

Why Do We Have Purkinje Fibers Deep in Our Heart?

D. SEDMERA^{1,2}, R. G. GOURDIE^{3,4,5}

¹Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic, ²Department of Cardiovascular Morphogenesis, Institute of Physiology Academy of Sciences of the Czech Republic, Prague, Czech Republic, ³Virginia Tech Carilion Research Institute, Center for Heart and Regenerative Medicine Research, Roanoke, Virginia, USA, ⁴Virginia Tech School of Biomedical Engineering and Sciences, Roanoke, Virginia, USA, ⁵Department of Emergency Medicine, Virginia Tech Carilion School of Medicine, Roanoke, Virginia, USA

Received October 21, 2013

Accepted October 29, 2013

Summary

Purkinje fibers were the first discovered component of the cardiac conduction system. Originally described in sheep in 1839 as pale subendocardial cells, they were found to be present, although with different morphology, in all mammalian and avian hearts. Here we review differences in their appearance and extent in different species, summarize the current state of knowledge of their function, and provide an update on markers for these cells. Special emphasis is given to popular model species and human anatomy.

Key words

Cardiac conduction system • Specialized tracts • Gap junctions • Connexin

Corresponding author

D. Sedmera, Department of Cardiovascular Morphogenesis, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague, Czech Republic. E-mail: dsedmera@biomed.cas.cz

Introduction

The cardiac conduction system (CCS) is defined as a network of specialized myocardial cells that generates the cardiac rhythm and assures a coordinated propagation of the electrical impulse for efficient contraction of the heart. In the adult mammalian heart, the CCS comprises the sinoatrial (SA) node, the

internodal tracts, the atrioventricular node, the atrioventricular (His) bundle, its right and left branches, and the network of Purkinje fibers. While the functional equivalent of these components are present in some form in all vertebrate hearts (Sedmera *et al.* 2003), all morphologically distinct parts are present only in the heart of mammals. While development of the CCS has been subject to numerous reviews (Gourdie *et al.* 2003a,b, Christoffels *et al.* 2010, Burggren *et al.* 2013), little was written on the comparative morphology of its components in different species, with a few notable exceptions (Davies 1930, Davies *et al.* 1952, 1994). The goal of this overview is to put the Purkinje fibers into context of other CCS components, briefly describe the history of their discovery, provide functional insight into equivalent structures in the lower vertebrates, and then to focus in detail on their structure and function in the most popular model species of mammals and birds.

Function of individual CCS components

The SA node is the principal pacemaker of the heart. Under normal conditions it is the cardiac tissue that autonomously sets the rhythm of the heart beat. It is also subject to neurohumoral regulation, in particular by autonomous nervous system. This allows the heart to change its frequency in reaction to the functional status of the organism. Morphologically, it is organized into a three dimensionally complex compact structure

(Mommersteeg *et al.* 2007, Fedorov *et al.* 2010), with specific connection points to the working atrial myocardium. In lower vertebrates, its function is contained in a compartment termed the sinus venosus (Koprla 1987, Jensen *et al.* 2012). New data on the cellular origin of the SA node from outside the traditional heart fields and role of Wnt signaling in recruitment of mesodermal cells into pacemaker lineage were recently reported by the Mikawa lab (Bressan *et al.* 2013).

Atrial special conduction pathways

The morphological distinction of specialized intra-atrial and internodal conduction pathways is a controversial topic. Some claim that there are tracts of Purkinje-like cells connecting the sinoatrial and atrioventricular node (James and Sherf 1971), while others agree that the conduction through the atria is anisotropic, see the main reason in holes caused by the entrance vessels (Betts *et al.* 2002, Ho *et al.* 2002). Our view is that these preferential conduction pathways, the best example of which is the interatrial bundle of Bachmann (Sedmera *et al.* 2006) can be explained by tissue geometry (pectinate muscles), in agreement with previous experimental data (Komuro *et al.* 1986), but are open to a marker that would distinguish cells within those tracts from the remaining atrial myocytes.

The AV node

The main function of the atrioventricular node is generation of a delay between activation (and ensuing contraction) of the atria and the ventricles. Due to its prolonged refractory period, it also serves as a filter against propagation of atrial tachyarrhythmias to the ventricles. In mammals, it shows a distinct morphological organization with specific cell phenotypes (Efimov *et al.* 1997, Aanhaanen *et al.* 2010). On the other hand, its morphological localization in birds is still obscure (Vicente-Steijn *et al.* 2011). In lower vertebrates, and during embryonic development, the function of delay generator is located in the atrioventricular canal region, a slowly proliferating, conducting and contracting portion of the cardiac tube that apparently retains its “primitive” phenotype – not following the pathway of chamber myocardium differentiation (Kirby 2007). The transcriptional regulation of these events has been recently uncovered, and Tbx2, BMP and Tbx3 are implicated in maintaining this transcription programme

(Mommersteeg *et al.* 2007, Aanhaanen *et al.* 2009, 2010). Electrophysiologically, this region shows a typical action potential shape (Arguello *et al.* 1986) and has a level of automaticity, which can manifest even in the embryo when the sinoatrial node is perturbed (Raddatz 1997). Interfaces between atria and node and node and His are marked by distinct transitions in connexin expression (Coppin *et al.* 1999, Gourdie and Sedmera 2008). Such abrupt changes in cellular coupling can play a role in AV delay generation in adult heart (Choi and Salama 1998).

The His bundle

Also known as the atrioventricular, or non-branching bundle, it forms under normal conditions the only conductive pathway between the atria and the ventricles. It is a rapidly conducting tissue, with propagation velocity an order of magnitude faster than the working myocardium. As it traverses the atrioventricular fibrous plane, the bundle is insulated from the rest of the myocardium except of its proximal connection with the AV node and distal bifurcation into the left and right bundle branch.

The bundle branches

These form continuation of the His bundle, sharing many of its characteristics – rapid conduction speed, expression of gap junction protein connexin40 (without co-expression of connexin43) and fibrous insulation from the working myocardium. This makes these bundles well suited to act as electrical cables, assuring rapid spread of the impulse through the ventricles. There is a notable asymmetry between the left and right bundle branch, the left being broad, in the mouse composed of multiple parallel isolated strands, while the right bundle being a narrow structure (Miquero *et al.* 2004). It is likely due to optimization of source:sink ratio due to a marked asymmetry of the myocardial mass between the left and right ventricle.

The Purkinje fibers

The Purkinje fibers are the terminal part of the cardiac conduction system. They form a three-dimensional subendocardial network originating from the bundle branches and their main function is to distribute the depolarization signal rapidly to the working myocardium.

Discovery of CCS

The historical sequence, in which various CCS components were discovered, is in reverse order to the functional sequence of activation. In 1839 the Czech scientist Jan Evangeliste Purkinje described a pale network of cells in the sheep heart, and noted their microscopic characteristics, including the presence of one or two nuclei and cross striations, which made him, after some discussion, consider them a special form muscular tissue (Eliska 2006). It took more than fifty years before a Swiss physiologist His found the elusive connection between the atrial and ventricular myocardium, first by a series of cuts in the beating heart, then by detailed histological examination of the region, where such cuts led to atrioventricular block (Suma 2001). Over a decade later, Tawara (1906) connected this bundle by describing the atrioventricular node proximally and the bundle branches distally. The last component discovered was the sinoatrial node, described by Keith and Flack (1907) only a year later.

Original description by Purkinje

The original 1839 description by Purkinje was a part of a larger volume dealing with innervation of various organs and published in Polish. He followed up six years later in 1845 with a more detailed account in German, then the universal language of morphologists (Eliska 2006). There are few interesting points in this description that pertain to the present review. First, while the original species was sheep, he noted similar structures in the cow, pig, and horse. On the other hand, he was unable to see his cells in the human, dog, rabbit, or hare heart. This is an important reminder of the dangers of expecting that a functionally similar structure will look the same in different species (Robb 1965). Second, already by then he stated with certainty that these structures are not nervous fibers, especially significant in the context of general focus of his 1839 text on nervous tissue. Third, although he did seriously consider (due to their appearance) that these fibers could be cartilage, the presence of striations made him to decide that most likely these tissues were muscular. Nevertheless, later studies using various neuronal markers re-ignited the issue (Gorza *et al.* 1988, 1994), and the final word on myogenic nature of the CCS was provided using genetic lineage tracing techniques – showing its common origin with working cardiomyocytes (Gourdie 1995, Cheng *et al.* 1999).

Differentiation of Purkinje fibers during development

It has been shown in experimental studies in the chick heart, that ventricular Purkinje fibers share common origin with the surrounding ventricular myocytes and terminally differentiate during fetal period (Gourdie *et al.* 1995). In the avian model, it was convincingly demonstrated that the differentiation towards the conduction phenotype is time-sensitive, and locally produced endothelin-1 was demonstrated to be a key signaling molecule (Gourdie *et al.* 1998, Hyer *et al.* 1999, Takebayashi-Suzuki *et al.* 2000). The clonal relationship between the working ventricular myocytes and Purkinje myocytes observed in birds was confirmed recently in the mammalian (mouse) model (Meilhac *et al.* 2003, Miquerol *et al.* 2010). However, transcription or any other regulation governing this process in mammals is still elusive. While it was long speculated that ventricular trabeculae, forming during chamber differentiation as a means to increase myocardial mass prior to presence of coronary perfusion, form the precursors of the Purkinje network (de Jong *et al.* 1992, Sedmera *et al.* 2004), it is clear that not all the trabeculae will turn into Purkinje fibers – in fact, the majority will form trabeculae carneae, or “meaty” trabeculations composed largely of working myocardium, that we find on the inside of the ventricles. As noted above, our knowledge of mechanisms governing these decisions is woefully incomplete.

Subendocardial and intramural network

The Purkinje network is composed of two components: the subendocardial fibers, which have connection to the bundle branches and assure the apex-to-base activation of the ventricle, and a variably present intramural component. While the first is invariably reported, although with different morphology, from all mammalian and avian hearts, the latter, presumably accelerating transmural conduction, is only morphologically distinguishable in some species – in particular sheep (Ryu *et al.* 2009) (Fig. 1), cow (Oosthoek *et al.* 1993), or pig (Fig. 2). In these animals, connection between the subendocardial and intramural network can be found at regular intervals (Fig. 6 in Oosthoek *et al.* 1993), and the extent in cow is about 80 % of left ventricular wall thickness. On the other hand, despite quite extensive searches, no intramural fibers

have been located in the mouse or human heart (Fig. 3). The presence or absence of the intramural component does not seem to be related to heart size, as some small animals – such as rats (Fazel *et al.* 1989, Thompson *et al.* 1990, Gourdie *et al.* 1992, 1993, Vuillemin *et al.* 1992) or chicken (Gourdie *et al.* 1993) (Fig. 4) do have intramural fibers – in the case of chicken clearly associated with the coronary arteries.

Functionally, the Purkinje fibers are characterized by a unique ion channel expression and faster conductivity in comparison to the working ventricular myocardium. This creates a local heterogeneity in conduction velocity and coupling, which can lead to re-entry. Their role as foci of arrhythmias was recently and extensively reviewed in (Boyden *et al.* 2010).

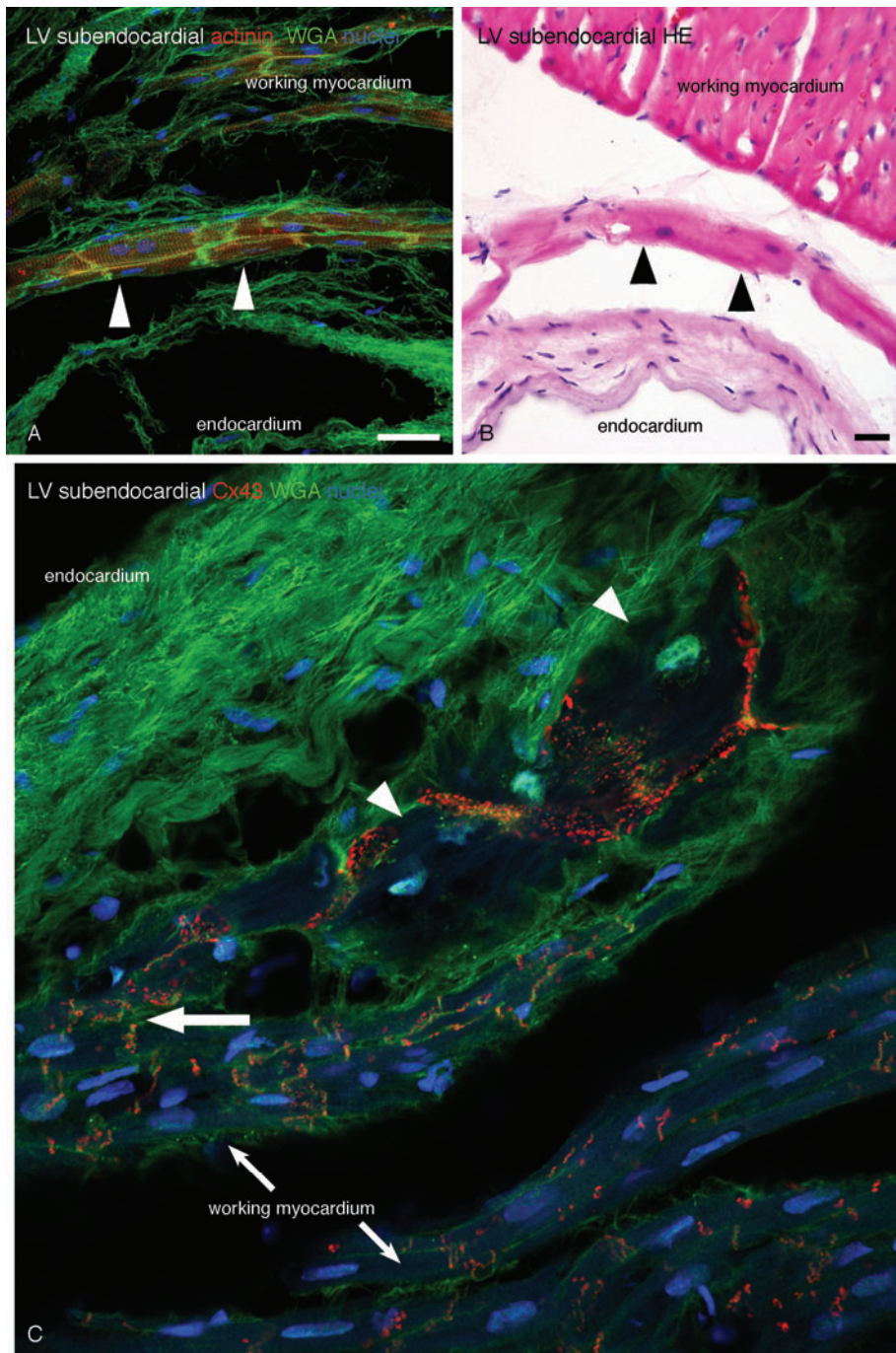


Fig. 1. Purkinje fibers in the sheep heart. **A.** Actinin staining shows that both the subendocardially located Purkinje fibers and the working cardiomyocytes have well organized contractile apparatus. **B.** Hematoxylin and Eosin staining shows clearly that they are larger than the working ventricular myocytes and their spatial and fibrous isolation. **C.** Staining for connexin43 shows very high density of these gap junction proteins on the entire cell membrane (compare to neighboring working myocardium where it is localized mostly at cell ends). Wheat germ agglutinin (WGA) staining highlights cell boundaries and fibrous tissue. Scale bars 25 μm.

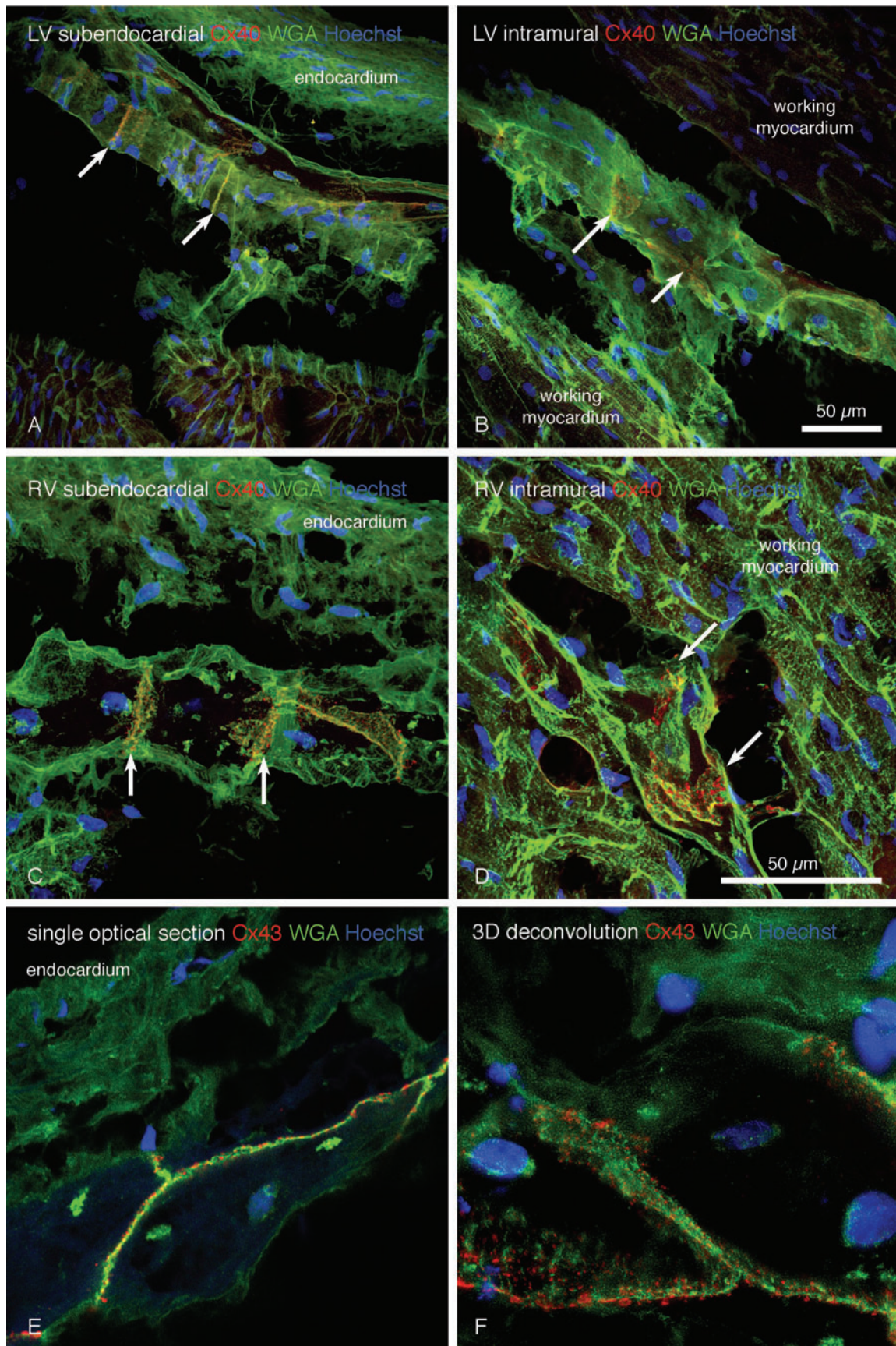


Fig. 2. Purkinje fibers in the pig heart. Connexin40 staining labels specifically both subendocardial (**A**) and intramural (**B**) Purkinje fibers. **C** and **D**: similar morphology with Connexin40 staining preferentially localized to cell ends and distribution of Purkinje cells is found also in the right ventricle. **E** and **F**: in contrast, Connexin43 staining is distributed in the entire cell surface.

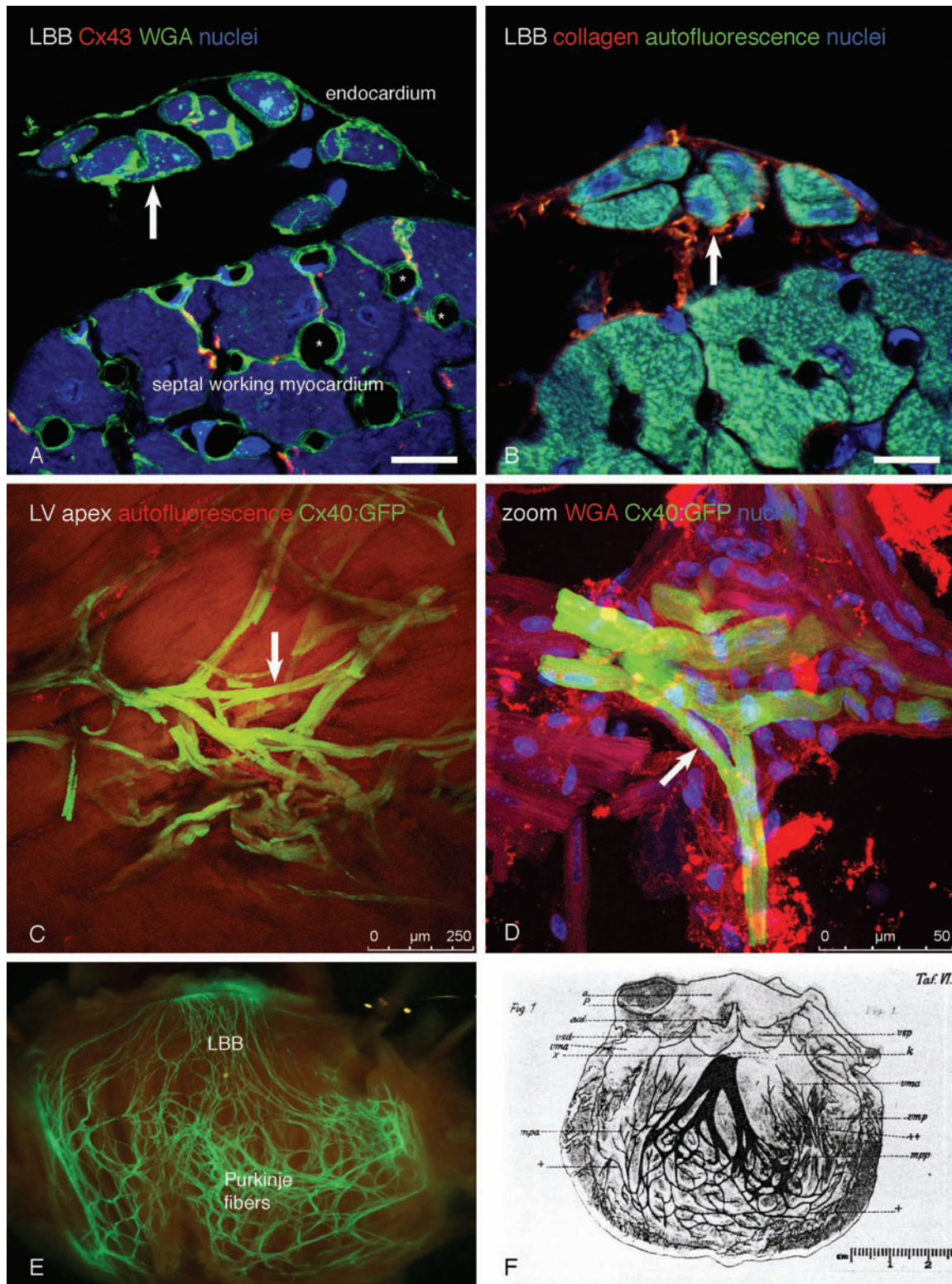


Fig. 3. Purkinje fibers in the murine heart. Panels **A** and **B** show the transition of Connexin43-negative left bundle branches into Purkinje fibers, which co-express both Connexin40 and 43. The tracts are spatially separated from the working myocardium and show a thin fibrous sheath. Scale bars 10 μ m. Panels **C** and **D** visualize the Purkinje network using Connexin40:GFP transgenic mouse. The Purkinje network is formed by 1-3 cells thick strands of myocytes that are thinner but longer than the working myocytes. Panels **E** and **F** compare the arrangement of the entire left ventricular network in mouse (from Miquerol *et al.* 2004, with permission) and human (Tawara 1906). In neither species were described any intramural Purkinje fibers.

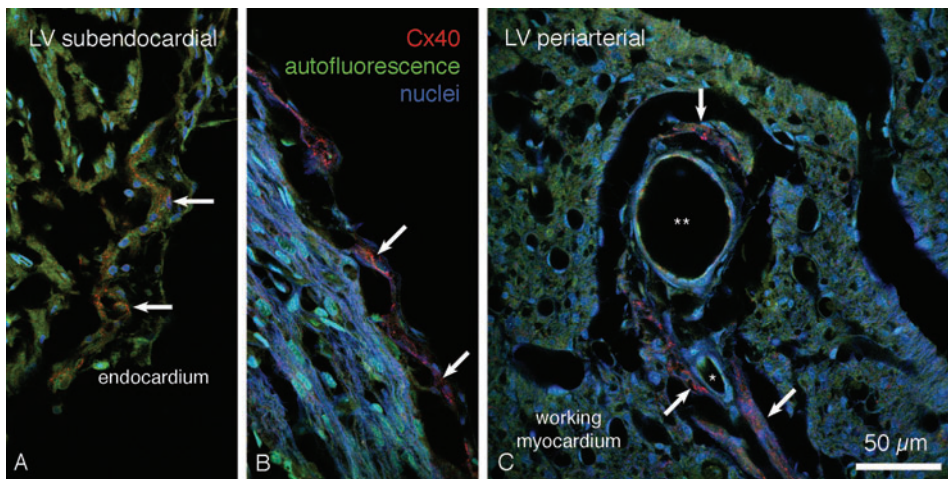


Fig. 4. Purkinje fibers in the ED17 chick embryonic heart. Similar to ungulates, there are both subendocardial (**A, B**) as well as intramural (**C**) Purkinje cells; the later are located periarterially. Coronary arteries are labeled with asterisks.

Current state of the art future perspectives

As a closing paragraph, we would like to provide some insight into some potentially fruitful areas of active research of Purkinje fibers. First, new morphological markers such as contactin (Pallante *et al.* 2010) allow us better delineation of the entire network across species (Ryu *et al.* 2009), and help to uncover signaling pathways directing their differentiation from the working myocytes in mammals such as notch signaling (Rentschler *et al.* 2012). This would be important to resolve the issue why some closely related species do or do not possess the intramural component (e.g. rat vs. mouse). Second little explored question is the morphological and functional imaging of contacts and conduction at the Purkinje-myocyte junction, that should be enabled by coupling of these markers with high-speed, high-resolution mapping techniques. Is there a gradual transition of phenotype from clear PF to working myocyte, or is the boundary sharply defined? The third question worthy of attention is

the origin and differentiation of Purkinje myocytes. Is the commitment to the conduction lineage irreversible, and if yes, at which point of development? What is the default program of ventricular myocytes – conduction, or working phenotype? Resolution of this particular question would be important piece of information necessary to production of larger pieces of implantable tissue-engineered myocardium.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Author's primary research is supported by the Ministry of Education of the Czech Republic PRVOUK-P35/LF1/5, and institutional RVO: 67985823 from the Academy of Sciences of the Czech Republic. Current support from the Grant Agency of the Czech Republic P302/11/1308 and 13-12412S is also gratefully acknowledged. RGG is supported by NIH RO1 HL56728.

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