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The *area composita* of adhering junctions connecting heart muscle cells of vertebrates. VI. Different precursor structures in non-mammalian species

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Abstract

Recent studies on the formation and molecular organization of the mammalian heart have emphasized the architectural and functional importance of the adhering junctions (AJs), which are densely clustered in the bipolar end regions (*intercalated disks*, IDs) connecting the elongated cardiomyocytes of the adult heart. Moreover, we learned from genetic studies of mutated AJ proteins that desmosomal proteins, which for the most part are integral components of ID-specific composite AJs (*areae compositae*, AC), are essential in heart development and function. Developmental studies have shown that the bipolar concentration of cardiomyocyte AJs in IDs is a rather late process and only completed postnatally. Here we report that in the adult hearts of diverse lower vertebrates (fishes, amphibia, birds) most AJs remain separate and distinct in molecular character, representing either *fasciae adhaerentes*, *maculae adhaerentes* (desmosomes) or – less frequently – some form of AC. In the mature hearts of the amphibian and fish species examined a large proportion of the AJs connecting cardiomyocytes is not clustered in the IDs but remains located on the lateral surfaces where they appear either as *puncta adhaerentia* or as desmosomes. In many places, these *puncta* connect parallel cardiomyocytes in spectacular ladder-like regular arrays (*scalae adhaerentes*) correlated with – and connected by – electron-dense plaque-like material to sarcomeric Z-bands. In the avian hearts, on the other hand, most AJs are clustered in the IDs but only a small proportion of the desmosomes appears as AC, compared to the dominance of distinct *fasciae adhaerentes*. We conclude that the fusion and amalgamation of AJs and desmosomes to ACs is a late process both in ontogenesis and in evolution. The significance and possible functional implications of the specific junctional structures in vertebrate evolution and the class-specific requirements of architectural and molecular assembly adaptation during regeneration processes are discussed.

Keywords: Intercalated disks; Adhering junctions; Desmosomes; Heart evolution; Cardiomyopathies

Introduction

Superficially seen, the morphological dominance of the sarcomeric organization in both cross-striated muscle cell systems, the skeletal and the cardiac muscles, together with their contractile nature may suggest a closer relationship of these cells than that actually

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justified by cell, molecular and developmental biological criteria (for reviews see, e.g., Manasek, 1970; Severs, 1989; Mikawa, 1999). In fact, the mono- or bi-nucleated, spontaneously, autonomously and rhythmically contractile cardiomyocytes are directly derived from epithelial precursor cell layers in early stages of heart formation and maintain some typically epithelial structures and molecules, including cell junction proteins of the desmosomal type and at least in some species, initially even certain cytokeratins (Franke et al., 1981, 1982; Kartenbeck et al., 1983; Atherton et al., 1986; Kuruc and Franke, 1988; van der Loop et al., 1995). This remarkable specificity of cardiomyocyte differentiation is not only obvious in comparison with the syncytial system of skeletal muscle cells but is also seen directly in the endocardium when the endothelial cells differentiate from cardiomyocytes, by morphology as well as by the synthesis of specific hallmark structures and molecules (for reviews see also Severs, 1989; Mikawa, 1999; Sedmera et al., 2000).

Recently, we have reported that the ultrastructural organization and the molecular arrangement of the adhering junctions (AJs) connecting cardiomyocytes in mature mammalian hearts are complex and special (Borrmann et al., 2006; Franke et al., 2006, 2007). Cardiomyocyte elongation, myofibril organization and extension, and the bipolar AJ accumulations, resulting in the formation of a complex cardiac coupling system, are late, partly postnatal processes (e.g., Markwald, 1973; Shibata et al., 1980; Angst et al., 1997; Hirschy et al., 2006; Pieperhoff and Franke, 2007). Finally, almost all junctions of the AJ category are clustered in the so-called ''intercalated disks'' (IDs) and often appear to be fused and molecularly amalgamated to *areae compositae* (ACs, ''composite junctions''), representing a mixture of desmosomal (e.g., plakophilin-2, desmoplakin, desmocollin-2 and desmoglein-2) and *fascia adhaerens* (e.g., N-cadherin, cadherin-11, α- and β-catenins, proteins p120 and ARVCF) components (Borrmann et al., 2006; Franke et al., 2006; for ultrastructure see also Tandler et al., 2006; see also Goossens et al., 2007). Composite junctions of this kind have also been described in primary cultures of cardiomyocytes from neonatal rat hearts but here indications of a pathological disintegration of these structures upon prolonged cultivation cannot be overlooked (Franke et al., 2007; see also Atherton et al., 1986). In recent years, studies of the formation and molecular organization of the cardiac AJ system have found special genetic and medical interest (Bierkamp et al., 1996; Ruiz et al., 1996; Grossmann et al., 2004; for indirect contributions see, e.g., Ehler et al., 2001), in particular since a number of hereditary cardiomyopathies have been reported to result from mutations in AC molecules (Beffagna et al., 2007; Kleber, 2007; Marcus et al., 2007; Otterspoor et al., 2007; Thiene et al., 2007; for further references see Pieperhoff et al., 2008; for reports of genetic causes of other cardiomyopathies see Ferrans et al., 1973; Towbin and Bowles, 2002).

In view of the late development of AC structures and the bipolar clustering in the IDs of mammalian hearts, it is surprising to note the different patterns of organization and development in lower vertebrates such as fishes, amphibia, reptiles and birds (e.g., Hibbs, 1956; Fawcett and Selby, 1958; Grimley and Edwards, 1960; Huang, 1967; Leak, 1967; Manasek, 1968; Hagopian and Spiro, 1970; Hirakow, 1970; Manasek, 1970; Midttun 1980, Pfeiffer et al., 1990; Burggren and Warburton, 1994; Mikawa, 1999; Sedmera et al., 2000; Laube et al., 2006). Therefore, we have studied the formation of AJs connecting cardiomyocytes in non-mammalian vertebrates. Surprisingly, we have found differences in the organization of cell–cell interactions in the hearts of lower vertebrates, which have led us to the conclusion that the formation of the AC junctions is not only a relatively late process in mammalian ontogenesis but also in vertebrate evolution.

Materials and methods

Tissues, cell cultures, fixations and processing

Mature cardiac tissue of chicken (*Gallus gallus*) was obtained from freshly slaughtered, ca. 1-year-old cocks and was fixed and processed essentially as described for mammalian tissues (e.g., Kuruc and Franke, 1988; Franke et al., 2006). Pigeon heart was obtained and treated similarly. Living adult rainbow trout (*Oncorhynchus mykiss*, 20–40 cm) and eels (*Anguilla anguilla*) were purchased from a local delicatessen shop (Feinkost Schlereth, Heidelberg, Germany), and living zebrafish (*Danio rerio*) specimens were from a local aquaculture shop. Trout and eels were killed directly or after anesthesia with MS 222 (Sigma, Taufkirchen, Germany). Adult animals of different amphibian species, including *Xenopus laevis*, *Rana pipiens*, *Ambystoma mexicanum* and *Pleurodeles waltl*, were obtained from the animal core facility of the German Cancer Research Center and were anesthesized with MS 222 (Sigma), followed by decapitation.

Various tissue samples from these species, notably heart, skeletal muscle, stomach, intestine, skin and liver, were processed immediately after death for immuno- and standard-electron microscopy of ultrathin sections, for paraffin-embedding and microwave-mediated antigen retrieval, or for cryosectioning as described (e.g., Franke et al., 2006; Borrmann et al., 2006; Pieperhoff and Franke 2007).

Tissue samples of $\sim$1 cm^2 and cell culture monolayer preparations were processed for SDS–PAGE and immunoblotting as described (e.g., Borrmann et al., 2006; Franke et al., 2007).