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Cell–cell junction remodeling in the heart: Possible role in cardiac conduction system function and arrhythmias?

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Abstract

Anchoring Cell–cell junctions (desmosomes, *fascia adherens*) play crucial roles in maintaining mechanical integrity of cardiac muscle cells and tissue. Genetic mutations and/or loss of critical components in these macromolecular structures are increasingly being associated with arrhythmogenic cardiomyopathies; however, their specific roles have been primarily attributed to effects within the working (ventricular) cardiac muscle. Growing evidence also points to a key role for anchoring Cell–cell junction components in cardiac muscle cells of the cardiac conduction system. This is not only evidenced by the molecular and ultra-structural presence of anchoring cell junctions in specific compartments/structures of the cardiac conduction system (sinoatrial node, atrioventricular node, His-Purkinje system), but also because conduction system-related arrhythmias can be found in humans and mouse models of cardiomyopathies harboring defects and/or mutations in key anchoring Cell–cell junction proteins. These studies emphasize the clinical need to understand the molecular and cellular role(s) for anchoring Cell–cell junctions in cardiac conduction system function and arrhythmias. This review will focus on (i) experimental findings that underline an important role for anchoring Cell–cell junctions in the cardiac conduction system, (ii) insights regarding involvement of these structures in age-related cardiac remodeling of the conduction system, (iii) summarizing available genetic mouse models that can target cardiac conduction system structures and (iv) implications of these findings on future therapies for arrhythmogenic heart diseases.

Keywords

Heart; Anchoring Cell–cell junction; Desmosomes; Fascia adherens junction; Cardiac conduction system; Cardiac arrhythmias; Aging heart; Arrhythmogenic heart disease; Mouse models

Introduction

The contractile and synchronous nature of the heartbeat requires robust mechanical and electrical coupling to maintain the physical and functional integrity of the heart. For tissues that undergo constant mechanical stress, like the heart, anchoring Cell–cell junctions are particularly relevant since they provide stability to cardiac muscle cells in the face of severe stress by mechanically ‘anchoring’ cells to one another while the heart expands and contracts. In adult ventricular and atrial cardiac muscle cells, these Cell–cell junctions are classically localized at the longitudinal end and in a step-wise fashion at lateral ends, in a

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Conflict of interest statement

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structure known as the intercalated disc (Shimada et al., 2004). Intercalated discs can be readily visualized through light microscopy as an eosinophilic band following hematoxylin/eosin staining of heart tissue sections. At the molecular level, the intercalated disc appears as a highly organized triad of junctions between cardiac muscle cells, which includes both adhesive as well as communicating structural complexes containing anchoring (e.g. desmosomes and adherens junctions) and communicating junctions (e.g. gap junctions), respectively (Sheikh et al., 2009). Considerable attention has focused on the importance of anchoring cell junctions within cardiac muscle cells of the working (especially ventricular) myocardium, mainly because human and mouse genetic studies show that loss/mutations of key components within these complexes are associated with and can recapitulate human right and left ventricular cardiomyopathies, such as arrhythmogenic right ventricular dysplasia/cardiomyopathy and dilated cardiomyopathy, respectively (Garcia-Gras et al., 2006; Kostetskii et al., 2005; Li et al., 2005; Pilichou et al., 2006). For this reason, anchoring cell junctions have become the subject of a great deal of attention in both basic and clinical cardiac research fields.

Growing evidence also suggests potential key roles for anchoring cell junctions within specialized cardiac muscle cell subpopulations that form structures of the cardiac conduction system (sinoatrial node, atrioventricular node, His-Purkinje system). These cell populations are critical for generating and propagating electrical signals across the heart, giving rise to normal heart rhythm. It has become increasingly clear that a more thorough understanding of these structures in cardiac conduction system cells is of clinical necessity. Arrhythmias are a key feature of cardiomyopathies associated with genetic defects and/or loss of anchoring cell junction components (Asimaki et al., 2009; Delmar and McKenna, 2010), yet there is limited information on their origin, triggers, and specific underlying mechanisms. Furthermore, this cardiomyocyte subpopulation is especially relevant in the aging population. Cardiac conduction system cells are particularly prone to age related structural and functional remodeling, increasing the likelihood of arrhythmias (Haqqani and Kalman, 2007; Yanni et al., 2009) and the requirement for pacemaker implantation (Mond et al., 2008) in the growing aging population. This review will provide an up-to-date overview of the anchoring cell junction complexes currently identified within the cardiac conduction system and discuss evidence regarding cross-talk between proteins of these junctions and electro-generating, propagating and coupling channels (e.g., connexins and ion channels) to highlight how defects in the anchoring cell junction protein complexes may play a pivotal and specific role in cardiac conduction system related arrhythmias.

Anchoring Cell-cell junction biology in cardiac muscle

The heart contains a number of cell types that contain a wide variety of Cell-cell junction complexes. These may be broadly classified according to function as anchoring and communicating junctions. Anchoring junctions bind one cell to another through direct association with cytoskeletal components of both cells and include the adherens junctions and desmosomes. Adherens junctions are mainly composed of *fascia adhaerentes/fascia adherens* junctions (Franke et al., 2009) which, in cardiac tissue, are formed by membrane spanning cadherins (N-cadherin) linking the actin microfilaments at the cytoplasmic end with cadherins from the neighboring cell at the extracellular end. The molecular components of *fascia adherens* junctions in cardiac muscle include the (i) main transmembrane protein N-cadherin (~88 kDa), as well as other more recently identified transmembrane and catenin-binding proteins: Coxsackievirus and adenovirus receptor (CAR; ~40 kDa) and lysosomal integral membrane protein 2 (LIMP-2; ~54 kDa) (Lim et al., 2008; Schroen et al., 2007), (ii) the catenins/armadillo proteins, α , β and γ (plakoglobin)-catenin (~102, ~88 and ~82 kDa respectively) as well as (iii) the cytoskeletal actin-binding proteins, vinculin/metavinculin (~117/124 kDa), zonula occludens-1 (ZO-1; ~220 kDa), Xin repeat containing protein,

mXinc (~155 kDa) and α -actinin (~110 kDa) (Borrmann et al., 2006; Choi et al., 2007; Franke, 2009; Gutstein et al., 2003; Itoh et al., 1997; Sheikh et al., 2006). Desmosomes (*maculae adherentes*) are considered the strongest anchoring junctions and are formed by specialized cadherins (desmocollin-2 ~100 kDa and desmoglein-2 ~122 kDa in the heart) of neighboring cells that bind to one another at the extracellular end (Franke, 2009). From the intracellular side, the desmosomal cadherins are anchored to the catenins/armadillo proteins, β and γ -catenin (plakoglobin), as well as plakophilin-2 (~97 kDa) which are in turn bound to the central cytoplasmic component, desmoplakin (~250 kDa, large isoform; ~210 kDa small isoform), that anchor directly to the intermediate filament network of cardiac muscle via desmin (55 kDa) (Sheikh et al., 2009) (Fig. 1). Recent studies also show that the localization of molecular components of desmosomes and *fascia adherens* junctions is not as distinct as previously thought and can overlap in a structure known as the '*area composita*', which is a hybrid junction combining components from both complexes and suggesting cross-talk between desmosomes and *fascia adherens* junction (Pieperhoff et al., 2010). Communicating junctions (gap junctions or *nexus*), are channels that allow passage of small molecules and ions between two cells and are formed by the connexin family of proteins. These tetraspan membrane proteins form hexamers ("connexons") at each cell membrane and give rise to a pore between two neighboring cells. A detailed review of specific connexins in the cardiac conduction system is summarized in Severs et al. (2008). The recent identification of ZO-1, and its presence at *fascia adherens junctions* have been proposed to actively target connexins to the gap junction (Barker et al., 2002; Toyofuku et al., 1998, 2001). However, there are more complex functions of ZO-1 in relation to gap junction biology since Rhett et al. (2011) have also proposed that ZO-1, which is present at the perinexus (surrounding the gap junction), might prevent connexon recruitment to the communicating *nexus*. Furthermore, Shaw et al. (2007) propose a model for adherens junction mediated targeting of connexin 43 (Cx43) to gap junctions, suggesting that proper gap junction positioning at the ends of cardiomyocytes depends on the presence and interaction of adherens junction proteins and the cellular trafficking machinery. A schematic representation of the major molecules that form the anchoring Cell-cell junctions in cardiac muscle alongside electro-propagating, generating and coupling channels (e.g., connexins, ion channels) is shown in Fig. 1.

Cardiac conduction system: Evidence of anchoring cell junction structures and molecular components

The generation and propagation of spontaneous action potentials by the cardiac conduction system (CCS) have stimulated decades of exciting research concerning the role of electrical channels (e.g., connexins and ion channels) in CCS structures. An additional level of complexity is brought to the CCS when increasing evidence suggests that anchoring Cell-cell junctions, thought to purely serve a mechanical scaffolding role, may also be involved in maintaining the stability and function of electro-generating, propagating and coupling channels. Therefore, understanding how CCS cells are anchored to one another may provide important insights on how anchoring structures may play an intimate role in the maintenance of electrical channels and a healthy CCS. The main components of the CCS include the sinoatrial node (SAN), atrioventricular node (AVN) and the His-Purkinje system (Boyett, 2009). These structures are classically formed by specialized cardiac cells that possess a rudimentary contractile apparatus but are rich in mitochondria and glycogen, as observed by transmission electron microscopy (Shimada et al., 2004). The developmental origin of the fine structures of the CCS has been elucidated through genetic and lineage tracing analyses (Christoffels et al., 2010; Mikawa and Hurtado, 2007). Agreement has been reached regarding the myogenic origin of these structures, however the patterning of the entire CCS network is still a matter of debate. This is most evident as regards to Purkinje network development. While some groups suggest that an "out-growth" mechanism by which distal

elements of the CCS are derived from proximal progenitors others suggest that there is local recruitment of cells, which are in turn coupled *in situ* (Mikawa and Hurtado, 2007). Evidence for the presence of specific anchoring junction structures and/or the proteins that form them in the CCS as well as relevant references is summarized in Fig. 2. The following sections will summarize key experimental findings, which demonstrate the ultra-structural and molecular presence of anchoring cell junctions and their components, respectively, in specific CCS structures.

Sinoatrial node—The SAN serves as the primary pacemaker of the heart and is thought to arise *de novo* from cardiac progenitor cells expressing the T-box transcription factor Tbx18, while not expressing the cardiac transcription factor, Nkx2.5, after embryonic day 9 to 9.5 (E9–E9.5) of mouse heart development (Wiese et al., 2009). Once fully developed in the postnatal heart, the mature SAN cells anatomically run parallel at the junction between the superior vena cava and the right atrium, as well as display evidence of end to end connections, which include the presence of anchoring and communicating junctions (Shimada et al., 2004). These nodal cells are described as spindle, elongated or spider-like according to their shape after isolation. They are found to have a few micro projections (spindle and elongated cells) but may also be ramified (spider cells) (Mangoni and Nargeot, 2008; Shimada et al., 2004). Although gap junction biology in the heart has been extensively reviewed elsewhere (Boyett, 2009; Severs et al., 2008), it is important to note that the connexins in cardiac muscle cells of the CCS differ from those found between working (atrial/ventricular) cardiac muscle cells, which has been a useful molecular marker in distinguishing specific CCS structures, such as the SAN, in the heart. Namely, Cx43 and Cx40, which form the large conductance gap junctions in the ventricular and atrial cardiac muscle cells, respectively, are largely absent from the central portion of the SAN (van Kempen et al., 1995, Van Kempen et al., 1996). Instead, centralized SAN cells express the gap junction proteins, Cx30, Cx30.2 (in mouse) (Gros et al., 2010; Kreuzberg et al., 2006) and Cx45 (in human and rabbit) (Coppin et al., 1999a,b, 2003; Davis et al., 1995). SAN cells can also be readily molecularly distinguished from working cardiac muscle in sectioned heart tissue by assessing for the combined expression of hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (Hcn4), which is responsible for the funny current and pacemaker action potential in heart (Alig et al., 2009; DiFrancesco, 2010), and the presence of Cx45 (Coppin et al., 1999a,b) which forms low conductance pores in the SA and AV nodes (Severs et al., 2008). Elegant scanning and transmission electron microscopy studies performed by Shimada et al. provide strong evidence for the presence of specific anchoring intercellular junctions and their associated molecular components in the CCS of different species, including monkeys and humans. These studies show that SAN cells display typical desmosomal and gap junction structures, which are visible at the ultra-structural level (Shimada et al., 2004). Although the specific proteins forming these complexes have not been systematically studied, various reports focusing on SAN function have identified key molecular components in these structures. The central desmosomal protein, desmoplakin, was identified to be localized in the guinea pig SAN by immunofluorescence microscopy studies (Dobrzynski et al., 2000). Although *fascia adherens* junction ultra-structures are not readily apparent, molecular components of the *fascia adherens* junction, β -catenin, have also been localized to specific regions of the SAN in rabbit hearts (Peters et al., 2009). The structural presence of key anchoring cell junction elements alongside gap junction components in SAN cells highlights their potential importance in maintaining SAN physiology and function. Since the SAN contains a gradient population of cells ranging from periphery to center (Boyett et al., 2000), it is also tempting to speculate that these structures may be important to tether macromolecular complexes in these cells and modulate the strength of electrical connectivity across these gradients. Further studies will be required to establish if Cell–cell junction structures are composed of

similar and/or distinct macromolecular complexes with similar and/or distinct functional roles in SAN cardiac muscle cells, when compared to cardiac muscle cells of the working myocardium.

Atrioventricular node—The AVN is the second major site of pacemaker function in the heart, but exhibits slower pacemaking activity than the SAN. It is thought to originate *de novo* from the cardiac transcription factor, *Tbx3*, positive precursor cells of the atrioventricular canal, which itself is largely derived from the cardiac crescent and the first heart field as early as E9.5 of the developing mouse heart (Aanhaanen et al., 2010; Christoffels et al., 2010). Recent studies also point to contributions from precursors, which stem from the atrial septum and the myocardium of the embryonic interventricular ring (Aanhaanen et al., 2010; Christoffels et al., 2010). In the adult heart, the AVN is insulated and localizes at the base of the interatrial septum in a region which is deeply embedded in the endocardial tissue. Molecular markers that define the AVN region include the expression of the ion channel, *Hcn4*, the transcription factor *Tbx3* and gap junction proteins, *Cx45* (Coppén et al., 1999a,b) and *Cx30.2* (mouse) as well as acetylcholine-esterase (AChE) enzyme activity (Aanhaanen et al., 2010). The absence of *Cx43* as well as the sodium channel, voltage-gated, type V, α subunit, *Scn5a*, also further distinguishes the AVN from the working myocardium (Christoffels et al., 2010). Similar to the SAN, the AVN cells contain few myofibrils but also contain Cell–cell junction ultra-structures, which include desmosomes and gap junctions (Shimada et al., 2004). In terms of molecular components, desmoplakin immunoreactivity at the AVN has been observed in rat hearts (Yoo et al., 2006). Studies in mouse hearts also revealed that the *fascia adherens* junction proteins CAR (Lim et al., 2008; Lisewski et al., 2008) and ZO1 (Lim et al., 2008) are expressed in the AVN of the adult heart, along with the adaptor protein plakoglobin (γ -catenin), which is dually present in both desmosomes and *fascia adherens* junctions (Lim et al., 2008). The importance of *fascia adherens* junctions in AVN function was demonstrated when genetic deletion of CAR in cardiac muscle cells *in vivo*, using the α -myosin heavy chain (MHC)-Cre mouse line, resulted in AV block (first, second and third degrees) in adult mice (Lim et al., 2008; Lisewski et al., 2008). Although the precise mechanisms remain unclear, it was demonstrated that CAR directly formed a complex with the gap junction protein, *Cx45*, and loss of this interaction at Cell–cell junctions within the AVN was suggested to be a contributing factor to the development of AVN functional defects (Lim et al., 2008). These studies highlight the importance of understanding the precise macromolecular complexes formed between anchoring Cell–cell junctions and electrical channels in fine CCS structures and the necessity to understand their importance in CCS biology and diseases.

His-purkinje network—The His-Purkinje network serves to enable rapid passage of electrical impulses into the contractile ventricular myocardium, and thus termed the ventricular conduction system (Boullin and Morgan, 2005). This network is thought to originate from the embryonic myogenic precursors of the ventricular myocardium as shown in experiments performed in developing chick hearts (Gourdie et al., 1995) and includes the lower cells of the AVN, His bundle (or Atrioventricular bundle), right and left bundle branches, and the Purkinje fiber conduction cells (Aanhaanen et al., 2010). In the adult heart, the localization of the His bundle extends from the AVN at the posterior right atrial wall near the atrial septum, while following the path of the upper part of the ventricular septum before bifurcating to become left and right bundle branches, which descend on either side of the septum to give rise to the Purkinje network of fibers (Boullin and Morgan, 2005). The atrioventricular/His bundle cells are mostly cylindrical in shape and are enriched in intercalated discs, which exhibit an irregular “stair-like” pattern (Shimada et al., 2004). Purkinje cells are larger in size than the surrounding cardiac muscle cells with less

myofibrils but are highly enriched in glycogen and also contain intercalated discs that exhibit a typical “stair-like” pattern, but appear less mature, in terms of microprojections, than the intercalated discs observed in working cardiac muscle cells (Shimada et al., 2004). Similar to the atrioventricular/His bundle, they can be cylindrical but also polygonal in shape and can connect to one another via end–end connections as well as unique lateral connections, a feature that has also been observed in bovine hearts by Pieperhoff et al. (2010). Specifically, these conductive cells are thought to remain in contact with one another through lateral connections via an extended plasma membrane region, which contains an exceptionally high proportion of junctions with desmosomal characteristics and components, some of which can be distinguished from *fascia adherens* junctions and others found in conjunction with *fascia adherens* junctions in the *area composita*, which is largely devoid of gap junctions (Pieperhoff et al., 2010). These specialized area composita junctions at lateralized regions, contain molecular components found in both desmosomes (plakophilin-2, desmoplakin, desmoglein2, desmocollin2) and *fascia adherens* junctions (N-cadherin, β -catenin) (Pieperhoff et al., 2010). The presence of anchoring cell junctions at both lateralized and end–end regions in these specific conductive cells further highlights the unique requirement for reinforcing mechanical strength in CCS cells of the ventricular myocardium. These data invite future studies to delineate the specific role for the macromolecular complexes underlying anchoring Cell–cell junctions (specifically desmosomes due to their enrichment in these structures) in His-Purkinje network biology and diseases.

Cross-talk between anchoring Cell–cell junctions and electro-generating, propagating and coupling channels?

While the heart acts as a powerful mechanical pump the synchronous nature of the heartbeat requires that each cardiac muscle cell intimately ‘communicates’ with its adjacent neighbors, suggesting a level of crosstalk between Cell–cell connections, Cell–cell communication and electrical synchrony that cannot be uncoupled. A growing body of data obtained from *in vitro* cell culture models, *in vivo* genetically engineered animal models and human disease causing mutations underscores the importance of anchoring Cell–cell junction structures in the formation and maintenance of proper electrical propagation of the action potential in the heart. Aside from the physical proximity of these complexes at the intercalated disc, previous studies highlight a functional association between anchoring Cell–cell junction components and coupling gap junction channels in working cardiac muscle. First there is the question of localization at the ends of cardiomyocytes of the three main structures of the cardiac ventricular intercalated disc: desmosomes, *fascia adherens* and gap junctions. The temporal distribution of intercalated disc components (desmosomes, *fascia adherens* and gap junction) from the embryonic to the adult mammalian heart follows what could be described as a hierarchy of events, first during embryonic development, desmosomes and *fascia adherens* junctions are localized at the ends of myocytes and later during the postnatal development gap junctions also form at those sites (Angst et al., 1997). Gap junction positioning seems to require the presence of at least *fascia adherens* proteins such as N-Cadherin as observed *in vitro* (Hertig et al., 1996). Both these studies highlight the interdependence between Cx43 and components of desmosomes (desmoplakin) and *fascia adherens* (N-Cadherin) for proper positioning of gap junctions at the intercalated disc. Physical interactions between Cx43 and N-Cadherin proteins have been reported by various groups. Studies by Shaw et al. highlight interactions between Cx43, tubulin and N-Cadherin during gap junction formation (Shaw et al., 2007). In addition, independent studies report that Cx43 and N-Cadherin form a multi-protein complex that could allow for the assembly of both gap and *fascia adherens* junctions (Wei et al., 2005). *in vivo* loss or decrease of N-cadherin from the mouse heart leads to a decrease in total Cx43 levels disrupting cardiac impulse propagation resulting in lethal ventricular arrhythmias (Li et al., 2005, 2008).

Further reinforcing this idea, recent studies by Cheng et al. (2011) highlighted that N-cadherin regulates Kv1.5 channel function at the intercalated disc in cardiac muscle cells, through a disruption of the actin binding scaffolding protein, cortactin, revealing a novel mechanistic link between N-cadherin and ion channel function. A potential molecular link between the desmosomal protein, desmoglein2 and the gap junction protein, Cx43 was recently suggested, when a novel Desmoglein 2a mutation identified in a patient exhibiting right ventricular conduction abnormalities, abolished a newly discovered interaction between desmocollin-2a and Cx43, resulting in defects in Cx43 protein expression and phosphorylation, suggesting a more direct level of cross-talk between these structures than previously observed (Gehmlich et al., 2011). *in vivo* removal of CAR from cardiac muscle cells lead to a decrease in the electro-coupling gap junction protein, Cx45 at Cell–cell junctions in the AVN leading to AV block, providing a role for CAR within the gap junction complex because of its physical association with Cx45, while highlighting specific cross-talk between anchoring Cell–cell junctions and electro-coupling channels in the CCS (Lim et al., 2008; Lisewski et al., 2008). These studies serve as an example to demonstrate that remodeling of electro-coupling channels such as gap junctions are very tightly linked to architectural remodeling of the heart and the molecules that anchor them. Growing evidence also points to new physical associations between anchoring Cell–cell junctional components and specific electro-generating and propagating channels (ion channels), which modulate ion currents in cardiac cells. Confocal microscopy and immunofluorescence labeling studies performed in SAN of guinea pig hearts, revealed preferential co-localization of the ion channel, Kv1.5, which is responsible for ultra-rapid delayed rectifying K⁺ current and action potential repolarization, with the desmosomal protein, desmoplakin and desmosomes (Dobrzynski et al., 2000). Although further studies are needed to explore this connection, it has been hypothesized that sites of anchoring Cell–cell junctions may provide a more suitable site for anchoring channel proteins to the cytoskeleton, which can result in less exposure to mechanical stress, or alternatively may be the first site of newly formed channels by the biosynthetic machinery (Dobrzynski et al., 2000; Mays et al., 1995). Similar studies performed in SAN of rabbit hearts, revealed co-localization of the SAN pacemaker, Hcn4 channel with the *fascia adherens* junction component, β -catenin (Peters et al., 2009) and possible interaction of these molecules. Furthermore, studies have identified that the major cardiac Na_v1.5 sodium channel, is localized at the lateral membranes, t-tubules and at the intercalated disc of cardiomyocytes (Malhotra et al., 2004; Petitprez et al., 2011). At the intercalated disc the localization is dependent upon AnkG and plakophilin-2 (Sato et al., 2009, 2011). Regulation of ion channel function by these same anchoring cell junction complexes was demonstrated when loss of plakophilin-2, via siRNA knockdown strategies, in isolated neonatal rat cardiomyocytes resulted in slowed propagation of the action potential in a monolayer of cultured cardiac muscle cells, as well as alterations in the properties of the sodium current (I_{Na}) as demonstrated by patch clamping techniques (Sato et al., 2009). Recent studies suggested that these electrophysiological defects associated with loss of plakophilin-2, may be sufficient to create a substrate for reentrant activity associated with increased arrhythmogenesis (Deo et al., 2011). These tight spatial relationships between the desmosomal and *fascia adherens* junction proteins to gap junction/ion channels in cardiac muscle cells encourage further exploration on how members of anchoring junctions may cross-talk and affect electrical channel function specifically in the CCS.

Do defects in anchoring Cell–cell junctions also underlie cardiac conduction system diseases?

Electron microscopy and immunohistochemistry studies have identified anchoring Cell–cell junction ultra-structures and molecular components in the CCS. Evidence for a functional connection between both was best noted by two independent groups which reported that cardiac muscle-specific loss of the anchoring Cell–cell junction component, CAR, in the

mouse *in vivo* using the α -MHC Cre mouse line, lead to defects in working cardiac muscle but also direct effects on CCS cardiac muscle cells as evidenced by the impaired AVN conduction and loss of CAR-Cx45 interaction in AVN Cell–cell junctions, as noted by *Lim et al.* (Lim et al., 2008; Lisewski et al., 2008). These studies highlight that conventional cardiac-muscle specific Cre mouse lines have the ability to target gene deletion in the CCS and the need for researchers to more closely examine cardiac phenotypes in genetic mouse models using these Cre lines, which may also unmask novel functional defects in the CCS. Human genetic studies have begun to have an impact in unmasking specific CCS dysfunction in family members of patients harboring mutations in anchoring Cell–cell junction components (e.g., desmosomal complex) associated with end-stage arrhythmogenic right ventricular cardiomyopathy (ARVC) and dilated cardiomyopathy (DCM) (Cox et al., 2011; Quarta et al., 2011). Interestingly, in asymptomatic relatives of ARVC patients, abnormal ECG findings (terminal activation delay and epsilon waves) were significantly correlated with the presence of a mutation in a desmosomal gene (Cox et al., 2011). In a Finnish study that tested the association between specific desmosomal mutations and electrocardiographic findings in a general population cohort, it was demonstrated that carriers of a *DSP* mutation T1373A had significant prolongation of the PR interval suggestive of first degree AV block (Lahtinen et al., 2011). Although specific desmosomal mutations were not assessed in this particular ARVC patient, a case report highlighted that atrial arrhythmias associated with SAN dysfunction in the form of Sick Sinus Syndrome (SSS) and not ventricular arrhythmias may be the cause of symptoms in some cases of ARVC (Takemura et al., 2008). Interestingly patients with familial/inherited DCM may also harbor mutations in desmosomal genes (Elliott et al., 2010; Perriard et al., 2003). This is relevant to CCS dysfunction because familial DCM can be classified according to the presence or absence of conduction system disease (Hershberger, 2011). The relationship between the genetics of the patient and the phenotypic manifestation of this familial form of DCM is still incompletely understood giving room for further areas of research that may explain phenotypes where only borderline DCM is present and yet there is evident conduction system disease (Hershberger and Siegfried, 2011).

Age-related cardiac conduction remodeling: A role for anchoring Cell–cell junctions?

The aging heart undergoes deleterious changes in CCS remodeling, which is thought to be a contributing factor for the relatively high prevalence of cardiac arrhythmias in the aging population. Atrial arrhythmias, including Sick Sinus Syndrome (SSS) and atrial fibrillation (AF), are an especially growing public health concern in the aging population (Iguchi et al., 2010). SSS incidence is higher in the elderly (>65 years of age) and results in a significant loss of SAN function and also increases the risk of AF in this growing population (Mohler and Anderson, 2008; Sweeney et al., 2007). Although the mechanisms underlying these age-related cardiac arrhythmias are not precisely known, there is growing evidence to suggest that molecular remodeling of electro-generating, propagating and coupling channels as well as structural remodeling of the CCS, are important contributors which coincide with functional alterations associated with these arrhythmias. In aged guinea pigs (26 months old) the peripheral SAN region loses L-type Ca^{2+} channel expression (Jones et al., 2007), and the atrial tissue that surrounds the SAN loses Cx43 expression, further isolating nodal conduction to the remainder of the heart (Jones et al., 2004). These changes not only coincide with age dependent transcriptional downregulation of pacemaker channels, *Hcn2* and *Hcn4* but also slowed conduction velocity from the SAN into the atrial tissue (Huang et al., 2007). Furthermore there is evidence of lengthened conduction distance of the action potential generated by the nodal cells within the senescent guinea pig heart (38 months) (Jones et al., 2004) suggesting age-related tissue remodeling. This has also been observed in aged rats (18–24 months) where the nodal cell size increases with age as does mean interstitial volume of the SAN region (de Melo et al., 2002; Yanni et al., 2009). Despite

these implications, there is a growing need to determine whether alterations in anchoring cell junction structures, which are ultra-structurally and molecularly present in CCS structures and known to functionally couple to electro-generating, propagating and coupling channels in CCS structures as described above, are major contributors of the arrhythmia landscape in the age related dysfunction of CCS structures. This question is very relevant since the mechanisms that lead to alterations in connexins and ion channels such as Cav1.3 and Hcn4 (Huang et al., 2007) in aged hearts and CCS structures have not been elucidated and may be important drivers of susceptibility towards these forms of cardiac arrhythmias. The intimacy and relevance of these connections are further reinforced in studies by *Li et al.*, which highlighted that haploinsufficiency of the anchoring cell junction component, N-cadherin, affects Cx43 expression in the adult heart and that double N-cadherin/Cx43 compound heterozygous mutants displayed increased susceptibility to atrial and ventricular arrhythmias, when compared to single mutant mice (Li et al., 2008; Mays et al., 1995).

Mouse models as molecular tools to dissect cellular origin of cardiac conduction arrhythmias *in vivo*

During the past decade genetically engineered mouse models have greatly increased our ability to identify the role of specific genes in specific subsets of cells and organs throughout development or adult life *in vivo*. Through the discovery of cardiac-specific promoters (e.g., α -myosin heavy chain (MHC), myosin light chain-2v (MLC2v)) and the advent of Cre-loxP technology, it has been possible to achieve tight spatial and temporal control of gene expression and ablation in the heart and particularly in cardiac muscle cells in mice *in vivo* (Sheikh and Chen, 2007). It is also apparent that cardiac-specific Cre recombinase mouse lines, which are typically used to achieve gene deletion in cardiac muscle cells of the working myocardium (e.g. α -MHC-Cre, MLC2v-Cre) can also target cells within the CCS and cardiac phenotypes. This is evident in the cardiac specific knockout mouse models described below where excision of the gene of interest *in vivo*, in this case CAR and Nkx2.5, was achieved using the α -MHC-Cre and MLC2v-Cre mouse lines, respectively. The authors observed that *in vivo* deletion of the CAR gene with α -MHC-Cre mouse line led to atrioventricular conduction block (Lim et al., 2008). Atrioventricular conduction block was also observed in mice with targeted ablation of the cardiac transcription factor Nkx2.5, using the MLC2v-Cre mouse line (Pashmforoush et al., 2004). These studies prompt the need for more refined CCS specific Cre recombinase mouse lines since mouse models generated using currently available cardiac-specific Cre recombinase mouse lines, may harbor cardiac muscle phenotypes that could mask direct effects on the CCS or be a mixture of effects on the CCS and working myocardium. Several studies point to the expression of a specific subset of ion channels and adapter molecules in the CCS that could be used to target cardiac muscle cells of the CCS in mice. Furthermore, attention must also be placed on the fact that the available 'cardiac specific' Cre recombinase mouse lines are cardiac myocyte specific and do not target other cell populations in the heart, such as cardiac fibroblasts, smooth muscle, endothelial cells, etc. These cell populations, and especially fibroblasts are abundant in the cardiac conduction system, proposed to also form direct connections to cardiac myocytes and are implicated to be important in the development of arrhythmias (Vasquez et al., 2011), and thus, may also have profound effects on the generation and/or propagation of the action potential giving rise to CCS related arrhythmias, warranting future studies in this area. For example, studies support the abundance of fibroblasts in the SAN and their potential to electrically couple with cardiac myocytes through the gap junction protein, Cx45 (Camelliti et al., 2004). Although the *in vivo* electrical coupling of myocytes to fibroblasts needs to be further explored (Vasquez et al., 2011), studies focused on targeting ablation of anchoring cell junction components specifically in cardiac fibroblasts, will be required to understand their specific contributory

role in relation to CCS related arrhythmias. Table 1 summarizes existing mouse models that target the cardiac conduction system.

Therapeutic implications

Current arrhythmia management, as in most pathologies, depends on the etiology of the disease, and when the etiology is unknown or there are no effective treatments available then the most effective method of restoring a rhythm that will support the physiological demands of the patient is pursued. These include pharmacological therapy, ablation of accessory pathways and pacemaker implantation. Anti-arrhythmic drugs have been organized in the Vaughan–Williams classification according to their effects on action potential duration, propagation and specific ion channel or receptor targeting (namely calcium channel blockers and beta receptors) (Makielski, 2011). Headway is being made to identify the mechanisms behind the beneficial effects of anti-arrhythmic drugs such as recently highlighted in studies by Zhou et al. (2011), which took advantage of the store overload-induced Ca^{2+} release inhibiting and selective (optimal) beta blocking properties of carvedilol to design a new drug compound (VK-II-86) that can be combined with more selective beta blockers to more effectively prevent stress-induced arrhythmias associated with heart failure in mice. However, some of the effects of anti-arrhythmic drugs are not completely understood with the current knowledge available, as is the case with the most widely prescribed anti-arrhythmic drug — amiodarone. In addition many arrhythmias are unresponsive to pharmacological treatment altogether, and thus require surgical intervention and implantation of pacemakers. Such is the case for cardiac conduction system diseases, such as sinus node dysfunction. As reviewed by Olshansky et al. (2010) current research in drug therapy has been broadened to include modulation of ion channel activity by targeting underlying macromolecular complexes. In other words target the interaction between the ion channel (“physiologic unit”) and adaptor molecules. An example of the latter is the ankyrin family of proteins (Le Scouarnec et al., 2008). In this regard, interactions between the macromolecular complexes that form anchoring junctions and ion channels might prove to be interesting candidates for drug discovery and therapeutic innovation. In the event of ion channel dysfunction, anchoring cell junction structures themselves can also be used as a potential strategy or target to reinforce the remaining functional channels within CCS structures. There is already evidence that the adaptor molecules, plakophilin2 and cortactin, are involved in Na^+ and K^+ channel regulation (Cheng et al., 2011; Sato et al., 2009). Both of these molecules have been shown to mediate interaction between the anchoring Cell–cell junction structures and the above-mentioned ion channels. More adaptor molecules and interesting interactions will certainly be discovered and may provide targets for a novel class of anti-arrhythmic drugs.

Future directions

Anchoring Cell–cell junctions are key components of cardiac conduction system and their co-localization with key regulators of electro-generating, -propagating and -coupling channels suggests they have important roles in cardiac conduction. Future experiments that define precisely how these classes of molecular components work in concert and affect specific fine cardiac conduction system structures (i.e. SAN, AVN, His-Purkinje network) and the arrhythmia landscape should provide important insights into the underlying causes and future therapeutic targets for cardiac conduction arrhythmias and arrhythmogenic heart diseases.

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Fig. 1. Schematic representation of the major molecular components found in anchoring cell junction complexes in the heart. These structures anchor the cytoskeleton of neighboring cells through their intermediate and microfilaments thus giving stability to these mechanically stressed cells at a region of the cell membrane rich in gap junctions. EC: extracellular, IC: intracellular, PM: plasma membrane.

Fig. 2. Schematic representation of the cardiac conduction system and the anchoring cell junction structures (circles) and molecular components (squares) as evidenced by electron microscopy and immunoreactivity, respectively. Key references which provided evidence for their ultra-structural or molecular inclusion within this figure are cited alongside each annotated structure and molecular component.

Table 1

Currently available mouse models that genetically delineate and can be used to study cardiac conduction system structure and function in vivo.

Target gene or region	Mouse model	Phenotype	Reference
<i>Hcn4</i>	Homologous recombination knockout	Hcn4-LacZ mouse has been used to delineate conduction	Garcia-Frigola et al. (2003),
	Homologous recombination knock-in Cre-ER ^{T2} recombinase.	system development in the mouse embryo. Hcn4 KO mice exhibit sinus node dysfunction with frequent sinus pauses.	Herrmann et al. (2007), and Hoesl et al. (2008)
<i>Hcn2</i>	Homologous recombination knockout	Hcn2 knock out mouse has a mild cardiac phenotype with increase HR variability but no sinus pauses.	Ludwig et al. (2003)
<i>minK</i>	Homologous recombination knock-in replacing <i>minK</i> gene by <i>LacZ</i> .	minK co-assembles with KvLQT1 which gives rise to the slow rectifier K current. minK expression genetically traces the cardiac conduction system only in the postnatal heart.	Kondo et al. (2003)
<i>Contactin-2</i>	<i>Cntn2-lacZ</i> knock-in and <i>Cntn2-EGFPBAC</i> transgenic reporter	LacZ and EGFP specifically label Purkinje network of cells (ventricular specialized cardiac conduction system).	Pallante et al. (2010)
<i>GATA6</i>	Transgenic Cre recombinase mouse line which is under the control of the chicken GATA6 promoter enhancer	Genetically delineates the atrioventricular conduction system with some expression in atrial cells. SAN and purkinje cells are not targeted.	Stroud et al. (2007)
<i>Tbx3</i>	Homologous recombination knock-in, which targets first 3 codons of Tbx3 coding region replaced by Cre. Transgenic overexpression of human TBX3 cDNA in the atria or whole heart.	Tbx3 is only expressed in the conduction system of the developing heart and is necessary for proper repression of the atrial program in the SAN cells.	Hoogaars et al. (2004, 2007)
Tbx18	Homologous recombination knockin of GFP in the coding sequence of Tbx18, Tbx18 ^{GFP} (or Tbx18 ^{tm2Aks}). Homologous recombination targeting Cre recombinase to Tbx18, Tbx18 ^{cre} (or Tbx18 ^{tm3Alds}).	<i>Tbx18</i> is required to form the head structure of the SAN.	Wiese et al. (2009)