Short Communication *J. Cell. Mol. Med. Vol 11, No 5, 2007 pp. 1175-1184*

Myocardial interstitial Cajal-like cells (ICLC) and their nanostructural relationships with intercalated discs: shed vesicles as intermediates

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Received: August 31, 2007; Accepted: August 31, 2007

Abstract

Intercalated discs (ID) are complex junctional units that connect cardiac myocytes mechanically and electrochemically. However, there is limited information concerning the cardiomyocyte interaction with interstitial non-muscle cells. Our previous studies showed that myocardial interstitial Cajal-like cells (ICLC) are located in between cardiomyocytes, blood capillaries and nerve fibres. Typically, ICLC have several very long, moniliform, cytoplasmic processes which establish closed contacts with nerve fibres, as well as each other. We report here ultrastructural evidence concerning the relationships of ICLC processes with ID. The ICLC cytoplasmic prolongations (tens micrometers length) preferentially pass by or stop nearby the ID. Transmission electron microscopy emphasized three distinct connecting features between the tips of ICLC extensions and myocytes at the 'mouth' of ID: free or budding shed vesicles, exocytotic multi-vesicular bodies and direct contacts. In the last case, electron-dense repetitive nanostructures ('pillars') (35–40 nm high and 100–150 nm wide, similar to adhesion molecules) fasten the ICLC to the myocytes. All these features suggest a juxtacrine and/or paracrine intercellular mutual modulation of ICLC and cardiomyocytes in the microenvironment of ID, possibly monitoring the cardiac functions, particularly the electrical activity.

Keywords: interstitial Cajal-like cells • myocardocytes • intercalated discs • shed vesicles • exosomes • nanocontacts • heterocellular communication • intercellular signaling

Introduction

One major objective of our laboratories during the last few years was to investigate the presence of the interstitial Cajal-like cells (ICLC) outside the musculature of the gastrointestinal tract [1, 2] and to define the ultrastructural characteristics of this type of cell [3–9]. We found that the main attribute of ICLC is the presence of several, thin (mainly ~50 nm, uneven caliber), extremely long (tens of micrometers), and moniliform cytoplasmic processes. These processes seem to form a cellular network connecting target cells. Anyway, our results seem to be accepted by a lot of authors from four continents [*e.g.*: 10–25].

Noteworthy, we also demonstrated in a series of papers [26–28] the unequivocal existence of ICLC in human and rat, atrial and ventricular myocardium. Our findings are not unexpected since it is quite well known that myocardial interstitial cells overpass

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doi: 10.1111/j.1582-4934.2007.00117.x

numerically the working myocytes. In addition, the myocardial ICLC have tens micrometers long processes which appear moniliform and share typical close contacts with nerve fibres [28]. Our findings about myocardial ICLC seem to be confirmed in very recent papers [29–34].

However, there is limited information regarding the cardiomyocyte interaction with interstitial non-muscle cells, although heterocellular close contacts between fibroblasts and adult cardiomyocytes have been reported in co-culture and experimental models [35, 36]. However, always remains some uncertainty about cellular phenotype and the results obtained in culture.

Up to now, the relationship between interstitial cells and intercalated discs of cardiomyocytes has not been investigated. Intercalated discs (ID), the hallmark of the heart muscle, are complex microstructures composed by gap junctions, desmosomes, fascia adherens and a recently documented structure named the 'transitional junction' [37]. This study investigates the relationship of ICLC with the ID of cardiomyocytes.

Material and methods

Tissue samples from Wistar rat myocardium were obtained and processed for ultrastructural investigation as previously described [28].

Ten Wistar rats, having a body weight of 200–250 g, with free access to food and water, maintained in a temperature-controlled facility with a 12-hrs light/dark cycle were used for this study. All animal experiments have been carried out in accordance with the ethical Guidelines for Animal Experimentation and the study was approved by the Bioethics Committee of 'Carol Davila' University of Medicine Bucharest.

Ventricular and atrial myocardium was harvested under anaesthesia after perfusion-fixation (1.5% buffered glutaraldehyde) followed by immersion in 4% buffered glutaraldehyde. Tissue samples were cut into 1 mm three small fragments and fixed for 4 hrs in 4% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4 at 4`B0C. The fragments were post-fixed for 1 hr in buffered 1% $OsO_4$, dehydrated in an ethanol series and then processed for Epon 812 embedding at 60`B0C for 48 hrs.

One-micron-thick sections stained with 1% toluidine blue were examined for a precise orientation of the subsequent thin sections. The ultrathin sections were cut using an LKB ultramicrotome with a diamond knife and double stained with 1% uranyl acetate and Reynolds lead citrate.

Electron microscopy examination was performed with both a Philips CM 12 and a Philips 301 transmission electron microscope at 60 kV. The images were recorded with Morada 11 megapixel CCD camera and analysed with iTEM SYS software. Data are expressed as mean `B1 SD. Digitally colour images were obtained using Adobe Photoshop software.

Results and discussion

The transmission electron microscopy (TEM) investigation showed a strong affinity of ICLC for the neighbouring area of ID. The tips of ICLC cytoplasmic processes have been observed in proximity of about 55% of ID (Figs. 1–8). Most of the ICLC processes end up in the extracellular myocyte pockets associated with the 'mouth' of ID (Figs. 1 and 3).

We have noticed free vesicles with electron-lucent content or even multi-vesicular body structures in the extracellular space between the ICLC endings and the periphery of ID (Fig. 4). The mean diameter of these free vesicles was 78 `B1 10 nm (min = 50 nm, max = 91 nm).

We have not seen any direct contact between ICLC tips and the external limit of the ID, but the distance in between was ranging between 80 and 500 nm (272 `B1 32 nm) which suggests some kind of paracrine signalling. Endorsing this hypothesis we have also observed shed vesicles (118 `B1 16 nm average diameter, min = 98 nm, max = 177 nm) between ICLC fingers and cardiomyocytes, next to the ID (Figs. 5–7). Some of these vesicles bud from the ICLC cytoplasmic processes (Fig. 5) and budding could be the mechanism of their formations. We have also observed round, dense granules (~50 nm diameter) placed either in contact with the ICLC plasma membrane, in the basal lamina thickness or in the cortical cytoplasm of the myocardocytes in the proximity of ID (Figs. 3 and 7).

Moreover, the ultrastructural analysis showed electron-dense structures connecting the ICLC cytoplasmic processes by cardiomyocytes on the ID areas (Figs. 7 and 8). These anchoring structures ('pillars') have 35–40 nm high and 100–150 nm wide and show a repetitive pattern.

We have previously showed that ICLC establish stromal synapses with immunoreactive cells [38]. These electron-dense structures connecting ICLC

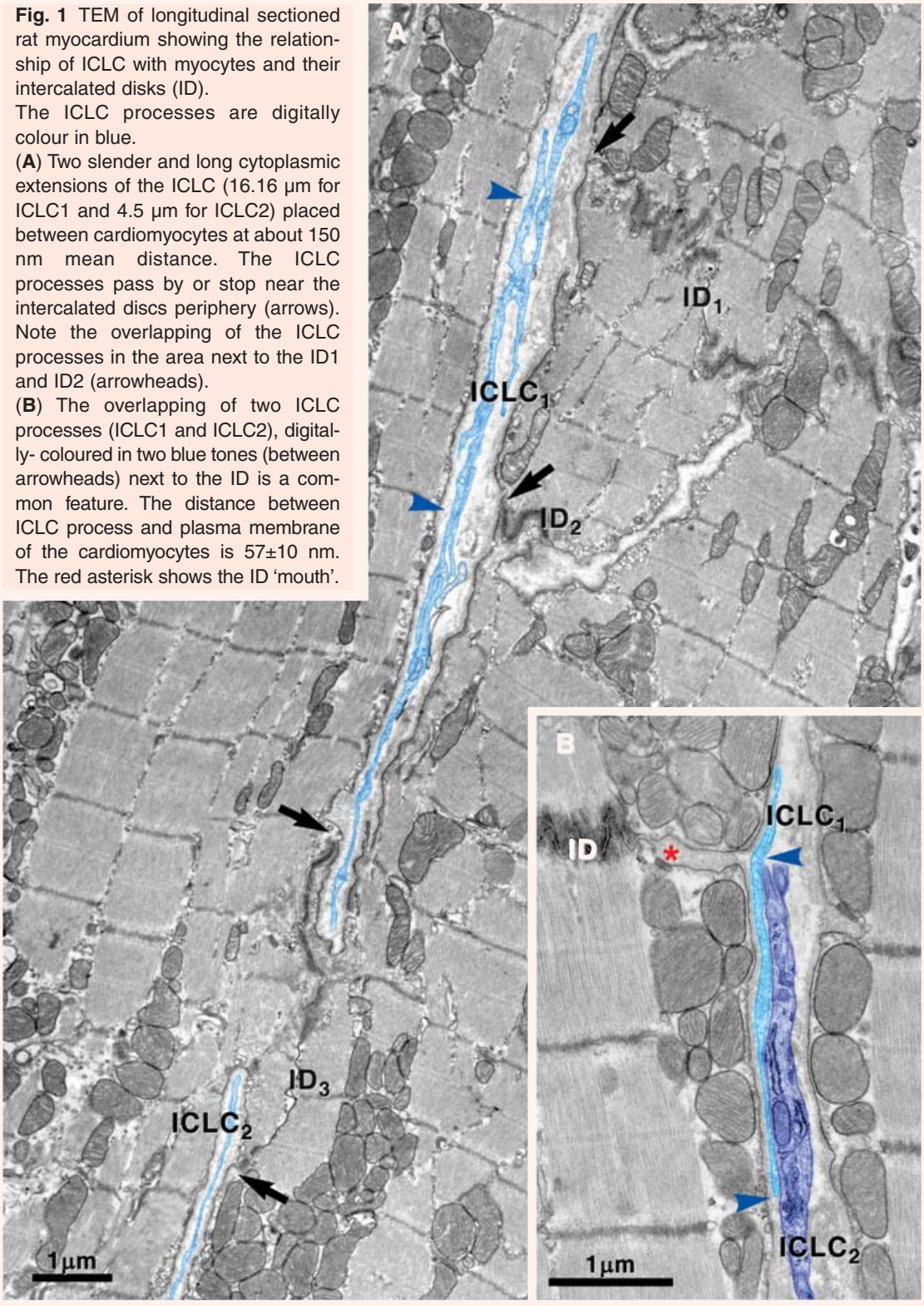

Fig. 1 TEM of longitudinal sectioned rat myocardium showing the relationship of ICLC with myocytes and their intercalated disks (ID).
The ICLC processes are digitally colour in blue.
(**A**) Two slender and long cytoplasmic extensions of the ICLC (16.16 µm for ICLC1 and 4.5 µm for ICLC2) placed between cardiomyocytes at about 150 nm mean distance. The ICLC processes pass by or stop near the intercalated discs periphery (arrows). Note the overlapping of the ICLC processes in the area next to the ID1 and ID2 (arrowheads).
(**B**) The overlapping of two ICLC processes (ICLC1 and ICLC2), digitally- coloured in two blue tones (between arrowheads) next to the ID is a common feature. The distance between ICLC process and plasma membrane of the cardiomyocytes is 57±10 nm. The red asterisk shows the ID 'mouth'.

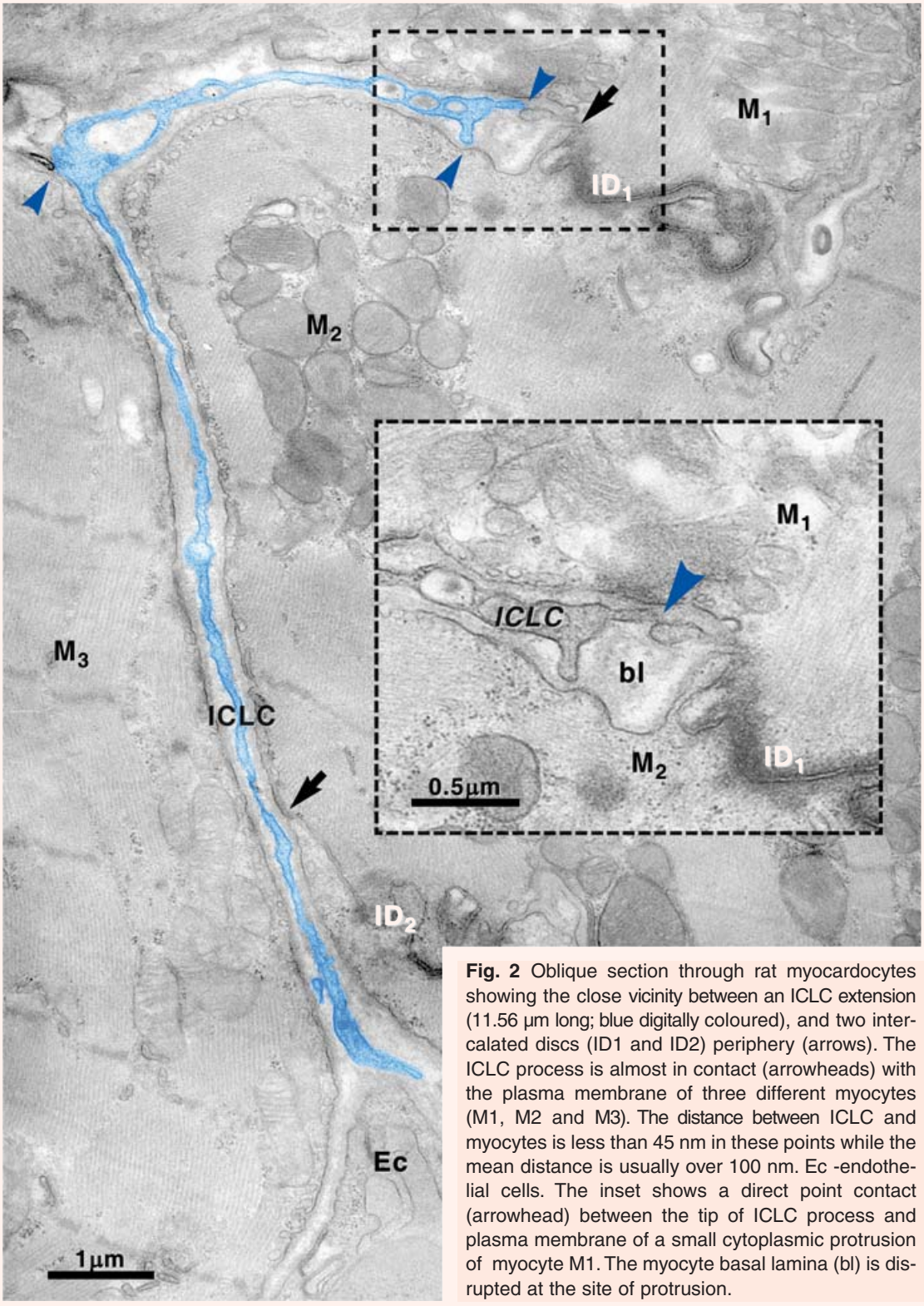

Fig. 2 Oblique section through rat myocardocytes showing the close vicinity between an ICLC extension (11.56 µm long; blue digitally coloured), and two intercalated discs (ID1 and ID2) periphery (arrows). The ICLC process is almost in contact (arrowheads) with the plasma membrane of three different myocytes (M1, M2 and M3). The distance between ICLC and myocytes is less than 45 nm in these points while the mean distance is usually over 100 nm. Ec -endothelial cells. The inset shows a direct point contact (arrowhead) between the tip of ICLC process and plasma membrane of a small cytoplasmic protrusion of myocyte M1. The myocyte basal lamina (bl) is disrupted at the site of protrusion.

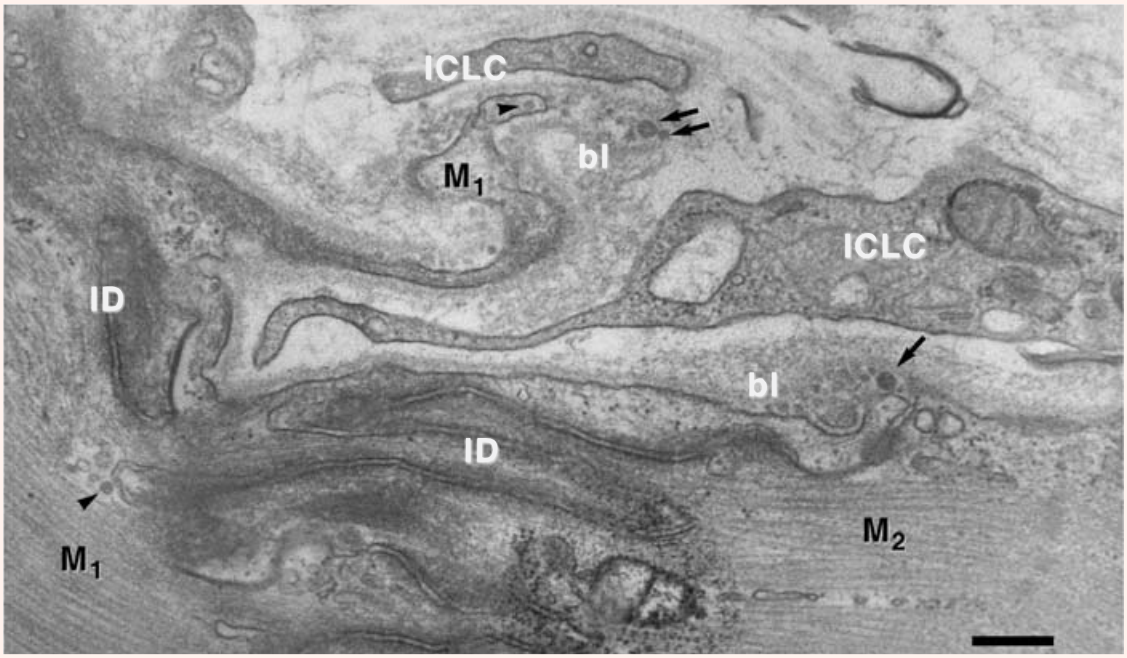

Fig. 3 An ICLC process ending in a myocytic pocket of myocardocyte M1 at the level of the intercalated disc (ID). Small electron-dense particles (50 nm or less) can be seen in the basal lamina thickness of the myocardocytes (arrows) or beneath plasma membrane in the cytoplasm of the myocardocyte M1 (arrowheads). Scale bar = 0.2 μm.

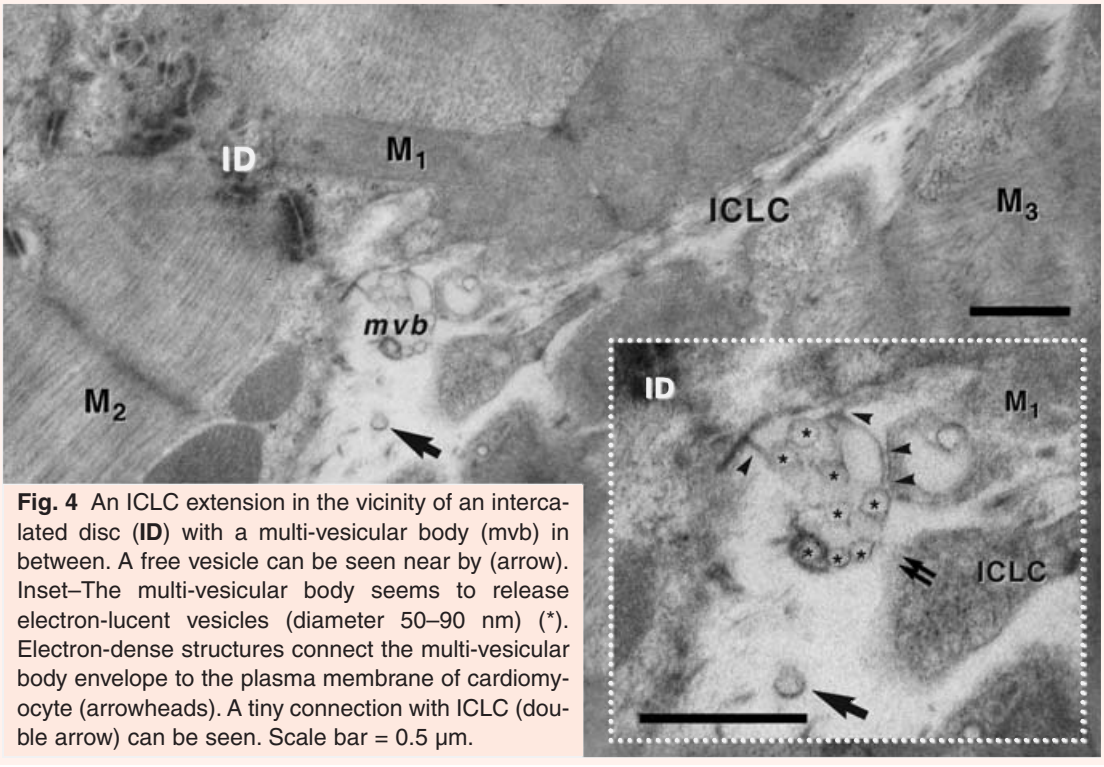

Fig. 4 An ICLC extension in the vicinity of an intercalated disc (**ID**) with a multi-vesicular body (mvb) in between. A free vesicle can be seen near by (arrow). Inset–The multi-vesicular body seems to release electron-lucent vesicles (diameter 50–90 nm) (*). Electron-dense structures connect the multi-vesicular body envelope to the plasma membrane of cardiomyocyte (arrowheads). A tiny connection with ICLC (double arrow) can be seen. Scale bar = 0.5 μm.

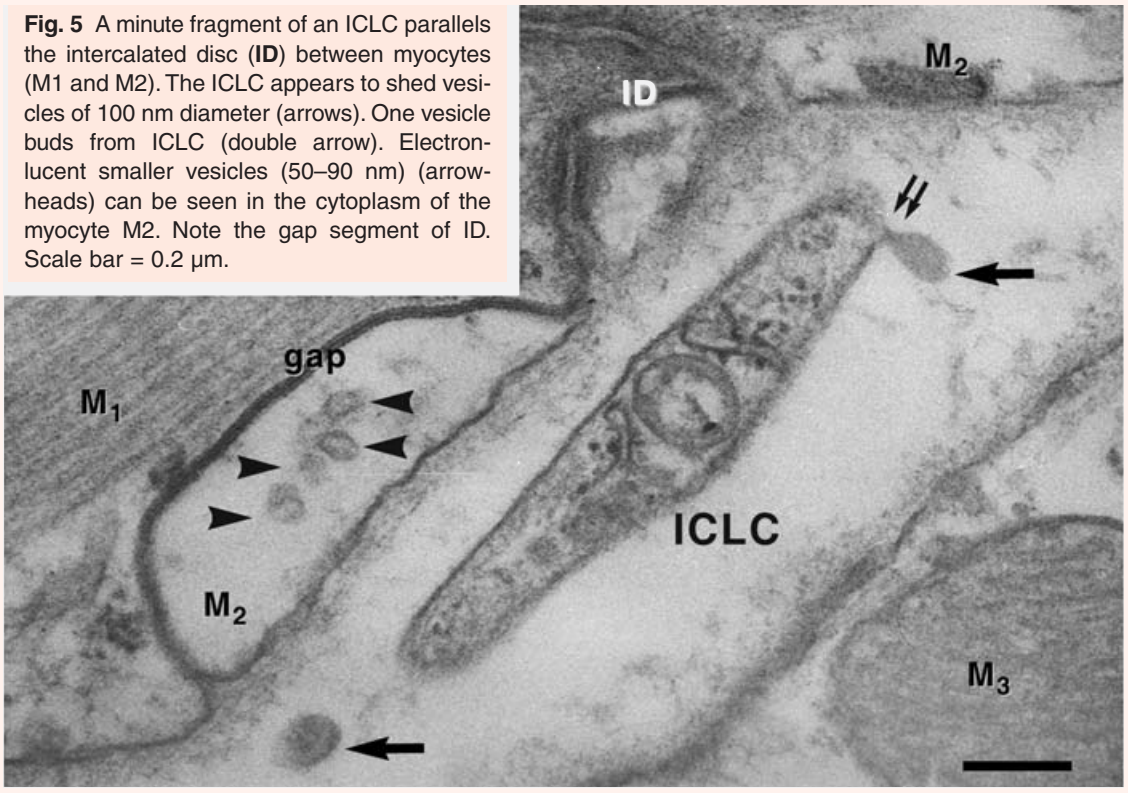

Fig. 5 A minute fragment of an ICLC parallels the intercalated disc (**ID**) between myocytes (M1 and M2). The ICLC appears to shed vesicles of 100 nm diameter (arrows). One vesicle buds from ICLC (double arrow). Electron-lucent smaller vesicles (50–90 nm) (arrowheads) can be seen in the cytoplasm of the myocyte M2. Note the gap segment of ID. Scale bar = 0.2 μm.

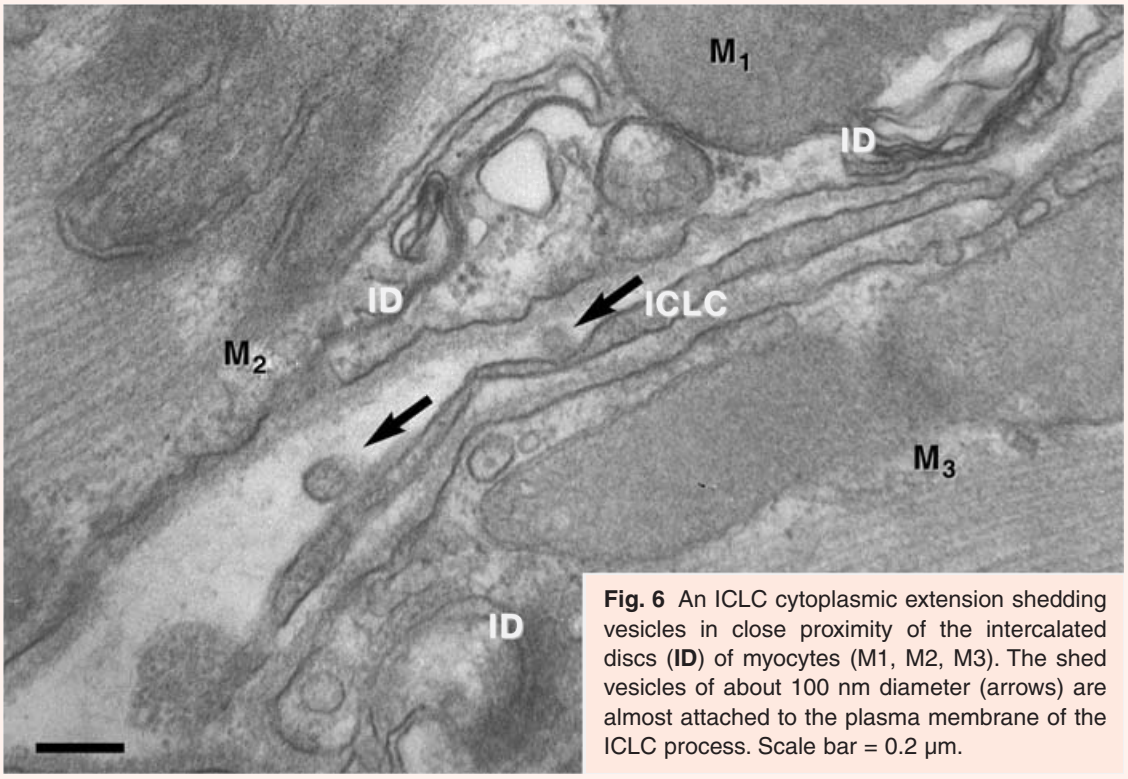

Fig. 6 An ICLC cytoplasmic extension shedding vesicles in close proximity of the intercalated discs (**ID**) of myocytes (M1, M2, M3). The shed vesicles of about 100 nm diameter (arrows) are almost attached to the plasma membrane of the ICLC process. Scale bar = 0.2 μm.

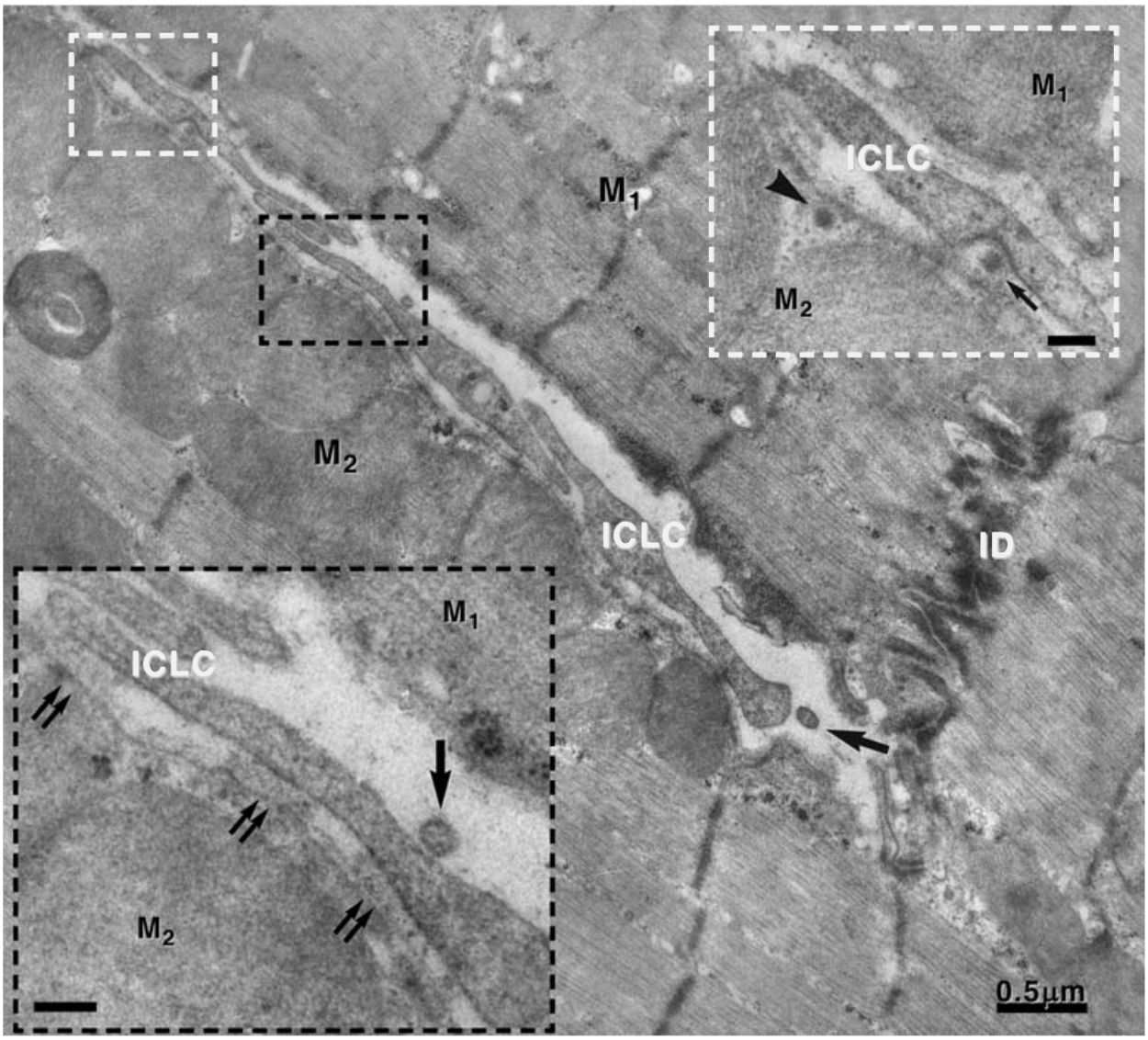

Fig. 7 A segmented ICLC extension shedding a vesicle (arrow) toward the intercalated disc (**ID**). The white-bordered inset shows two electron-dense particles with about a 50 nm diameter: one of them in a pocket of the ICLC process (arrow) and next to the plasma membrane of the myocyte (M2), the second in the cytoplasm of the myocyte (arrowhead). Another shed vesicle (arrow) can be seen in the black-bordered inset. Note the electron-dense nanostructures (double arrows) that connect the ICLC and the myocyte (M2). The distance in between the two plasma membranes at the level of attachments structures is about 35–40 nm, similar to adhesion complexes. Scale bar for insets = 0.1 µm.

with cardiomyocytes have a size comparable with adhesion molecules [39] and they could be involved in a juxtacrine intercellular signalling process or could facilitate a paracrine signalling process.

All these features suggest a paracrine and/or juxtacrine intercellular mutual modulation of ICLC and cardiomyocytes in the microenvironment of ID.

Exosomes and shed vesicles [40–45] have been described in a variety of physiological and pathological conditions, but they continue to be under a thoroughly investigation. Microvesicles, exosomes and shed vesicles are produced and secreted by tumour and normal cells with an important role in intercellular communication and immune response [45].

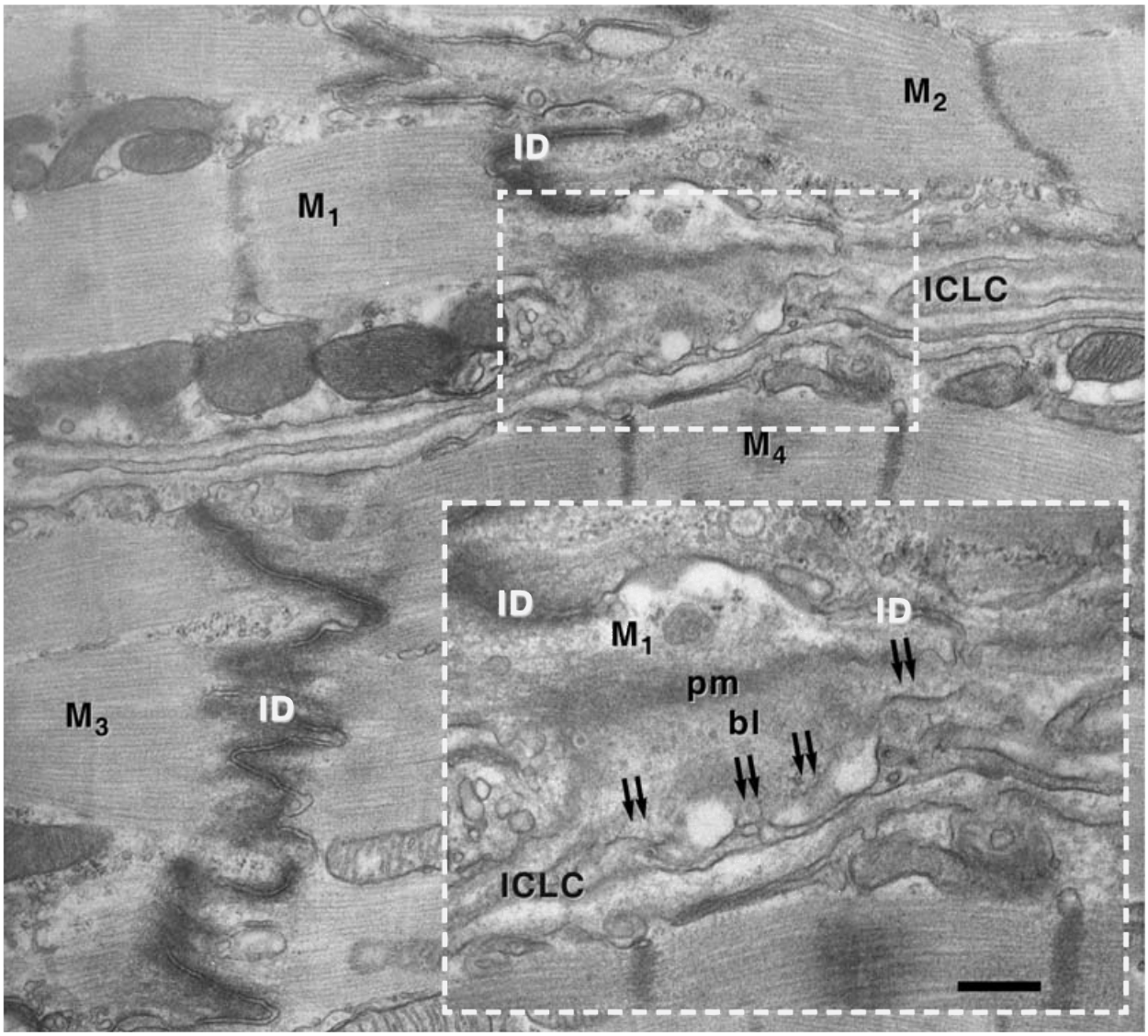

Fig. 8 ICLC cytoplasmic extension in apposition with a myocyte in the intercalated discs (**ID**) area. The inset shows in an oblique-sectioned segment of plasma membrane (pm), four electron-dense attachment structures connecting the basal lamina (bl) of cardiomyocyte (M1) with the ICLC cytoplasmic process. Scale bar = 0.2 μm.

Intercellular communication entails not only huge structures with a distinctive architecture (as ID), but more elusive mobile nanostructures with limited time-life which mediate the information among different cellular types (nanovesicles, exosomes, shed vesicles). The immediacy of intercalated discs and ICLC long processes which are connected to each other and with nerve fibres and other interstitial cells could affect in a paracrine or juxtacrine manner myocar-dial contraction.

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