arm domain contains 13 imperfect repeats of 42 amino acids that mediate numerous protein-protein interactions. The central arm domain is the most conserved region of the two proteins with 83% amino acid similarity while the N- and C-terminal tails share 57% and 15% similarity, respectively. The relatively low homology between the terminal regions of these proteins likely contributes to the differences in binding partners and functional activity of these otherwise closely related arm family members. In addition to their adhesive roles as part of the junctional complex, these proteins regulate gene expression by interacting with members of the T-cell factor (TCF)/lymphoid enhancing-binding factor (LEF) family of transcription factors (Zhurinsky et al., 2000). In contrast to β-catenin, current opinions regarding the physiological significance of PG as a transcriptional regulator remain controversial. It has been proposed that PG functions primarily as a competitive inhibitor of β-catenin transcriptional activity by sequestering TCF/LEF. This review will focus on β-catenin and PG as both structural proteins of the ID

as well as their roles in signal transduction in the heart. Evidence from animal models illustrates the functional interdependence of β-catenin and PG in cardiac homeostasis and disease.

β-catenin function in the heart

Although β-catenin interacts with N-cadherin, there is no evidence supporting an essential role for β-catenin as a structural protein in the heart. Cardiac-specific deletion of β-catenin in mice has revealed important signaling roles for β-catenin at different stages of cardiac development, however these lethal phenotypes appear to be independent of any myocardial cell adhesion defect (Qu et al., 2007, Piven et al., 2011). To overcome the requirement for β-catenin during cardiac morphogenesis, an inducible cardiac-specific β-catenin (β-cat) CKO was generated similar to the N-cad CKO model. Induced deletion of the *β-catenin* (*Ctnnb1*) gene in the adult heart has no apparent effect on ID structure, cardiac pathology, or cardiac function. The authors suggested that the lack of a structural defect in the β-cat CKO mice was due to replacement of β-catenin by PG in AJs (Zhou et al., 2007). This hypothesis was recently confirmed by generating a β-cat/PG double knockout (DKO) mouse model (Swope et al., 2012). In the absence of both arm proteins, the N-cadherin/catenin complex is no longer stable at the plasma membrane leading to disassembly of the ID structure and SCD of the β-cat/PG DKO mice.

β-catenin is a well-established downstream effector of the Wnt signaling cascade (Clevers and Nusse, 2012). The cytoplasmic pool of β-catenin is regulated by canonical Wnt signaling and ubiquitin-proteasome-dependent degradation. The targeting of β-catenin degradation is achieved through the phosphorylation of N-terminal serine residues by a multi-protein “destruction complex” containing glycogen synthase kinase 3β (GSK3β) and scaffold proteins adenomatous polyposis coli (APC) and axin. Activation of the Frizzle receptor by Wnt increases the stability of β-catenin and its cytosolic accumulation. β-catenin is then able to translocate into the nucleus where it can interact with TCF/LEF transcription factors and promote the transcription of specific genes involved in cellular growth, differentiation and cell fate.

Upon pathological stress, the heart reactivates several signaling pathways involved in cardiac development including Wnt/β-catenin signaling (Bergmann, 2010). Several groups have subjected β-catenin mutant mice to various stress or injury protocols in order to investigate the requirement for β-catenin in pathological remodeling (Table I). Following transverse aortic constriction (TAC) to induce pressure overload, β-catenin mice exhibit reduced Wnt target gene expression and the pathological hypertrophy response is blunted (Chen et al., 2006). Consistent with a transcriptional mechanism, cardiac-specific expression of dominant negative Lef1 also failed to exhibit hypertrophy following TAC. The role of β-catenin signaling in angiotensinII-induced hypertrophy appears to differ from those reported for TAC or ischemic injury (i.e. myocardial infarction) suggesting involvement of other signaling pathways (Baurand et al., 2007). Although β-catenin inhibition appears to be cardio-protective following injury, the mechanism behind increased cardiac function and reduced infarct size is controversial. A different group reported that loss of β-catenin results

in enhancement of cardiac progenitor cell differentiation post injury as opposed to inhibition of hypertrophy (Zelarayan et al., 2008).

Plakoglobin function in the heart

Plakoglobin (PG, γ-catenin, JUP) was initially described as a component of multiple intercellular mechanical junctions (Cowin and Burke, 1996). PG is a unique ID protein as it binds to the cytoplasmic domain of both classical cadherins and desmosomal cadherins thus mediating linkage to the actin and intermediate filaments, respectively. Although PG shares many binding partners with β-catenin, its function outside the junctional complex is less well understood compared to β-catenin.

The first genetic mutation identified in AC patients was a homozygous 2 base pair deletion (2057del2) in the *JUP* gene causing a premature truncation of the PG protein (McKoy et al., 2000). The disease was originally identified in patients on the Greek island of Naxos hence it is referred to as “Naxos disease” (Protonotarios et al., 1986). In addition to heart problems, Naxos patients exhibit palmoplantar keratoderma and woolly hair. Despite the fact that *JUP* was the first AC mutation identified, *JUP* mutations are the least common (<1%) among all the desmosomal mutations identified in AC patients over the past 15 years (Fressart et al., 2010, van Tintelen et al., 2007). The relatively low mutation frequency compared to the other desmosomal genes supports a critical role for this multi-junctional protein in tissue homeostasis. Interestingly, PG is usually diminished at the ID of AC patients regardless of the desmosomal gene mutation making PG a potential diagnostic tool for AC in affected individuals (Asimaki et al., 2009).

Over the past several years, several groups have studied various animal models to understand the molecular mechanisms underlying PG dysfunction in the heart. Interestingly, both cardiac-restricted PG loss-of-function (LOF) and gain-of-function (GOF) studies have shown that altering PG expression can cause AC-like pathology in mice. Two groups recently reported that cardiac-specific deletion of the *PG* gene cause altered desmosome ultrastructure and decreased cardiac function as determined by echocardiography (Li et al., 2011a, Li et al., 2011b). Expression of AJ proteins is not affected by loss of PG presumably due to upregulation of β-catenin in the mutant hearts. PG CKO mice recapitulate many features of AC pathogenesis that include progressive loss of myocytes, extensive inflammatory infiltration, and replacement fibrosis. In contrast to human AC, adipocytes were not observed in the PG CKO hearts. A more severe phenotype including arrhythmias was observed in one of the two models where PG was deleted early in the perinatal period instead of the adult heart (Li et al., 2011a).

A hallmark of AC is the loss of cardiomyocytes that are replaced with fibroblasts and adipocytes. The molecular mechanism(s) underlying this unique fibro-fatty pathology highlight the dual function of these arm proteins as both structural components of mechanical junctions and transcriptional regulators. The canonical Wnt/β-catenin signaling is an important regulator of myogenesis versus adipogenesis (Ross et al., 2000). The Marian lab showed that DP knockdown caused translocation of PG to the nucleus of cardiomyocytes where it interferes with β-catenin/TCF transcriptional activity resulting in an adipogenic

switch (Garcia-Gras et al., 2006). In a subsequent study, the same group performed genetic fate-mapping experiments to demonstrate that adipocytes in the DP CKO model originate from a cardiac progenitor cell population (Lombardi et al., 2009). The current LOF and GOF animal models have provided important insight into the pathogenesis of AC. However, the next generation murine models will require knock-in of specific human mutations to further investigate the molecular mechanism(s) responsible for this unique cardiac pathology.

α-catenin function in the heart

α-catenins are AJ proteins that directly link the cadherin cytoplasmic domain at the sarcolemma to the actin/tubulin cytoskeleton, and have been shown to act as tension transducers that translates mechanical stimuli into a chemical response (Twiss and de Rooij, 2013). In the heart, there are two α-catenins expressed: the ubiquitously expressed αE-catenin and the cardiac-restricted αT-catenin. Both α-catenin isoforms contain three vinculin homology domains, and share 57% amino acid identity (Janssens et al., 2001). The N-terminal domain of α-catenin binds β-catenin (or PG) while its C-terminal domain interacts with F-actin allowing the linkage of the classical cadherins with the actin cytoskeleton. α-catenin binds directly with actin-binding proteins that include vinculin, ZO-1, afadin, and EPLIN. α-catenin functions as a platform for F-actin remodeling machinery and is required for the mechanical coupling between cadherin and actomyosin (Twiss and de Rooij, 2013).

The cardiac-specific αE-catenin CKO model presents with progressive left ventricular dilatation associated with a thinning right ventricular anterior wall leading to a high susceptibility to cardiac rupture post-MI (Sheikh et al., 2006). Loss of αE-catenin did not affect the expression of junctional components located in the AJ, Des or GJ and no arrhythmias were reported in these mice. However, vinculin, a binding partner of αE-catenin, was decreased in the αE-catenin CKO heart. The relatively late onset of cardiomyopathy (>8 months of age) suggests αT-catenin may compensate, at least to some degree, for loss of αE-catenin. In another study, significant mortality was observed in αE-catenin heterozygous null mice post-MI (van den Borne et al., 2008).

αT-catenin is the newest member of the α-catenin family of proteins. Using yeast two-hybrid and co-immunoprecipitation, αT-catenin was shown to bind the desmosomal protein PKP2 (Goossens et al., 2007). By contrast, αE-catenin lacks PKP2 binding capacity. As demonstrated by immunoelectron microscopy αT-catenin and PKP2 localize in the area composita but not the Des (Figure 2). The unique ability of αT-catenin to interact with PKP2 provides a new paradigm for understanding the molecular integration of the junctional components including GJs and ion channels.

Recently, an αT-catenin KO mouse model confirmed the link between αT-catenin and PKP2 in the area composita and its essential role in cardiac function (Li et al., 2012). The AJ components discussed earlier are all required for embryonic development, however αT-catenin-null mice are viable and fertile. Loss of αT-catenin in the area composita leads to early onset of dilated cardiomyopathy, gap junction remodeling and an increased susceptibility to ventricular arrhythmia in the setting of ischemia/reperfusion injury. The

expression and distribution of AJ and Des components are not affected in the αT-catenin KO heart, with the exception of PKP2. The more severe cardiac phenotype in the αT-catenin KO compared to the αE-catenin CKO model reveals a unique role for αT-catenin in cardiac homeostasis (Li et al., 2012). The disruption of the αT-catenin-PKP2 interaction may affect the spatial organization of additional junctional components located in the area composita. The Delmar lab has shown that PKP2 interacts with Cx43 as well as the sodium channel Nav1.5 in cardiomyocytes (Oxford et al., 2007, Sato et al., 2011, Sato et al., 2009). Further characterization of the αT-catenin KO model is warranted to determine the molecular mechanism(s) responsible for arrhythmogenesis in these animals.

Recently, two mutations in the human *αT-catenin* (*CTNNA3*) gene were identified in AC patients suggesting that perturbation of the area composita may play a critical role in the etiology of this disease (van Hengel et al., 2012). One *αT-catenin* mutation found in this screen of 76 AC patients inhibits the interaction between αT-catenin and β-catenin (or PG) leading to a mislocalization of αT-catenin into the cytoplasm of HL1 myocardial cells. The second *αT-catenin* mutation increases dimerization of αT-catenin, which might create aggresomes and disturb its function. Animal models will be necessary to elucidate the consequences of these mutations in the working myocardium.

Conclusions

The paramount importance of N-cadherin in the regulation of ID structure and function in the normal heart are well established. It is likely that the N-cadherin/catenin complex and its associated cytoskeleton will be altered during cardiac remodeling and progression to heart failure. Loss-of-function studies have been informative, however new approaches will be necessary to investigate changes in N-cadherin function during disease progression. The N-cadherin haploinsufficiency model may be useful in this regard, since the mice are healthy and only show signs of arrhythmia after programmed stimulation.

The recent discovery of a novel, exclusive type of ‘hybrid adhering junction’ or ‘area composita’ in the mammalian heart has important implications for our understanding of the molecular mechanisms underlying AC as well as other forms of heart disease. The exact mechanism(s) by which adhesion proteins influence connexon trafficking, channel assembly and/or stability at the ID cannot be fully understood until the functional interdependence of the junctional proteins is known. A ‘snap shot’ of the junctional protein interactome illustrates the complexity of the protein-protein interactions that occur at the ID (Figure 4). Most proteins such as Cx43 are capable of interacting with multiple proteins and thus have the potential to form large macromolecular complexes. Recent data indicate that oxidative stress can disrupt EB1/Cx43 interactions critical for connexon transport to the ID (Smyth et al., 2010). It will be of interest to examine the effects of stress on other junction protein interactions illustrated in this interactome.

Finally, AC may be considered a disease of the area composita where the AJ and Des join forces to create a unique hybrid junction found exclusively in the four-chamber heart.

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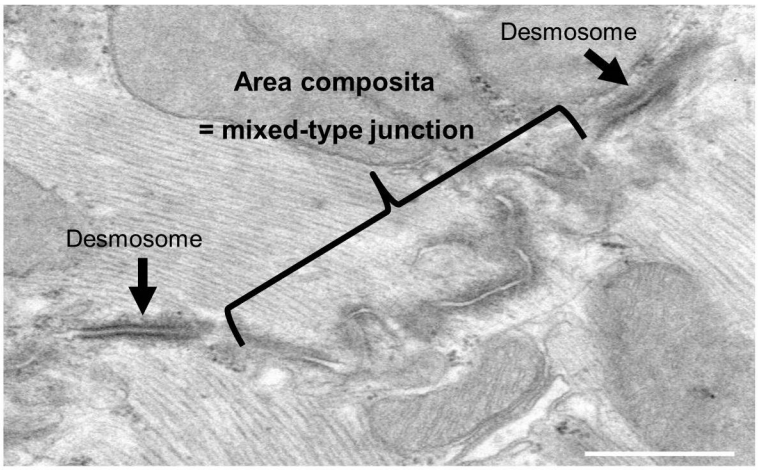

Figure 1: Transmission electron microscopy of a cross section through intercalated disc of a mouse heart.

TEM image showing the intercalated disc with a large mixed-type junction (area composita) surrounded by two dense desmosome structures (black arrows).

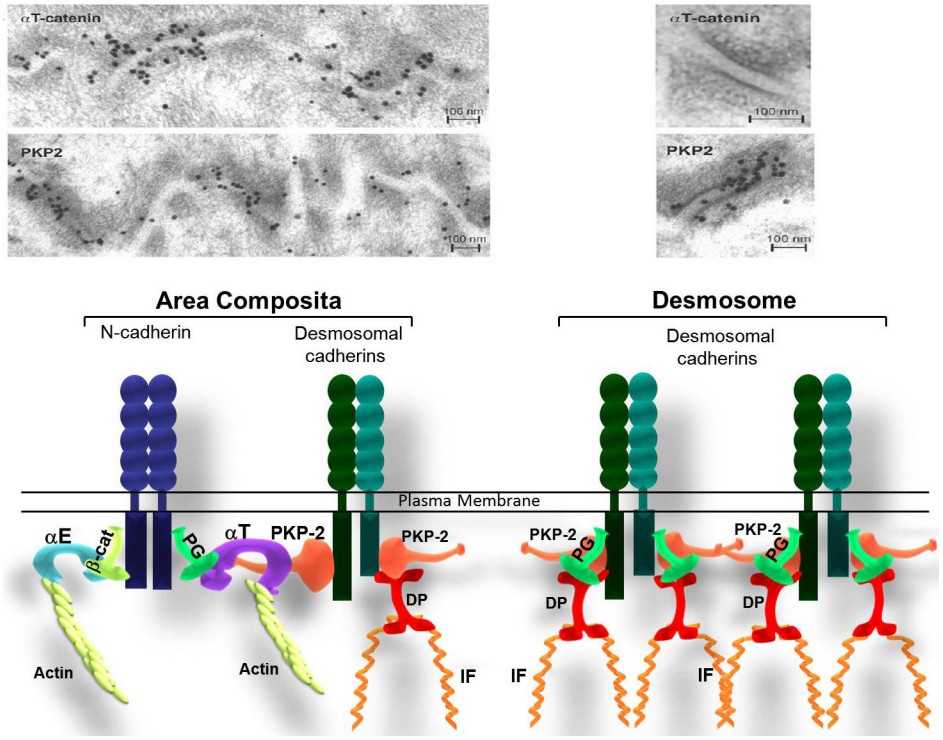

Figure 2: Molecular organization of Area composita vs. desmosome in the heart.

a) Immunoelectron microscopy images of the myocardium of a mouse heart showing the co-localization of PKP2 and αT-catenin at the area composita. Note the absence of αT-catenin in the desmosome. These images were originally published in "J Cell Sci., Goosens et al. 2007". b) Model for cadherin-based cell-cell adhesion in the heart. This model represents the composition of a hybrid junction compared to a desmosome. αT-catenin recruits desmosomal proteins through its interaction with plakophilin-2 (PKP2), forming a hybrid junction (named area composita), reinforcing the intercalated discs resistance. (αE: αE-catenin; αT: αT-catenin; β-cat: β-catenin; PG: Plakoglobin; PKP2: Plakophillin-2; IF: Intermediate Filaments).

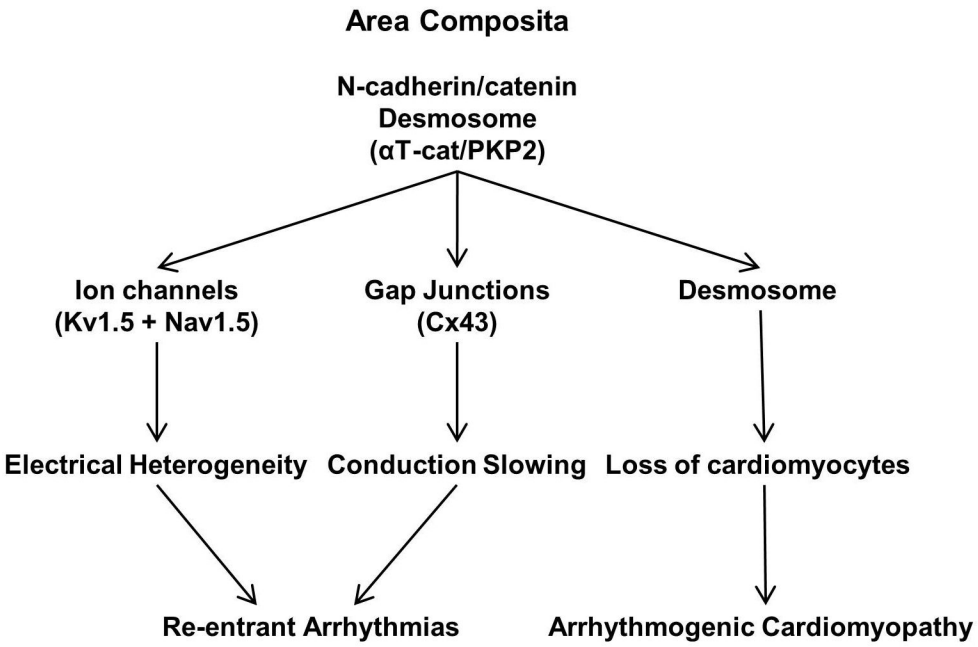

Figure 3: Functional Hierarchy at the Intercalated Disc.

Scheme depicts the relationship between the different junctions and cardiac dysfunction.

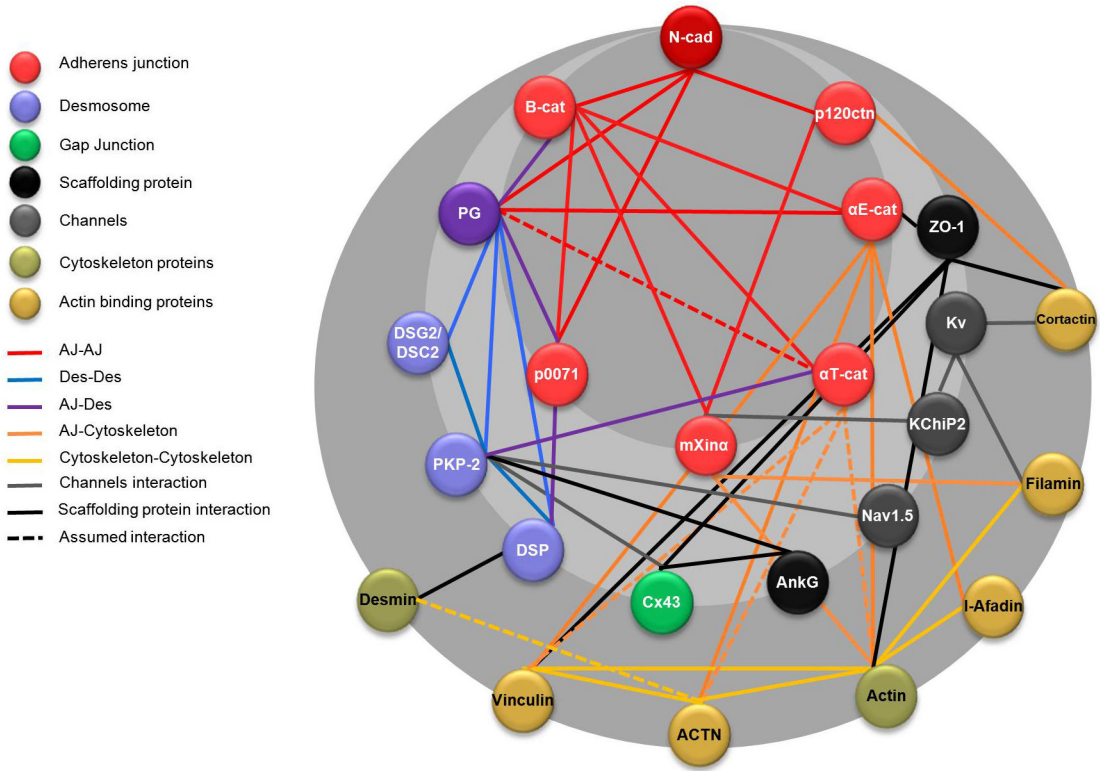

Figure 4: Junctional proteins interactome.

The intercalated disc protein-protein interaction network is based on STRING v9.1 analysis (http://string-db.org/) using the default settings (Franceschini et al., 2013). Black lines represent direct interactions, while dotted lines represent assumed interactions based on studies of the structures homology. (Ncad: N-cadherin; B-cat: β-catenin; p120ctn: p120-catenin; PG: Plakoglobin; PKP2: Plakophillin-2; DSG2: Desmoglein-2; DSC2: Desmocollin-2; DSP: Desmoplakin; Cx43: Connexin-43; AnkG: Ankyrin G; Kv: Potassium channels; kChiP2: Kv channel-interacting protein 2; ACTN: Actinin).

Table I

Genetic manipulation of adherens junction proteins in the adult myocardium

Mouse model	Stress	Cardiac Phenotype	Reference
N-cadherin LOF	None	Cardiomyopathy, GJ remodeling, arrhythmia, SCD	(Cheng et al., 2011, Franceschini et al., 2013, Kostetskii et al., 2005, Li et al., 2005)
N-cadherin HET	PS	Normal cardiac function, GJ remodeling, inducible arrhythmia	(Li et al., 2008)
β-catenin LOF	None	Normal cardiac function, increase PG	(Zhou et al., 2007)
β-catenin LOF	TAC	Block hypertrophy, decrease Wnt genes	(Chen et al., 2006)
β-catenin LOF	AngII	No change in hypertrophy response	(Baurand et al., 2007)
β-catenin LOF	MI	Improve LV function, increase cpc	(Zelarayan et al., 2008)
β-catenin HET	TAC	Block hypertrophy, increase fetal genes	(Qu et al., 2007)
$\beta\text{-catenin}^{\Delta ex3}$ GOF	None	Cardiomyopathy, no change Wnt genes	(Hirschy et al., 2010)
$\beta\text{-catenin}^{\Delta ex3}$ GOF	MI	No improvement in LV function, decrease cpc differentiation	(Zelarayan et al., 2008)
$\beta\text{-catenin}^{\Delta ex3}$ GOF	AngII	Block hypertrophy, decrease cardiac function	(Baurand et al., 2007)
PG LOF	None	AC-like pathology except adipocytes	(Li et al., 2011a, Li et al., 2011b)
PG Hypomorph	None	Normal cardiac function, increase β-catenin	(Swope et al., 2012)
PG GOF	None	AC-like pathology including adipocytes	(Lombardi et al., 2011)
PG^{Naxos} GOF	None	AC-like pathology including adipocytes	(Lombardi et al., 2011)
αE-catenin LOF	None	Cardiomyopathy late onset	(Sheikh et al., 2006)
αE-catenin LOF	MI	Ventricular wall rupture	(Sheikh et al., 2006)
αT-catenin LOF	None	Cardiomyopathy early onset, GJ remodeling	(Li et al., 2012)
αT-catenin LOF	I/R	Arrhythmia	(Li et al., 2012)

LOF, loss-of-function; GOF, gain-of-function; HET, heterozygous; SCD, sudden cardiac death; PS; program stimulation; GJ, gap junction; TAC, transverse aortic constriction; AngII, angiotensinII; MI, myocardial infarction; cpc, cardiac progenitor cells; I/R, ischemia/reperfusion.