Heterocellular communication in the heart: electron tomography of telocyte–myocyte junctions

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Received: November 26, 2010; Accepted: December 10, 2010

Abstract

Myocardium is composed of two main cell populations: cardiomyocytes (CMs) and interstitial cells (*e.g.* fibroblasts, immunoreactive cells, capillaries). However, very recently we have showed that a novel type of interstitial cell called telocytes (TCs) does exist in epi-, myo- and endocardium. They have very long and thin telopodes (Tp) formed by alternating podomeres and podoms. Heterocellular communication between TCs and CMs it is supposed to occur by shed vesicles and close apposition. If TCs have to play a role in cardiac physiology it is expected to develop direct and unambiguous contacts with CMs. Because a clear membrane-to-membrane junction has not been reported by electron microscopy we have investigated the heterocellular communication in the mouse heart by electron tomography. This advanced technique showed that small dense structures (10–15 nm nanocontacts) directly connect TCs with CMs. More complex and atypical junctions could be observed between TCs and CMs at the level of intercalated discs. This study proves that TCs and CMs are directly connected and might represent a 'functional unit'.

Keywords: telocytes • cardiomyocytes • myocardium • heterocellular junctions • nanocontacts • electron tomography

Introduction

The heart is a heterogeneous organ composed of different types of cardiomyocytes (CMs), capillaries, nerves and a large variety of interstitial cells. Among interstitial cells, telocytes (TCs) [1] have been recently described as a novel type of cell in the mammalian heart [2–15]. TCs are interstitial cells with exceptionally long cellular processes – telopodes (Tp) – which form an interstitial network connecting different types of cell from myocardium [4–8], epicardium [9, 10, 13], endocardium [14] and cardiac stem cell niches [3, 11]. The concept of TCs was adopted by other laboratories [15–30].

TCs seem to be involved in *long-distance intercellular signalling*, taking into account the 3D network made by coupled long Tp and the release of shed vesicles/exosomes [1]. Direct junctions between TCs and cardiac progenitors have been reported in cardiac stem cell niche [3, 11]. The proximity of Tp with intercalated discs has been reported [7] but a clear membrane-to-membrane junction between TCs and CMs has not been observed in normal heart by transmission electron microscopy. If TCs have to play a role in cardiac physiology it is expected to develop direct and unambiguous contacts with working CMs.

We used electron tomography (ET) to clarify the connection between TCs and CMs considering the advantage of 3D exploration offered by this advanced technique.

Material and methods

This study was performed on mouse heart samples obtained from 8-month-old C57BL/6 mice. The tissue samples were processed according to routine Epon-embedding procedure, as previously described [5, 7]. The thin and thick sections were cut with a diamond knife using an RMC ultramicrotome (Boeckeler Instruments, Inc., Tucson, AZ, USA) and double stained with 1% uranyl acetate and Reynolds lead citrate.

1. Transmission electron microscopy was performed on 60-nm-thin sections using a Morgagni 286 transmission electron microscope (FEI Company, Eindhoven, The Netherlands) at 60 kV. Digital electron micrographs were recorded with a MegaView III charged-coupled device (CCD) using iTEM-SIS software (Olympus, Soft Imaging System GmbH, Münster, Germany).

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doi:10.1111/j.1582-4934.2011.01299.x

2. ET was performed was performed on 250-nm-thick sections using a Tecnai G2 Spirit BioTwin electron microscope with single-tilt specimen holder (FEI Company) at 100 kV and electron tomographic data sets were recorded with a MegaView G2 CCD camera (Olympus) in ET mode [31, 32]. Tomographs were acquired at 1° angular increment from –65° to +65° about an axis perpendicular to the optical axis of the microscope, at 36,000× magnifications (1.64 nm/px). After data alignment, the data sets were reconstructed into 3D volume (data collection, reconstruction and visualization) using Xplore3D Tomography Suite software (FEI Company). Amira 5.0.1 software (Visage Imaging GmbH, Berlin, Germany) was used for 3D imaging.

Results

The ET study of 250-nm-thick sections showed that TCs have junctions with contractile CMs (Figs 1–4). Tp were located beneath the basal lamina of CMs (Fig. 1B, C) and established direct membrane-to-membrane contacts. Serial sections showed that a TC has more contact sites with the same CM (Fig. 1D).

Snapshots of digital sections from ET reconstructed volumes did not suggest the existence of any kind of classic junctions between TCs and CMs.

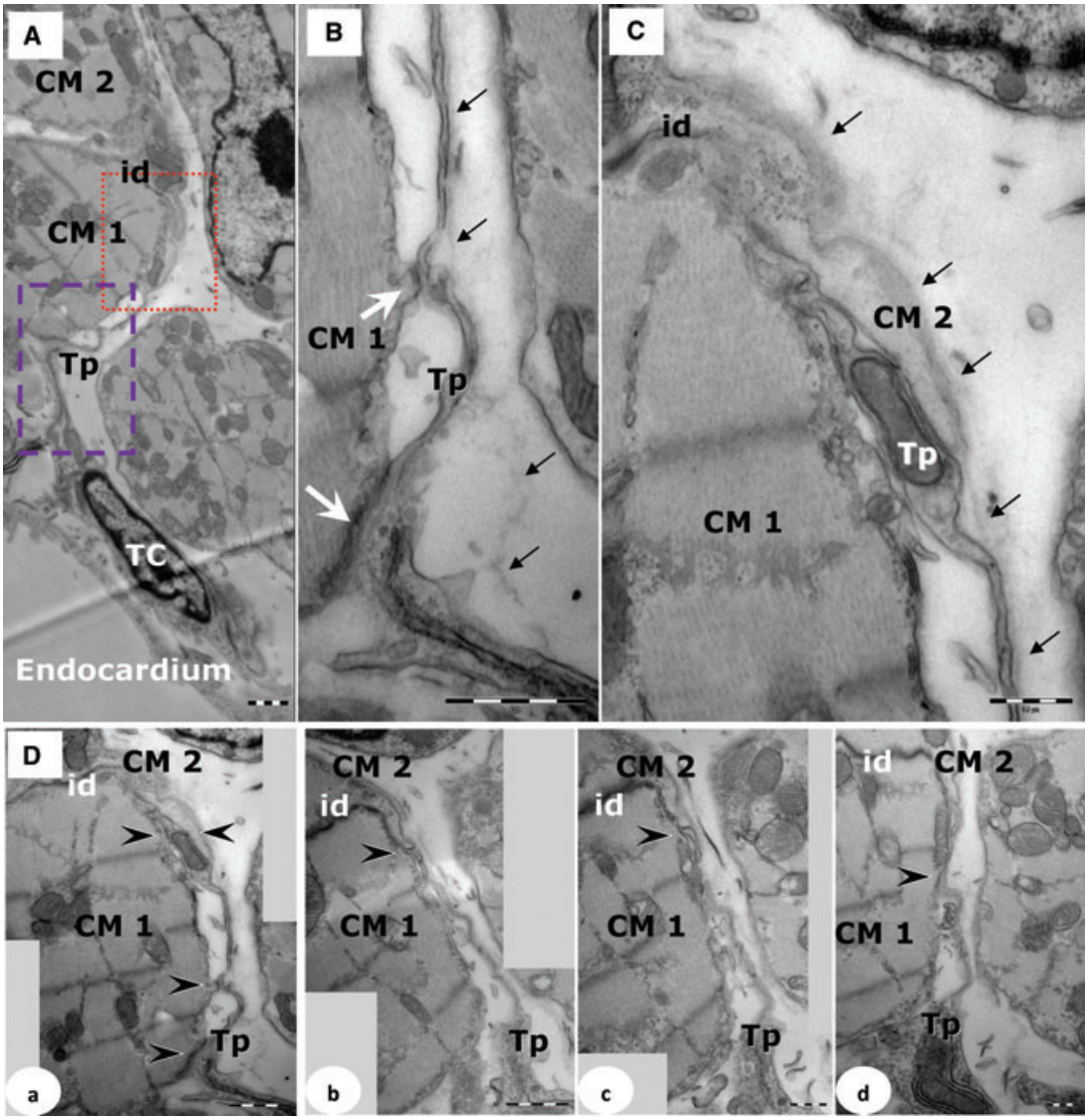

Fig. 1 (**A**)–(**C**) Electron microscopy on 250-nm-thick sections shows a TC extending a Tp beneath basal lamina (black arrows) of the CMs (CM1, CM2). id: intercalated disc. (**B**) Higher magnification of dashed rectangle in (**A**). The membrane of Tp has two distinct contact points (white arrows) with the membrane of CM. (**C**) Higher magnification of red dotted rectangle in (**A**). The Tp is enclosed by CM1 and a small process of CM2. Basal lamina (black arrows) goes over CM2 and covers the Tp. No basal lamina is visible in between CM1 and Tp. (**D**) Transmission electron microscopy images (a–d) of serial sections show that contacts between Tp and CMs (CM1, CM2) are discontinuous and limited to small areas (arrowheads). id: intercalated disc. Scale bars: (**A**), (**B**), (**D**) – 1 μm; (**C**) – 0.5 μm.

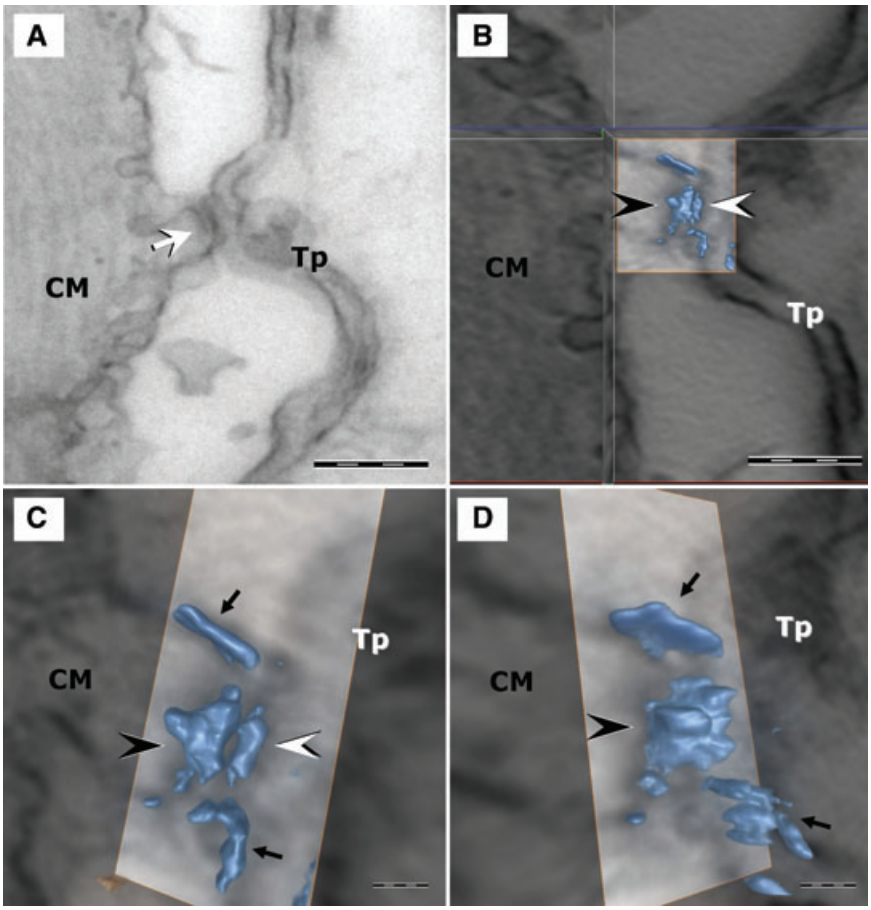

Fig. 2 (**A**) Digital section from tomographic volume shows a junction between the Tp and a discrete projection of the CM. (**B**) Top view of the 3D isosurface reconstruction of the junction shows a macromolecular complex connecting Tp and CM (arrowheads). (**C**) Lateral view of the 3D isosurface reconstruction shows that macromolecular connecting structure is formed by two asymmetric components. Black arrowhead indicates the bigger subunit (about 50 nm) which belongs to the CM membrane. The cytoplasmic segment has a bulb-like appearance. White arrowhead indicates the smaller subunit (about 15 nm) sited into the TC membrane. (**D**) The oblique view shows that contact surface of CM subunit (black arrowhead) has a square shape contact surface with four corner protuberances. (**C**), (**D**) Dense structures reinforce the cellular membranes of both cells (arrows) lateral to the macromolecular assembly. Scale bar: (**A**), (**B**) – 200 nm; (**C**), (**D**) – 40 nm.

One reconstructed contact (Fig. 2) showed a complex structure formed by two separate subunits sited in the cellular membranes of TCs and CMs. The CM subunit was composed of a bulb-like cytoplasmic domain which expands into a larger square-shaped membranar domain. The membranar face of CM subunit appeared square with four corner protuberances. The dimensions of this component were about 50 nm high and 40 nm wide, but the real dimensions cannot be exactly measured because of the chemical procedure used for fixation and embedding. The TC unit was flat (15 nm) and square (40 nm/40 nm) and matched the membranar surface of the CM unit. The Tp (45 nm wide) bent at the level of contact site with a small CM protuberance (100 nm emergence). The CM membrane had caveolae in the vicinity of connecting protuberance.

Another reconstructed contact between one TC and two CMs had a more complex appearance (Figs 3 and 4). A dilation of Tp (podom) accommodating a mitochondrion and a cisterna of endoplasmic reticulum was located in a niche made by two CMs connected by the intercalated disc (Fig. 1). CM membrane had caveolae and endoplasmic reticulum cisternae and multivesicular body were located beneath the membrane. The connection between TCs and CMs showed no typical features for different types of junctions known, but small nanopillars (10–15 nm / 20–30 nm) connecting the cellular membranes (Fig. 4). The bridging structures suggest a molecular interaction between the Tp and CMs but their electron density was below the density of membranes and isosurface reconstruction was not enough appropriate to imagine these nanostructures. Different sections through the reconstructed volume showed clusters of nanocontacts (Fig. 3) with symmetrical distribution fastening the connection between TCs and CMs.

Discussion

Homocellular communication is essential for cardiac physiology [33, 34]. Heterocellular communication is also important for heart physiology and pathology and we demonstrate here that TCs are directly involved in the myocardial function throughout molecular interactions.

Structural coupling of cardiac myocytes and fibroblasts was reported, especially in pathological sates [35]. Fibroblasts are

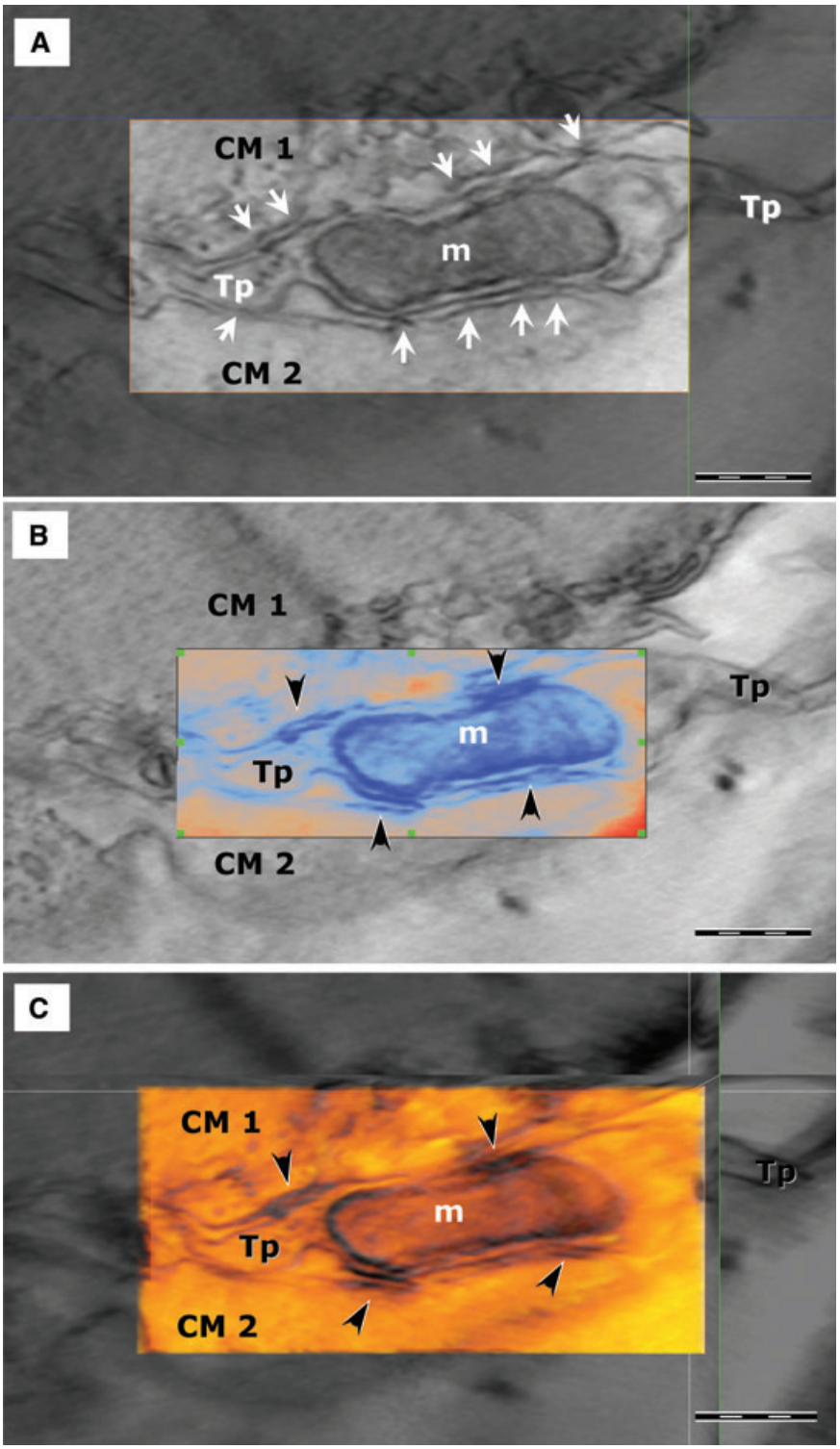

Fig. 3 ET shows small electron-dense structures (arrows) connecting the cellular membranes of a TC (Tp) and two CMs (CM1, CM2). A mitochondrion (m) is visible in the dilation of the Tp (podom). (**A**) A digital slice shows small electron-dense structures connecting Tp and both CMs. (**B**) A projection view and (**C**) a multiplanar view of the same region shows that nanocontacts are clustered (arrowheads). Scale bars: 250 nm.

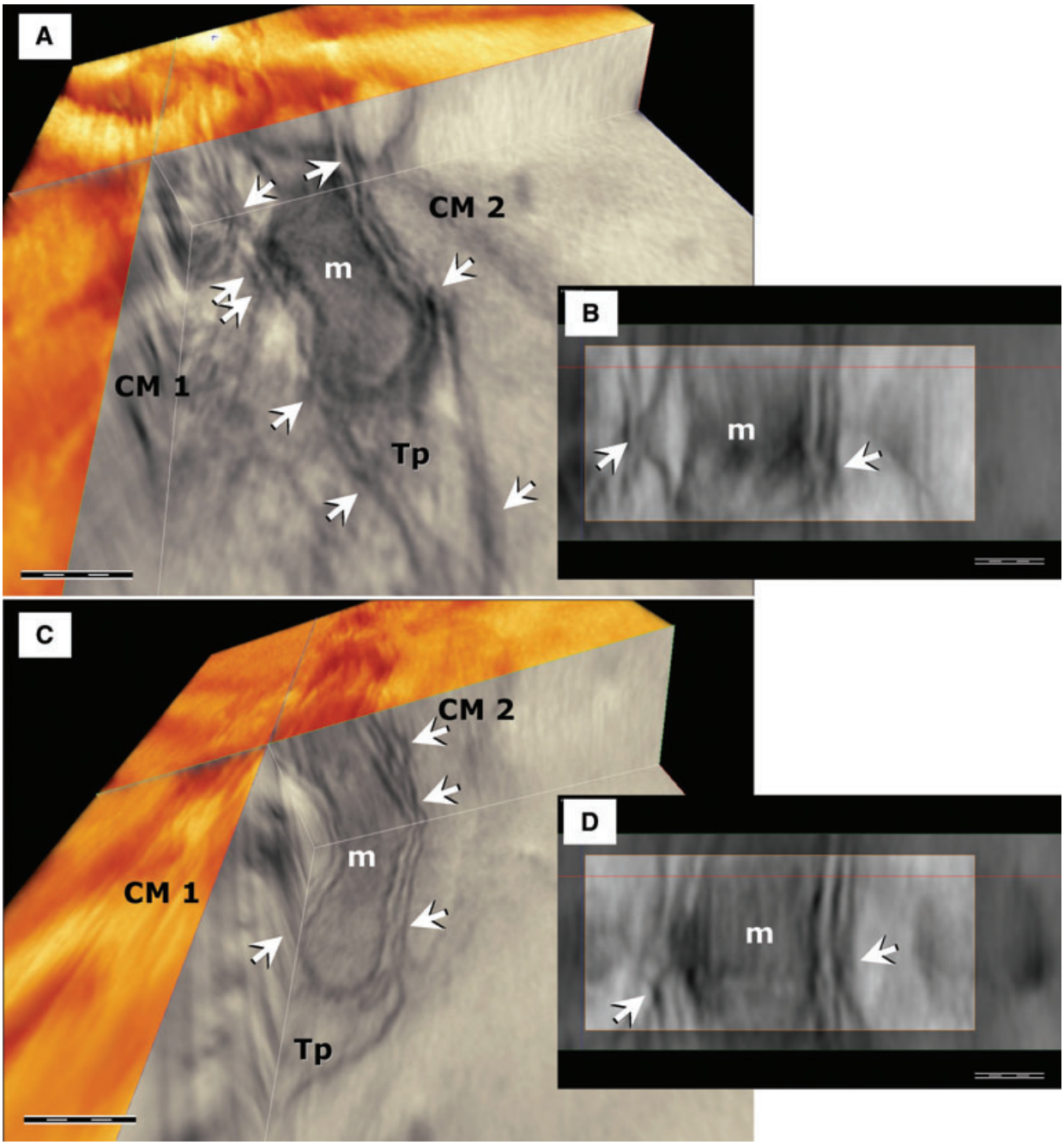

Fig. 4 ET shows nanocontacts (arrows) connecting the cellular membranes of the Tp and CMs (CM1, CM2). The mitochondrion (m) is visible in the dilation of Tp. Images in (**A**) and (**C**) illustrate nanocontacts at different levels into the reconstructed volume (cropped corners). (**B**) and (**D**) show nanocontacts in lateral views (*yz*-axis) of the reconstructed volume. Scale bars: (**A**), (**C**) – 200 nm; (**B**), (**D**) – 50 nm.

spindled cells with a cytoplasm dominated by rough endoplasmic reticulum [36] and we were unable to find fibroblasts in normal heart to investigate the heterocellular junctions between fibroblasts and CMs. In normal heart, TCs seem to be the main interstitial cell [2–14] and we believe that one of the most important roles of TCs is long-distance heterocellular communication [1, 11, 37]. However, it should be emphasized that, for the moment, (too) many people are focused on myocardial

fibroblasts [38]. TCs appear to be involved in some cardiac pathological events [39, 40].

The heterocellular communications between TCs and CMs do not belong to classic types of junctions found in textbooks [41]. More likely there are molecular interactions which need to be identified. For instance, the macromolecular assembly we have described in one type of TC–CM connection (Fig. 2) suggests the presence of the ryanodine receptor (see for instance Figure 4 in [42]). We assume that TC–CM interaction is essential not only for cardiac development [15, 20], renewal [2, 3, 11] and repair [2] but also plays a significant role in cardiac physiology. In conclusion, this study proves that TCs and CMs are unequivocally physical connected and might represent a 'functional unit' of the heart.

Acknowledgements

This work was supported by ANCS project number PN 09.33/2009 and partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109.

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