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Electron microscope tomography: further demonstration of nanocontacts between caveolae and smooth muscle sarcoplasmic reticulum

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Abstract

A spatial relationship between caveolae and sarcoplasmic reticulum (SR) in smooth muscle cells (SMC) was previously reported in computer-assisted three-dimensional reconstruction from transmission electron microscope serial sections. The knowledge of the three-dimensional organization of the cortical space of SMC is essential to understand caveolae function at the cellular level. Cellular tomography using transmission electron microscopy tomography (EMT) is the only available technology to reliably chart the inside of a cell and is therefore an essential technology in the study of organellar nanospatial relationships. Using EMT we further demonstrate here that caveolae and peripheral SR in visceral SMC build constantly spatial units, presumably responsible for a vectorial control of free Ca^{2+} cytoplasmic concentrations in definite nanospaces.

Keywords: electron microscope tomography • caveolae • sarcoplasmic reticulum • smooth muscle • calcium signaling

Electron microscope tomography (EMT) it is known for decades but it is actually used for three-dimensional (3D) imaging of sub-cellular structures in recent years [1–6]. Latest technical advances have improved the methods making it more reliable in description of 3D details of macromolecular and subcellular structures. Fully automated tomography is easy to use and allows the visualization of the 3D organization of the cortical space of smooth muscle cells (SMC) and this is essential to understand caveolae function at the cellular level.

We have shown that there are strategic caveolae-SR or caveolae-mitochondria contacts at the nanoscale level in the cortical cytoplasm of SMC [7, 8]. These complexes could be responsible for a vectorial control of free Ca^{2+} cytoplasmic concentrations in definite nanospaces and for selective activation of specific Ca^{2+} signaling pathways [7–10].

Wistar rat urinary bladder smooth muscle was Epon embedded as formerly described [7, 8]. The thin sections were cut at 500 nm using an RMC ultramicrotome and double stained with 1% uranyl acetate and Reynolds lead citrate. EMT was performed in FEI's NanoPort–Eindhoven (The Netherlads) using a Tecnai G2 F20 scanning transmission electron microscope (S/TEM) at 200 kV. Electron tomographic data sets were recorded in TEM mode on 500 *nm thick sections* Epon embedded smooth muscle cells. The images

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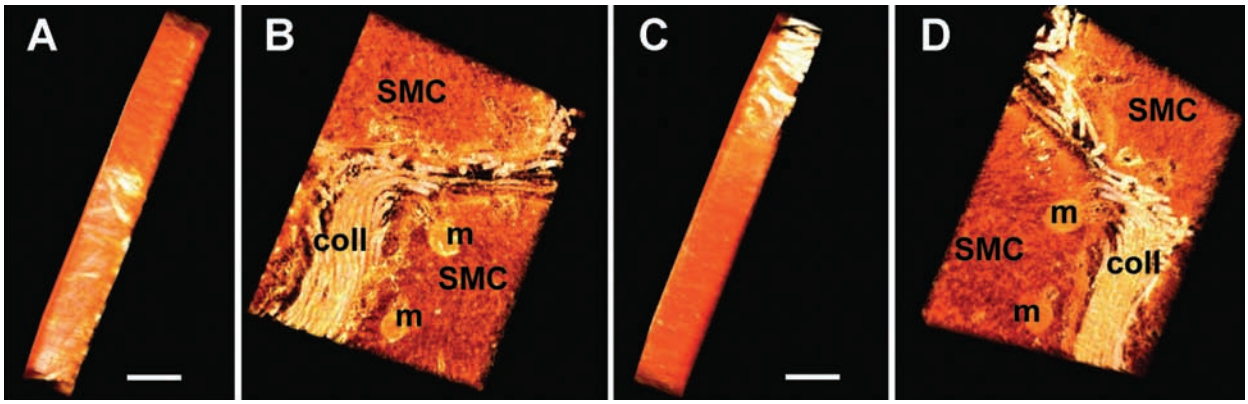

Fig. 1 The 3D reconstructed volume of smooth muscle cells (SMC) obtained by electron tomography and digitally colored in red is rotated with 90°. **A**, **C** illustrate the thickness of section which is 500 nm (scale bar = 0.5 μm). In the front (**B**) and back (**D**) views could be seen tissue features: SMC with a crowded cytoplasm and mitochondria (m) and a bunch of collagen fibers.

were acquired at 1° increment over an angular range of –70° to +70°, at magnification 8600x with a Gatan CCD camera. After data alignment, the data set was reconstructed into a 3D volume. FEI Xplore3D Tomography Suite software was used for 3D imaging (data collection, reconstruction and visualization).

EMT emphasized tremendously complex contractile system of SMC (Fig. 1 and *on-line supplement*) which appear as 'background noise' in a classical picture from transmission electron microscopy (Fig. 2). Focusing on caveolar domains (Fig. 3), EMT showed that each caveolae have at least one contact point with SR. Our previously results, particularly the 3D reconstructions from serial ultrathin sections highlight the idea that caveolae and SR form a unique feature, cortical **continuum compartment** in SMC. Cellular electron tomography of the cortical space of SMC augments the idea of a structural unit formed by caveolae and SR which could be regarded as a **'super-Ca^{2+} release/storage unit'.**

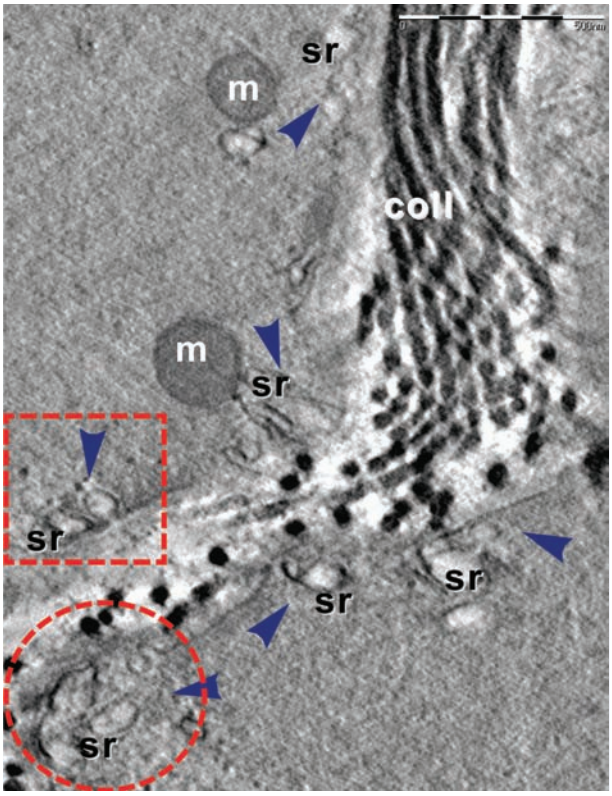

Fig. 2 A slice from tomographic volume shows in the SMC six caveolar domains (arrowheads), mitochondria (m) and sarcoplasmic reticulum (sr) and collagen fibers (coll) in the interstitial space. Scale bar = 0.5 μm.

Online supplementary material

Video 1. A series of 141 tilt images of the SMC obtained from a 500 nm thick section.

Video 2. Tomographic reconstructed volume of SMC and digitally red colored rotating volume.

[See also the cover of this issue *J Cell Mol Med*. 2007; 11(6).]

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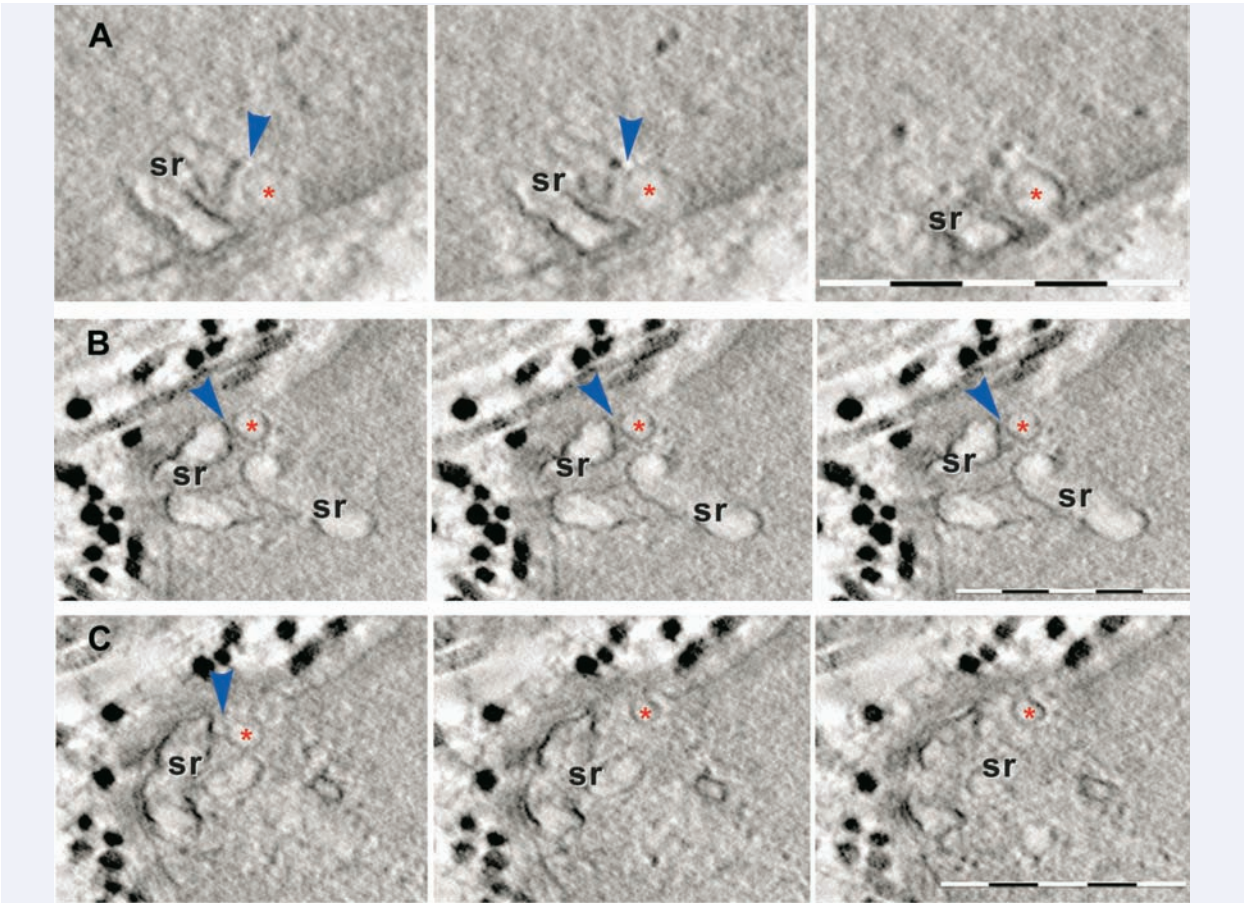

Fig. 3 Details of two caveolar domains from electron tomography of SMC. (**A**) Detail from square marked area in Fig. 2. (**B**) and (**C**) Details from round marked area in Fig. 2 at different levels of tomography. These images show that caveolae (asterisks) establish contacts with sarcoplasmic reticulum (sr) in at least one point (arrowheads). Scale bar = 500 nm.

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