Chapter from the book *Cardiomyopathies - From Basic Research to Clinical Management*

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1. Introduction

Proper cardiac function requires the synchronous mechanical and electrical activity of individual cardiomyocytes to ensure the coordinated excitation and contractile performance of the heart, as an organ. The intercalated disc (ID), a unique membrane structure forming at the edges of mammalian cardiomyocytes (Li and Radice, 2010), fulfills this role by allowing the transmission of mechanical and electrical activity between neighboring cells; (reviewed in Delmar and McKenna, 2010; Noorman et al., 2009).

1.1 A brief history of the ID

The ID was first depicted in 1866 by Karl Josef Ebhert et al. as “verdichtungsstreifen”, which literally translates to “compression strips”, but was later referred to as a homogeneous “cementing material” found at the ends of cardiac myocytes (Saphir and Karsner, 1924). A decade later, Engelman described the heart as a continuous syncytium, while a century later Weidmann suggested the presence of “membrane areas of synchronicity”, characterized by low resistance that allows the transmission of electrical potential (Engelmann, 1875; Weidmann, 1952).

The idea of a continuous region connecting two cells was challenged in the mid 1950’s by several groups who used electron microscopy to show that cardiac cells are separated from one another by a specialized extension of the sarcoplasm oriented transversely with respect to the cell’s boundaries (Sjostrand and Andersson, 1954; Van Breemen, 1953). Since the middle of the 20th century, we have significantly advanced our understanding of the structure and composition of the ID. Accordingly, the ID was found to be a highly organized structure composed of three main junctional complexes; the gap junctions, which enable the propagation of electrical stimuli throughout heart cells, the adherens junctions, and the desmosomes, which provide mechanical coupling and stability to cardiomyocytes, respectively. The advent of electron microscopy in the 1950’s to 1970’s further provided detailed visualizations of the regions connecting two cardiomyocytes (Fawcett and McNutt, 1969; McNutt et al., 1970; Muir, 1957; Rayns et al., 1969; Sjostrand and Anderssson, 1954; Van Breemen, 1953). Recently, novel cellular isolation techniques combined with scanning or transmission electron microscopy (SEM and TEM, respectively) have yielded three-
dimensional images of the ID (Hoyt et al., 1989; Shimada et al., 2004; Tandler et al., 2006), showing that in the mammalian ventricular heart, IDs are arranged both transversely and longitudinally in a stairwell like fashion with steps and risers. Transverse or plicate segments, resembling the steps, run in a zigzag arrangement with finger-like micro-projections, and contain mainly adherens junctions and desmosomes with smaller regions of gap junction plaques. Longitudinal or interplicate segments resemble the risers and contain mainly desmosomes and larger areas of gap junction plaques. The many folds and projections found within this region, increase the surface area of the ID, providing the cardiac cells with superior intercellular communication.

1.2 Spatiotemporal distribution of ID components

During cardiomyocyte development and maturation, major changes occur in structures associated with the ID. Studies using human myocardium showed that during embryonic development adherens junction and desmosomal organization follows that of gap junctions (Pieperhoff and Franke, 2007). However, during postnatal development proteins of the adherens and gap junctions appear to orient themselves at IDs simultaneously (Peters et al., 1994). Moreover, in vivo studies of lower mammals (including rodent, bovine and canine) have shown that at embryonic stages and postnatal day 1, components of gap junctions, desmosomes and adherens junctions are uniformly distributed throughout the sarcolemma, mutually exclusive from one another (Angst et al., 1997; Hirschy et al., 2006). However, at later postnatal stages (days 6-20), proteins of the adherens junctions and desmosomes begin to concentrate towards the termini of cardiomyocytes, leaving proteins of gap junctions uniformly distributed at the plasma membrane. By postnatal day 90, all components of the three junctions are segregated and organized at IDs. These findings were also supported by in vitro studies using primary cultures of rat and mouse cardiomyocytes (Geisler et al., 2010; Kostin et al., 1999). Interestingly, the latter further demonstrated that when individual cardiocytes are allowed to make contact in culture, proteins of the adherens junctions are the first to assemble and “mark” the location of the developing ID, closely followed by desmosomal proteins and finally proteins of gap junctions (Geisler et al., 2010; Kostin et al., 1999). Supporting this, the organization of adherens junctions and desmosomes is independent of gap junctions; however, gap junction organization requires that of adherens junctions and desmosomes (Gutstein et al., 2003; Wei et al., 2005). Taken together, these observations suggest that proteins necessary for mechanical coupling, i.e. components of adherens junctions and desmosomes, create the appropriate environment for proteins mediating electro-chemical coupling, i.e. those associated with gap junctions.

1.3 Organization of the ID

Gap junctions mediate the direct communication between neighboring cells by forming a low resistance pathway for the transmission of signals and electrical current (Rohr, 2004). A gap junction is composed of twelve connexin proteins, with connexin-43 being the most prominent in mammalian cardiomyocytes, along with low amounts of connexin-45 and 40 (Beyer et al., 1987; Vozzi et al., 1999). Each cardiomyocyte contributes six connexin molecules to form a hemi-channel, or a connexon; two connexons join to form a pore or gap junction channel, which is isolated from the extracellular space and connects the cytosol of two neighboring cells (Sohl and Willecke, 2004). These channels are responsible for the occurrence of synchronous contractions throughout the heart (Sohl and Willecke, 2004).
Consequently, in the absence of connexin-43 channels, normal propagation of contraction is disrupted, and lethal arrhythmias develop (Gutstein et al., 2001a; Gutstein et al., 2001b). Adherens junctions facilitate the transmission of contractile force from one cell to the next and are crucial in maintaining mechanical strength uniformly across the heart (Tepass et al., 2000). They are mainly composed of transmembrane cadherins and cytosolic catenins (Niessen, 2007). N-cadherin, the main cardiac isoform, is a transmembrane protein, with extracellular and intracellular components (Niessen, 2007). Its extracellular portion forms homodimers bringing together the membranes of two opposing cells, while its intracellular segment forms a complex with various members of the catenin family (α-, β-, γ- and p120) present in the cytosol, which in turn are linked to the actin cytoskeleton (Bass-Zubek et al., 2009). Consequently, adherens junctions serve as anchors between the extracellular space and the actin cytoskeleton (Noorman et al., 2009).

Desmosomes provide structural support to cardiomyocytes, which are subjected to strong contractile stress (Delmar, 2004). Desmosomes, similar to adherens junctions, are composed of intercellular and intracellular components (Rayns et al., 1969). The intercellular component consists of desmosomal cadherins, desmocollin and desmoglein, which form a hetero-complex within the extracellular space joining together two bordering cells (Green and Simpson, 2007), while the intracellular component consists of proteins of the armadillo/catenin (plakoglobin and plakophilin) and plakin (desmoplakin) families (Bass-Zubek et al., 2009). Desmoplakin directly interacts with intermediate filaments to stabilize the desmosomal structure. Importantly, a high incidence of mutations within genes encoding desmosomal proteins has been linked to the development of arrhythmogenic right ventricular cardiomyopathy (ARVC).

Although, the ID has been traditionally described to contain three distinct structures (i.e. gap junctions, adherens junctions and desmosomes), recent technological advancements indicate that they are more intertwined than originally proposed (Delmar and McKenna, 2010). Consistent with this, adherens junctions and desmosomes are intimately associated in the “area composita” where proteins from both structures are present (Borrmann et al., 2006; Franke et al., 2006). Similarly, proteins of the adherens and gap junctions have been shown to interact directly (Delmar, 2004). Taken together, these observations suggest that the ID is actually a single functional unit where macromolecular complexes interact to maintain structural integrity and synchronous contraction throughout the heart.

Bridging the gap between the ID and the sarcomeric cytoskeleton is a newly defined region termed the transitional junction. This area is rich in structural proteins, including spectrin, ankyrin-G, α-actinin and the NH2-terminal region of titin, which typically localizes to the Z-disc (Bennett et al., 2006). The transitional junction is suggested to connect the ID with the contractile apparatus, mediating the transmission of force between adjacent cardiocytes.

The high degree of complexity and organization of junctions at the ID suggests a tight interplay between mechanical and electrical activities. Disruption of either mechanical or electrical coupling leads to irregular conduction of electrical impulses and deterioration of cardiac function, subsequently resulting in the development of cardiac arrhythmias. Various mutations in genes encoding for ID proteins have been causatively linked to these complex disorders, many of which manifest themselves as ARVC; (recently reviewed in Protonotarios et al., 2011).

There are ~200 known proteins that are associated with the ID (Dowling et al., 2008; Estigoy et al., 2009; Geisler et al., 2007; Lin et al.; Kargacin et al., 2006; Satomi-Kobayashi et al., 2009;
Schroen et al., 2007; Seeger et al., 2010). Herein, we provide a summation of the current knowledge on the junctional structures present in the ID, focusing on their most prominent and influential components, and how these relate to each other and the sarcomeric cytoskeleton in normal and disease states.

2. Gap junctions

Gap junctions were first described by Revel and Karnovsky in 1967, as “hexagonal arrays” that localize to the ID and mediate the electrical and metabolic coupling of adjacent cardiomyocytes by allowing the diffusion of small molecules (<1000 Da) (Elfgang et al., 1995; Ravel and Karnovsky, 1967). At gap junctions, the distance between opposing membranes is ~3 nm (Fig. 1; Perkins et al., 1997). Gap junction plaques can contain from a few up to 200,000 connexon channels (Evans et al., 2006).

Fig. 1. Gap junctions in ventricular cardiomyocytes are composed of two homo-hexameric hemi-channels, forming a channel or a connexon. Each hemi-channel consists of six connexin-43 monomers (shown in dark purple), allowing the transmission of electrical current and small signalling molecules from adjoining cardiomyocytes. Zona Occludens-1 (ZO-1) (depicted in light purple) interacts directly with connexin-43. In addition, the connexin-43 complex interacts with members of the caveolin family (shown in light grey) that target gap junctions to lipid rafts, and cytosolic α/β tubulin heterodimers (shown in dark grey) that link gap junctions to the microtubular network.

2.1 Structural organization of connexons: Connexin-43

Connexin-43: The human connexin super-family is composed of at least twenty-one members. Connexin-43 is the predominant form expressed in the human heart, while connexins 40 and 45 are present in lower amounts (reviewed in Sohl and Willecke, 2004). Connexin-43 is a four-pass transmembrane protein that contains a cytoplasmic loop and two extracellular loops (Fig. 2A). Notably, both its NH₂- and COOH- termini are located in the cytosol (reviewed in Sohl and Willecke, 2004). Three conserved cysteine residues, located in
the extracellular loops, have been implicated in disulfide bond formation between neighboring connexins of adjacent cells, and contribute to the development of a tight seal that prevents the exchange of materials with the extracellular matrix (Unger et al., 1999). Consistent with this, a constitutive connexin-43 null murine model is embryonic lethal (Reaume et al., 1995), while a cardiac-specific knock-out model exhibits sudden cardiac death by 2 months of age (Gutstein et al., 2001a; Gutstein et al., 2001b).

Fig. 2. Schematic representation of the domain structure of major ID proteins. Grey ovals denote protein specific domains.
NMR studies have demonstrated the presence of short, flexible $\alpha$-helical segments in the cytoplasmic loop and the COOH-terminus of connexin-43, which provide binding sites for several proteins and mediate gating of the connexon (Duffy et al., 2002; reviewed in Gonzalez et al., 2007). Consequently, connexons can exist in a closed or open conformation; at high Ca$^{2+}$ concentrations (i.e. 1.8 mM), they tend to adopt a closed conformation, however, in the absence of Ca$^{2+}$ they exist in an open state (Thimm et al., 2005). Importantly, the gating of connexons is regulated by additional factors, including pH, levels of Mg$^{2+}$, voltage as well as the phosphorylation status of connexins (please see below; Bukauskas and Verselis, 2004; Delmar, 2004; Ek et al., 1994; Ek-Vitorin et al., 1996; Gonzalez et al., 2007; Matsuda et al., 2010).

### 2.2 Propagation of electrical stimulation throughout the heart

The propagation of electrical stimulation is the driving force for heart contraction. It originates at the sinoatrial (SA) node, traverses through the atria, crosses the atrioventricular (AV) node and propagates through the bundle of His and the Purkinje fibers before it activates the ventricles. The coordinated contraction of the atria and ventricles is achieved by conduction of the electrical impulse at variable speeds, mediated by the different forms of connexins, which confer to gap junction plaques distinct electrophysiological properties. As such, connexin-45 is preferentially expressed in the SA and AV nodes, but co-expressed with connexin-43 in the bundle of His and the Purkinje fibers. Conversely, connexin-43 is primarily present in the ventricles, but also co-expressed with connexin-40 in the atria; (reviewed in Severs et al., 2008).

Confering low conductance in a single homotypic channel, connexin-45 is distributed at SA node in a sparse and scattered pattern that ensures poor coupling between adjacent cardiocytes. Similarly at the AV node, connexin-45 contributes to the sequential activation of the atria and ventricles reducing the occurrence of arrhythmias; (reviewed in Severs et al., 2008). On the contrary, the rapid propagation of electrical signals through the Purkinje fibers is mediated by gap junctions mainly consisting of connexins 43 and 40, which confer relatively large conductance, and to a lesser extent connexin-45, thus maintaining the regular contractions of the heart (Gonzalez et al., 2007; Kirchhoff et al., 1998).

### 2.3 Phosphorylation regulates the permeability of connexons

Several kinases modulate the function of connexons. Although a complete listing of all identified kinases is beyond the scope of this chapter, we will refer to major ones, highlighting their roles during normalcy and stress. Connexin-43 is a substrate of Src tyrosine kinase, which phosphorylates Tyr-265 to disrupt its interaction with ZO-1 (discussed below, Toyofuku et al., 2001), and suppress gap junction communication in the failing heart (Giepmans et al., 2001a; Toyofuku et al., 2001). Similarly, mitogen-activated protein kinase (MAPK) phosphorylates connexin-43 at Ser-255, Ser-279 and Ser-282 to repress gap junction communication (Warn-Cramer et al., 1998; Warn-Cramer et al., 1996). Conversely, phosphorylation of Ser-365 by protein kinase A (PKA) promotes gap junction assembly and communication (Burghardt et al., 1995; Solan et al., 2007; TenBroek et al., 2001). Although several isozymes of protein kinase C (PKC) phosphorylate connexin-43 in diverse cell types and tissues, PKC$\varepsilon$ is the only isoform that phosphorylates it at the ID (Bowling et al., 2001; Doble et al., 2000; Lampe et al., 2000; Lin et al., 2003; Saez et al., 1997). Consistent with this, PKC$\varepsilon$ suppresses gap junction communication in the ischemic heart.
through phosphorylation of connexin-43 at Ser-368 (Ek-Vitorin et al., 2006; Hund et al., 2007; Hund et al., 2008).

Several phosphorylation sites (i.e. Ser-306 and Ser-325/Ser-328/Ser-330) on connexin-43 are non- or de-phosphorylated in ischemic and hypertrophic hearts (Lampe et al., 2006; Procida et al., 2009). The absence of phosphorylation at these sites has been suggested to correlate with reduced cardiac conductance (Lampe et al., 2006; Procida et al., 2009). In agreement with this, transgenic mice in which Ser-325/Ser-328/Ser-330 were substituted by glutamic acid (phosphomimetic residue) were less susceptible to arrhythmia. Yet, the kinase(s) that is responsible for these phosphorylation events remain(s) to be identified. Importantly, Ser-325/Ser-328/Ser-330 are substrates of casein kinase 1 (CK1) in normal rat kidney cells (Cooper and Lampe, 2002), however, its role in cardiac muscle remains to be defined.

Moreover, Ca²⁺/Calmodulin-dependent protein kinase II (CaMKII) is capable of phosphorylating many Ser residues on connexin-43 in vitro, including Ser-306, Ser-325, Ser-328 and Ser-330 (Huang et al., 2011), however the physiological significance of these results requires further investigation.

The phosphatases acting upon and regulating the activities of connexin-43 have been also long sought after. Receptor protein tyrosine phosphatase μ (RPTPμ) has been suggested to dephosphorylate Tyr residues present in connexin-43 in lung cells (Giepmans et al., 2003), however, its physiological relevance in the myocardium remains to be established. Moreover, serine/threonine phosphatase type 1 and type 2A (PP1 and PP2A, respectively) have been implicated in the dephosphorylation of connexin-43 (Ai and Pogwizd, 2005; Duthe et al., 2001; Jeyaraman et al., 2003). For instance, PP1, but not PP2A, modulates the phosphorylation status of Ser-368 (Jeyaraman et al., 2003). Conversely, PP2A exists in a complex with connexin-43 in homogenates prepared from patients suffering from dilated cardiomyopathy (DCM) or idiopathic dilated cardiomyopathy (IDCM), as well as from a non-ischemic heart failure rabbit model (Ai and Pogwizd, 2005; Ai et al., 2011). Consistent with this, application of specific PP2A inhibitors prevented uncoupling of cardiocytes in the rabbit failing heart (Ai and Pogwizd, 2005).

2.4 Connexin-43 interacts with ZO-1, caveolins and microtubules at the ID

Zona occludens-1: Zona occludens-1 (ZO-1) interacts with connexin-43 in cardiac myocytes via its PDZ2 domain that directly binds to the last five residues present in the COOH-terminus of connexin-43 (Giepmans and Moolenaar, 1998; Giepmans et al., 2001a; Toyofuku et al., 1998). Interestingly though, their interaction is not abolished in a transgenic murine model that expresses a truncated form of connexin-43 that is missing the last 124 amino acid residues (Maass et al., 2007), suggesting that additional domains contribute to binding.

The interaction of ZO-1 and connexin-43 mainly takes place at the periphery of the gap junctional plaque (Hunter et al., 2005; Zhu et al., 2005). Notably, their binding is suppressed in the presence of Src (Sorgen et al., 2004; Toyofuku et al., 2001). A number of early studies suggested that ZO-1 targets or retains connexin-43 to the ID, while others proposed that it regulates the size of gap junctions or the internalization of connexin-43 (Barker et al., 2002; Hunter et al., 2005; Rhett et al., 2011; Toyofuku et al., 1998). Intriguingly, recent studies from failing human hearts have provided conflicting results. Bruce et al. reported that in hearts of DCM and IDCM patients, ZO-1 interacts more extensively with connexin-43 compared to healthy ones (Bruce et al., 2008), whereas Laing et al. and Kostin, described diminished
colocalization of connexin-43 and ZO-1 in hearts from patients with DCM, ischaemic cardiomyopathy and end-stage heart failure (Kostin, 2007; Laing et al., 2007). In support of this, transgenic mice lacking ZO-1 are embryonic lethal, exhibiting cardiac developmental challenges (Katsuno et al., 2008; Xu et al., 2008). Recently, Rhett et al. proposed a model, whereby ZO-1 interacts with connexin-43 to inhibit the incorporation of additional connexons into gap junctional plaques (Rhett et al., 2011).

**Caveolin-1:** Caveolins are the main scaffolding components of caveolae in lipid rafts, and have been found to interact with connexin-43 in different cell types (Langlois et al., 2008; Liu et al., 2010; Schubert et al., 2002). While the caveolin scaffolding domain along with the COOH-terminus of caveolin-1 are sufficient to support binding to connexin-43, the respective interacting region of the latter has yet to be defined (Schubert et al., 2002). Contrary to epithelial cells, the interaction between caveolins and connexin-43 in the myocardium is less understood. Along these lines, caveolin-3, which is specifically expressed in heart and skeletal muscle (Tang et al., 1996), has been shown to interact with connexin-43 in a yeast-two-hybrid study and confirmed by co-immunoprecipitation assays using heart homogenates (Liu et al., 2010). As caveolin-3 is present at the sarcolemma, but not the ID, the physiological relevance of this interaction remains to be examined (Abi-Char et al., 2007; Yarbrough et al., 2002). Moreover, a murine model lacking caveolin develop DCM at early stages (Zhao, 2002).

**Microtubules:** First characterized as binding partners of connexin-43 in RAT-1 cells and other fibroblast and epithelial cell lines, α/β-tubulins have been shown to specifically interact with the tubulin-binding motif present in the COOH-terminus of connexin-43 (Giepmans et al., 2001a; Giepmans et al., 2001b). Immunofluorescence studies and live-cell imaging further demonstrated that connexin-43 co-localizes with tubulins along microtubule tracks, as it traverses from the Golgi apparatus to other membranes (Giepmans et al., 2001b; Lauf et al., 2002; Shaw et al., 2007). To date, only a handful of studies have described the interaction between tubulins and connexin-43 in the heart. Accordingly, a recent study by Smyth et al. proposed that EB1, a microtubule plus-end tracking protein, is required to deliver connexin-43 to the ID (Smyth et al., 2010). Consistent with this, disruption of the interaction between EB1 and microtubules (e.g. during ischemia) significantly decreases the surface expression of connexin-43 at the ID (Smyth et al., 2010).

### 3. Adherens junctions

Adherens junctions are specialized structures necessary for cell-cell adhesion that provide uniform mechanical strength to the heart. Unlike gap junctions where membranes remain relatively close, opposing membranes at adherens junctions are separated by ~20 nm (Niessen, 2007). Adherens junctions frequently alternate with gap junctions along the sarcolemma at the ID, and are typically oriented perpendicular to the long axis of cardiomyocytes, optimizing the transmission of mechanical force (Hoyt et al., 1989). In addition, they can be found with desmosomes at the *area composita*. Functional adherens junctions require two main anchor points, one within the extracellular space where cadherins from adjacent cells tightly interact in a homophilic manner, and the other within the cytoplasmic region linking the adherens junction complex to the actin cytoskeleton through direct interactions with members of the catenin family (Fig. 3; reviewed in Niessen, 2007; Noorman et al., 2009).
Fig. 3. Adherens Junctions connect neighbouring cardiomyocytes through homophilic dimers of N-cadherin. Connections within the intracellular space link N-cadherin to the actin cytoskeleton (depicted in grey), via additional adherens junction proteins (shown in hues of teal), i.e. p120 catenin, β-catenin, α-catenin and mXina. Proteins not traditionally considered as components of adherens junctions, but localizing to the ID are shown in grey. Proteins of other ID structures, ZO-1 (gap junctions) and γ-catenin (desmosomes), are depicted in light purple and gold, respectively.

3.1 N-cadherin

N-Cadherin: Cadherins are a super-family of transmembrane glycoproteins that mediate Ca^{2+}-dependent adhesion between neighbouring cells. In the early 1980s, N-cadherin was identified as a major component of the myocardium, that localizes to the ID (Volk and Geiger, 1984). Five extracellular domains are present in N-cadherin, with the first three possessing Ca^{2+} binding sites; each domain is composed of up to ~110 amino acids (Fig. 2B). The extreme NH\textsubscript{2}-terminus of N-cadherin contains a highly conserved ligand recognition
site composed of repeating motifs of His-Ala-Val residues, necessary for homophilic dimer formation (Nose et al., 1990). Two cadherin monomers, one from each adjacent myocyte, form a Ca\textsuperscript{2+}-dependent, zipper-like homodimer (Takeichi, 1994). Although Ca\textsuperscript{2+} is necessary for maintaining cadherin homodimers, it does not mediate their initial interaction (Nagaraj et al., 1996). Following the extensive extracellular domain, N-cadherin contains a single-pass transmembrane region and a short cytoplasmic segment that associates with the actin cytoskeleton via proteins of the catenin family (Ozawa et al., 1990).

The importance of N-cadherin in the stability of IDs is evidenced in various animal models. A murine systemic knock-out model of N-cadherin resulted in embryonic lethality due to improper development of the heart tube among other abnormalities, despite the development of primitive myocardial tissue (Radice et al., 1997). Interestingly, isolated murine myocytes from the same model were able to weakly contract and aggregate in culture (Radice et al., 1997). Taken together, these results suggested that N-cadherin is critical for embryonic development of the heart and other tissues, however it is not required for electrical coupling and cell adhesion at this stage. Moreover, in a murine conditional cardiac-specific knock-out model where N-cadherin was deleted in 6-10 week old mice, sudden death occurred after ~2 months (Li et al., 2005). A significant decrease in the expression levels of gap junction proteins was also observed in this model, which was accompanied by the development of dilated cardiomyopathy and impaired left ventricular function (Li et al., 2005). In addition, the amounts of other proteins at ID were reduced, including plakoglobin and \( \alpha-, \beta-, \) and p120 catenins, resulting in dissolution of the ID structure (Kostetskii et al., 2005; Li et al., 2005). Similarly, mice overexpressing N-cadherin developed early onset dilated cardiomyopathy (Ferreira-Cornwell et al., 2002), and N-cadherin/connexin-43 compound heterozygous mice were prone to cardiac arrhythmias (Li et al., 2008). Collectively, these studies suggested that N-cadherin is necessary for the maintenance and stabilization of the ID, while its absence may lead to the development of heart failure and ultimately death.

3.2 Proteins of the catenin family

Within adherens junctions, N-cadherin associates with the actin cytoskeletal network through direct interactions mediated by members of the catenin/armadillo family; these include \( \beta-, \alpha-, \) p120 and \( \gamma- \) catenin (also called plakoglobin). \( \beta- \) and p120 catenin bind directly to the cytoplasmic domain of N-cadherin, whereas \( \alpha- \) catenin links the actin cytoskeleton to N-cadherin, via its direct interactions with both components (reviewed in Aho et al., 1999; Butz and Larue, 1995; Niessen, 2007).

\( \beta- \) Catenin: \( \beta- \) Catenin, like other members of the catenin/armadillo family, is characterized by a series of central domains, referred to as armadillo (arm) repeats, each composed of 42 amino acids, that form an elongated superhelix when repeated in tandem (Huber et al., 1997). \( \beta- \) Catenin contains twelve arm repeats (Fig. 2C; Peifer et al., 1994); deletion mutagenesis has mapped the binding site for N-cadherin to the central repeat region of \( \beta- \) catenin (Hulsken et al., 1994). Flanking the arm repeats are small, ~100 amino acids long, NH\textsubscript{2}- and COOH- termini that mediate the regulatory functions of \( \beta- \) catenin.

p120 Catenin: p120 Catenin shares a similar organization with \( \beta- \) catenin, and a ~22% identity within the arm repeats region (Peifer et al., 1994; Reynolds et al., 1992). Alternative splicing gives rise to four similar p120 catenin isoforms (Keirsebilck et al., 1998). Each isoform is composed of ten arm repeats that are responsible for their direct
interaction with the COOH-terminus of cadherins (Fig. 2C; Daniel and Reynolds, 1995; Finnemann et al., 1997; Reynolds et al., 1992; Shibamoto et al., 1995; Staddon et al., 1995; Thoreson et al., 2000). p120 catenin does not interact with α-catenin or the actin cytoskeleton (Daniel and Reynolds, 1995), suggesting a novel, yet unidentified, function within adherens junctions.

α-Catenin: α-Catenin is a subfamily of proteins that differs significantly in both primary sequence and structural organization from the other members of the traditional catenin/armadillo family (reviewed in Kobielak and Fuchs, 2004). Instead of arm repeats, α-catenin contains three vinculin homology (VH) domains, therefore sharing considerable homology with vinculin (Fig. 2C; Rudiger, 1998). Of the main α-catenin isoforms, αT-catenin is the most prominent in the mammalian heart and localizes to the ID (Janssens et al., 2001). Through its most NH$_2$-terminal VH domain, α-catenin dimerizes and interacts directly with β- and γ-catenin (Koslov et al., 1997; Pokutta and Weis, 2000), while through its middle VH domain supports binding to vinculin and α-actinin, both of which are present within the transitional junction of the ICD (McGregor et al., 1994; Weiss et al., 1998). Similar to vinculin, α-catenin associates with filamentous actin through its last VH domain and its COOH-terminus (Rimm et al., 1995). In addition, its COOH-terminus interacts with ZO-1, which is also complexed with connexin-43 at gap junctions (Imamura et al., 1999; Talhouk et al., 2008). Taken together, these observations indicate that α-catenin functions as an intracellular adhesion protein.

It is well established that β- and p120 catenins play essential roles in diverse signaling pathways, including modulation of cell-cell adhesion; (reviewed in Anastasiadis and Reynolds, 2000; Niessen, 2007). Recently, α-catenin was also implicated in the regulation of cell adhesion and proliferation (reviewed in Kobielak and Fuchs, 2004). Although many of their suggested signaling roles originate from studies in non-cardiac cells, it is presumed that catenins may have similar regulatory activities at the ID of cardiomyocytes. In support of this, transgenic mice lacking either β- or α-catenin result in detrimental effects on the longevity of the animals, with phenotypes ranging from embryonic lethality to the development of early onset DCM (Haegel et al., 1995; Piven et al., 2011; Sheikh et al., 2006). Future studies are necessary to continue addressing this question.

4. Desmosomes

Similar to adherens junctions, desmosomes are also symmetrical protein complexes with intercellular elements connecting adjacent cells, and intracellular components associating with intermediate filaments. First identified as adhesive structures of epithelial cells by Giulio Bizzozero in the late 19th century, the term desmosomes was initially coined in 1920 by Josef Schaffer from the Greek words “desmo” and “soma” meaning bond or fastening and body, respectively; (reviewed in Delva et al., 2009). In the middle of the 20th century, desmosomes were identified as a major component of the cardiac ID (Fawcett and McNutt, 1969; Grimley and Edwards, 1960; Muir, 1957; Sjostrand and Andersson, 1954), where its main function is to provide structural support to neighboring cardiomyocytes (reviewed in Delmar and McKenna, 2010; Delva et al., 2009; Thomason et al., 2010). Desmosomes bring apposing cells within 20-35 nm of each other (Noorman et al., 2009), and are typically found in close proximity to gap junctions, although recent studies indicate that they are also present next to adherens junctions within the area composita. They consist of proteins from
three families: the desmosomal cadherins, the catenin/armadillo family and the plakins (Fig. 4).

Fig. 4. Desmosomes connect neighbouring cardiomyocytes through heterophilic dimers of desmocollin-2 and desmoglein-2 (shown in hues of orange) forming within the extracellular space. Interactions with plakophilin-2, plakoglobin (a.k.a. γ-catenin) and desmoplakin-1 (depicted in hues of gold and orange) link the desmosomal complex to the intermediate filament protein desmin (shown in gray) in cardiomyocytes.

4.1 Desmosomal cadherins
Desmosomal cadherins are a superfamily of Ca\(^{2+}\)-dependent adhesion molecules, which form dimers to make up the core of desmosomal junctions (Dusek et al., 2007). Desmogleins and desmocollins, the two main types of desmosomal cadherins, possess several isoforms (4 and 3 respectively in humans, Green and Simpson, 2007; Lorimer et al., 1994; Schmelz et al., 1986) with desmoglein-2 and desmocollin-2 being the main isoforms expressed in mammalian cardiomyocytes (Garrod and Chidgey, 2008).

*Desmoglein and Desmocollin:* These classical cadherins are highly homologous; desmogleins and desmocollins share ~30% identity with each other and with other members of the cadherin family (Garrod et al., 2002). Much of their homology is found within their extracellular domains. They possess five extracellular domains or cadherin repeats of ~110
amino acids and are separated by Ca\textsuperscript{2+} binding motifs, which are necessary for dimerization (Fig 2B; Pokutta and Weis, 2007). A single-pass transmembrane domain and an intracellular anchoring segment follow the extracellular domains (Green and Simpson, 2007; Kowalczyk et al., 1999). Within their intracellular regions, desmogleins and desmocollins possess a cadherin-like sequence capable of binding catenins, or in the case of desmosomal cadherins, plakoglobin (Mathur et al., 1994).

Desmoglein and desmocollin differ significantly within their COOH-termini, however. In particular, the COOH-terminal region of desmoglein contains a proline-rich linker region, a series of short (~29 amino acids long) repeats and a glycine-rich terminal domain (Garrod and Chidgey, 2008; Holthofer et al., 2007), which likely mediates weak interactions with other desmosomal proteins (Kami et al., 2009). Conversely, alternative splicing within the COOH-terminus of desmocollin gives rise to two forms (Collins et al., 1991; Parker et al., 1991); the “b” or shorter form does not contain the traditional catenin-binding domain, however, the longer “a” form possesses a normal catenin-binding domain and has been shown to bind plakoglobin with high affinity (Troyanovsky et al., 1993).

Many studies suggest that both desmoglein and desmocollin are necessary for desmosomal formation (Getsios et al., 2004; Marcozzi et al., 1998; Tselepis et al., 1998). However, it is unclear if homophilic or heterophilic interactions maintain desmosomal adhesion. Although heterophilic complexes between desmoglein-2 and desmocollin-2 have been reported, it has been suggested that homophilic interactions between desmogleins mediate complex formation (Heupel et al., 2008; Syed et al., 2002; Waschke et al., 2005). Nonetheless, the importance of both desmoglein and desmocollin in cardiac function is further evidenced by the numerous mutations identified in their respective genes that lead to cardiomyopathies, mainly manifested as ARVC. Consistent with this, mice harbouring a mutation resulting in a truncated form of desmoglein-2 develop ARVC (Krusche et al., 2011), while a systemic knockout mouse model of desmoglein-2 is embryonic lethal (Eshkind et al., 2002).

4.2 Proteins of the catenin/armadillo family

Desmosomal cadherins form cytoplasmic connections with intermediate filaments in part through proteins of the armadillo family. Armadillo proteins include plakoglobin (also called γ-catenin) and plakophilin, which are found at desmosomal structures (Cowin et al., 1986; Hatzfeld, 2005; Hatzfeld, 2007; Mertens et al., 1996; Mertens et al., 1999; Peifer et al., 1992), in addition to β-catenin, α-catenin and p120 catenin, which are mainly associated with adherens junctions (Hatzfeld, 2005; Hatzfeld, 2007). In addition to facilitating the anchoring of desmosomes to intermediate filaments, desmosomal armadillo proteins function in diverse signal transduction pathways.

Plakoglobin: Plakoglobin contains 12 arm repeats, which share 65% identity with the ones present in β-catenin, and are flanked by Pro-Lys-Gly rich NH\textsubscript{2}- and COOH-terminal domains (Fig. 2C; Garrod and Chidgey, 2008; Huber et al., 1997; Peifer et al., 1992). Mutation analysis suggested that plakoglobin interacts with desmosomal cadherins through its NH\textsubscript{2}-terminal domain as well as the arm repeats near its COOH-terminus (Chitaev et al., 1996; Wahl et al., 1996). Although the Pro-Lys-Gly motif interacts with both desmosomal and adherens junction cadherins, it has a higher affinity for desmoglein supporting plakoglobin’s mainly desmosomal localization (Chitaev et al., 1996; Choi et al., 2009).

Moreover, through its central arm repeats plakoglobin interacts with desmoplakin, which in turn binds to intermediate filaments.
**Plakophilin:** Plakophilins undergo alternative splicing giving rise to four products, referred to as plakophilin 1-4; (reviewed in Bass-Zubek et al., 2009), with plakophilin-2 being the most prominent form in mammalian cardiomyocytes (Mertens et al., 1996). Plakophilins contain 9 arm repeats flanked by an NH$_2$-terminal head and a short COOH-terminal region (Fig. 2C; Bass-Zubek et al., 2009). In addition, plakophilins 1-3 possess a flexible insertion between repeats 5 and 6, which introduces a major bend to their overall structure (Choi and Weis, 2005). Plakophilins bind to several desmosomal proteins through their NH$_2$-terminal regions, including desmocollin, desmoplakin and plakoglobin as well as actin and the intermediate filament proteins keratin and desmin (Hofmann et al., 2000). Notably, plakophilin-2 also interacts with ankyrin-G at the ID, a sodium channel anchoring protein and with connexin-43 (Sato et al., 2011). Consequently, loss of plakophilin-2 leads to a decrease in the level of the α-subunit of the sodium channel (Na$_v$1.5) at the membrane, which results in slow propagation of the action potential in cardiocytes (Sato et al., 2009). In addition to ankyrin-G, plakophilin-2 interacts with PKCa, which is necessary for phosphorylation and recruitment of desmoplakin to newly forming desmosomes in the developing heart and during repair of myocardial injury (reviewed in Garrod and Chidgey, 2008). Thus, through its multiple interactions, plakophilin-2 may serve as a scaffold to contribute to adhesion and signalling at the ID by facilitating the lateral interaction between desmosomes and adherens junctions (Kowalczyk et al., 1999).

The critical roles of both plakoglobin and plakophilin-2 in desmosomal assembly and maintenance is evidenced by the severe phenotypes that relevant transgenic mice models exhibit and the different forms of heart disease associated with mutations in their respective genes (please see Tables 1 and 2). Consistent with this, both plakoglobin and plakophilin-2 null mice show premature death during embryogenesis because of myocardial fragility (Bierkamp et al., 1996; Grossmann et al., 2004; Ruiz et al., 1996). Similarly, cardiac-specific knockout of plakoglobin results in progressive development of cardiac dysfunction (Li et al., 2011).

### 4.3 Plakins

**Desmoplakin:** Plakins are large multi-domain proteins that mediate the interaction of intermediate filaments (desmin in heart) with desmosomes. Desmoplakin, the main plakin protein expressed in heart, is characterized by a central α-helical coiled-coil rod domain, which is flanked by globular NH$_2$- and COOH-termini (Fig. 2D; Franke et al., 1982). Through its coiled-coil region, desmoplakin has been suggested to form homodimers (Kowalczyk et al., 1994), while its NH$_2$-terminal region binds to plakoglobins and plakophilins, targeting them to desmosomes (Bornslaeger et al., 1996; Bornslaeger et al., 2001; Holthofer et al., 2007; Kowalczyk et al., 1999). Its COOH-terminal tail is composed of three plakin-repeat domains and a Gly-Ser-Arg rich motif; both shown to mediate binding to desmin (Choi et al., 2002; Getsios et al., 2004). Interestingly, mice lacking desmoplakin exhibit embryonic lethality characterized by reduced number of desmosomes with residual structures separated from intermediate filaments (Gallicano et al., 1998). These results, along with the various desmoplakin mutations associated with human genetic disorders (please see below) support a strong role for desmoplakin in the assembly and interlinking of desmosomes to desmin intermediate filaments in cardiomyocytes.
<table>
<thead>
<tr>
<th>Major Proteins</th>
<th>References</th>
<th>Animal Models</th>
<th>Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connexin-43</td>
<td>Beyer et al., 1987</td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Reaume et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac Specific KO</td>
<td>Sudden cardiac death ~2 months</td>
<td>Gutstein et al., 2001b</td>
</tr>
<tr>
<td>ZO-1</td>
<td>Giepmans et al., 1998; Toyofuku et al., 1998</td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Xu et al., 2008; Katsuno et al., 2008</td>
</tr>
<tr>
<td>Caveolin</td>
<td>Schubert et al., 2002</td>
<td>Systemic KO</td>
<td>Development of DCM</td>
<td>Zhao et al., 2002</td>
</tr>
<tr>
<td>Microtubule</td>
<td>Shaw et al., 2007</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>N-Cadherin</td>
<td>Volk et al., 1984</td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Radice et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac specific KO</td>
<td>Sudden cardiac death ~2 months</td>
<td>Li et al., 2005; Kostetskii et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dual heterozygote with connexin-43</td>
<td>Develop arrhythmias</td>
<td>Li et al., 2008</td>
</tr>
<tr>
<td>β-catenin</td>
<td>Butz et al., 1995</td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Haegel et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac specific KO</td>
<td>Low survival rate</td>
<td>Piven et al., 2011</td>
</tr>
<tr>
<td>α-catenin</td>
<td>Butz et al., 1995</td>
<td>Cardiac specific KO</td>
<td>Development of DCM</td>
<td>Piven et al., 2011; Sheikh et al., 2006</td>
</tr>
<tr>
<td>P120 catenin</td>
<td>Aho et al., 1999</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Desmocollin-2</td>
<td>Lorimer et al., 1994</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Desmoglein-2</td>
<td>Schmelz et al., 1986</td>
<td>Transgenic lacking extracellular domains</td>
<td>Develop ARVC</td>
<td>Krsuche et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Eshkind et al., 2002</td>
</tr>
<tr>
<td>Plakoglobin</td>
<td>Peifer et al., 1992; Cowin et al., 1986</td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Birkamp et al., 1996; Ruiz et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac Specific KO</td>
<td>Premature death due to cardiac dysfunction</td>
<td>Li et al., 2011</td>
</tr>
<tr>
<td>Plakophilin-2</td>
<td>Mertens et al., 1996; Mertens et al., 1999</td>
<td>Systemic KO</td>
<td>Embryonic lethal</td>
<td>Grossmann et al., 2004</td>
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<tr>
<td>Desmoplakin</td>
<td>Franke et al., 1982</td>
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<td>Embryonic lethal</td>
<td>Gallicano et al., 1998; Uzumcu et al., 2006</td>
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<tr>
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<td></td>
<td>Tetraploid rescue of systemic KO</td>
<td>Embryonic lethal</td>
<td>Gallicano et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Val30Met &amp; Gln90Arg cardiac specific mutations</td>
<td>Embryonic lethal</td>
<td>Yang et al., 2006</td>
</tr>
</tbody>
</table>

Table 1. Listing of major proteins found at the ID and associated animal models with appropriate references; DCM: Dilated Cardiomyopathy, N/A: not applicable, and KO: knock-out.
<table>
<thead>
<tr>
<th>Gene Product</th>
<th>Mutations</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plakophilin-2</td>
<td>Arg79Stop</td>
<td>ARVC</td>
<td>Gerull et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Arg735Stop</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVSAS10, G-C, -1 (nt 2146)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS12, G-A, +1 (nt 2489)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desmocollin-2</td>
<td>1bp deletion, 1430C</td>
<td>ARVC</td>
<td>Syrris et al, 2006</td>
</tr>
<tr>
<td></td>
<td>1bp deletion, 1841G</td>
<td></td>
<td>Simpson et al., 2009</td>
</tr>
<tr>
<td></td>
<td>2bp deletion, 2687GA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS5AS, A-G, -2 (nt 631)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desmoglein-2</td>
<td>Arg45Gln</td>
<td>ARVC</td>
<td>Awad et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Arg48His</td>
<td></td>
<td>Pilichou et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Val56Met</td>
<td></td>
<td>Syrris et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Asn266Ser</td>
<td></td>
<td>Posch et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Glu331Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trp305Stop</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cys506Tyr</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Gly811Cys</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS12AS, A-G, -2 (nt 1881)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plakoglobin</td>
<td>3bp deletion, 118GCA</td>
<td>ARVC</td>
<td>McKoy et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Ser39Lys40insSer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2bp deletion, 2157TG</td>
<td>Naxos disease</td>
<td>Asimaki et al., 2007</td>
</tr>
<tr>
<td>Desmoplakin</td>
<td>Val30Met</td>
<td>ARVC</td>
<td>Norgett et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Ser299Arg</td>
<td></td>
<td>Rampazzo et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Lys959Met</td>
<td></td>
<td>Norman et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Arg1255Lys</td>
<td></td>
<td>Yang et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Arg1267X</td>
<td></td>
<td>Uzumcu et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Arg1775Ile</td>
<td></td>
<td>Bolling et al., 2010</td>
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<tr>
<td></td>
<td>Arg2834His</td>
<td></td>
<td>Bauce et al., 2010</td>
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<tr>
<td></td>
<td>Gly2375Arg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2034insA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg1934Stop</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1bp deletion, 7901G</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>IVS, G-A, +1 (nt 423)</td>
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</tbody>
</table>

Table 2. Listing of mutations found in desmosomal genes that have been causally linked to the development of ARVC or variations of it; bp: base pair, IVS or AVSAS: denotes a splice site mutation, IVS: intervening sequence, AS: acceptor splice site, nt: nucleotide, ins: insertion.
5. ID proteins in human heart disease

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a progressive disease characterized by loss of the right ventricular myocardium, and at advanced stages of the left ventricular myocardium as well, accompanied by fibro-fatty tissue infiltration and replacement. Its clinical manifestations include ventricular arrhythmias, syncope, heart failure and sudden cardiac death (Delmar and McKenna, 2010; Estigoy et al., 2009; Lombardi and Marian, 2011). ARVC has an estimated prevalence of 1 in 5,000 (Sen-Chowdhry et al., 2010), although in some regions (e.g. northern Italy) it reaches 1 in 2,000 (Thiene et al., 2007). Genetic studies have indicated that ~50% of the diagnosed ARVC cases are familial, with an autosomal dominant inheritance (Marcus et al., 1982). Accordingly, a number of mutations have been identified in the genes that encode cardiac desmosomal proteins, and thus ARVC is also referred to as “a disease of the desmosome” (Li and Radice, 2010). These mutations not only affect the number, structural integrity and proper localization of desmosomes, but also of gap junctions, resulting in impaired intercellular conductance and thus development of arrhythmias. To date, five desmosomal genes have been identified that carry inherited mutations causing different variations of ARVC, including: plakophilin-2, desmocollin-2, desmoglein-2, plakoglobin and desmoplakin. Table 2 includes a comprehensive list of mutations identified to date in these five desmosomal genes; for updated listing please refer to: http://www.ncbi.nlm.nih.gov/omim.

More than 70% of the identified desmosomal mutations associated with the development of familial ARVC are present in the gene encoding plakophilin-2 (Gerull et al., 2004; Sen-Chowdhry et al., 2010; van Tintelen et al., 2007). These account for ~20% of diagnosed ARVC cases, while mutations in the genes encoding desmocollin-2 (Simpson et al., 2009; Syrris et al., 2006) and desmoglein-2 (Awad et al., 2006; Posch et al., 2008; Syrris et al., 2006) account for ~10-15% of cases each (Lombardi and Marian, 2011; Plichou et al., 2006).

Plakoglobin was the first desmosomal protein to be causally associated with a cardiocutaneous subtype of ARVC, known as Naxos disease, which was first characterized by Protonotarios et al. (Protonotarios et al., 1986). Genetic studies of patients from the Greek island Naxos, where the syndrome took its name from, revealed a homozygous two-base-pairs deletion (2157-2158delGT) in the gene encoding plakoglobin that was inherited in an autosomal recessive manner (McKoy et al., 2000). In addition to developing ARVC, these individuals also suffered from palmoplantar keratoderma and woolly hair. Recently though, a variation of the Naxos syndrome was diagnosed in a German family that carried a dominantly inherited mutation in the plakoglobin gene (Ser39Lys40insSer) that caused ARVC without the accompanying cutaneous abnormalities (Asimaki et al., 2007).

Importantly, the reduced expression or complete absence of plakoglobin from the ID of ARVC patients is a consistent feature, making it a valuable marker for its diagnosis, which still remains problematic with many cases being un- or misdiagnosed.

Mutations in the gene encoding desmoplakin have been identified as the underlying cause of a variation of Naxos disease, referred to as Carvajal syndrome that is also characterized by woolly hair, palmoplantar keratoderma and cardiac disease (Kaplan et al., 2004a; Kaplan et al., 2004b; Norman et al., 2005; Rampazzo et al., 2002; Saffitz, 2009; Yang et al., 2006; Bause et al., 2010; Bolling et al., 2010; Norgett et al., 2000; Uzumcu et al., 2006). Notably, cardiac disease is presented as a generalized hypertrophy and dilation, involving both the right and left ventricles, and accompanied by focal ventricular aneurysms without any apparent fibro-fatty tissue replacement (Kaplan et al., 2004a; Yang et al., 2006). A major feature of the
Carvajal syndrome is the virtual absence of desmoplakin in the affected hearts, indicating that the missense or nonsense mutations identified result in truncated and/or unstable forms of the protein (Norman et al., 2005; Rampazzo et al., 2002). Alterations in the amounts, localization and functional properties of desmosomal proteins not only affect intercellular adhesion, but also promote remodelling of gap junctions by leading to abnormal expression and distribution of gap junctional proteins, and primarily connexin-43, which in turn induces defects in the electrochemical coupling of neighbouring cardiocytes and leads to the development of severe arrhythmias (Kaplan et al., 2004a; Pieperhoff et al., 2008; Saffitz, 2009). On the contrary, changes in gap junctions do not affect the structural integrity or proper function of desmosomes and adherens junctions, and thus mechanical coupling of adjacent cardiocytes is not disrupted (Delmar and McKenna, 2010; Li and Radice, 2010; Noorman et al., 2009).

A number of mutations have also been identified in the gene encoding connexin-43, which are associated with the development of ocudontodigital dysplasia (ODDD) that is frequently accompanied by hair and skin defects, too (Kelly et al., 2006). Some of these mutations have been further linked to the development of cardiac disturbances. In such patients, the expression levels of connexin-43, and thus the number of gap junctions, are moderately decreased (Manias et al., 2008); however, cardiac conduction is not affected. Thus, sole mutations in the gene encoding connexin-43 cannot be the primary inducers of electrical or mechanical defects underlying arrhythmogenesis. Interestingly, neither loss- nor gain-of-function mutations have been identified in proteins of adherens junctions that are causally associated with the development of cardiac disease. A plausible explanation is that dysfunctional adherens junctions may be detrimental to the developing myocardium and thus may result in embryonic lethality. Consistent with this, a constitutive null model of N-cadherin was embryonic lethal, while a developmental and cardiac tissue specific model developed dilated cardiomyopathy and died 2 months following excision of the gene, due to mechanical and electrical abnormalities (Li et al., 2005; Radice et al., 1997); for review of available animal models of N-cadherin and their phenotypic characterization, please refer to (Li et al., 2006).

6. Concluding remarks: The intercalated disc is a single functional unit

Although traditionally depicted as a composition of three separate units, data from the last decade suggest that the ID of cardiomyocytes is in fact a single functional unit. Several studies have begun to describe *area composita* as a hybrid between proteins of adherens junctions and desmosomes that form a single anchoring unit (Delmar, 2004; Franke et al., 2006; Pieperhoff and Franke, 2007; Saffitz, 2005). Consistent with this, plakophilin-2 and desmoglein, which typically localize to desmosomes, interact with β- or α-catenin and p120 catenin, respectively, present in adherens junctions (Chen et al., 2002; Goossens et al., 2007). Similarly, molecular linkages between desmosomes and gap junctions have also been identified (Rohr, 2007; Saffitz, 2005). As such, desmocollin-2 directly interacts with connexin-43 (Gehmlich et al., 2011). Taken together, these studies clearly suggest that there is a three-way exchange and cross-talk of junctional proteins, supporting the idea of the ID being a single functional unit.

During the last decade, there have been significant advancements concerning the structural composition of the ID. A plethora of new proteins has been identified as integral or peripheral components of the ID that directly or indirectly contributes to the mechanical and
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electrical coupling of neighbouring cardiocytes. The challenge of the future lies in the characterization of the precise roles that these proteins play to ensure the synchronous contraction of the myocardium. A combination of sophisticated molecular, genetic and cellular approaches will be needed to address this unequivocally important question.

7. Acknowledgments

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Cardiomyopathy means "heart (cardio) muscle (myo) disease (pathy)". Currently, cardiomyopathies are defined as myocardial disorders in which the heart muscle is structurally and/or functionally abnormal in the absence of a coronary artery disease, hypertension, valvular heart disease or congenital heart disease sufficient to cause the observed myocardial abnormalities. This book provides a comprehensive, state-of-the-art review of the current knowledge of cardiomyopathies. Instead of following the classic interdisciplinary division, the entire cardiovascular system is presented as a functional unity, and the contributors explore pathophysiological mechanisms from different perspectives, including genetics, molecular biology, electrophysiology, invasive and non-invasive cardiology, imaging methods and surgery. In order to provide a balanced medical view, this book was edited by a clinical cardiologist.

How to reference
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