Membrane contact site detection (MCS-DETECT) reveals dual control of rough mitochondria–ER contacts

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Abstract

Identification and morphological analysis of mitochondria–ER (MERCs) by fluorescent microscopy is limited by subpixel resolution and morphological distance. Here, the membrane contact site (MCS) detection algorithm, MCS-DETECT, reconstructs subpixel resolution MERCs from 3D super-resolution image volumes. MCS-DETECT shows that elongated ribosome-studded riboMERCs present in HT-1080 but not COS-7 cells, are morphologically distinct from smaller smooth contacts and larger contacts induced by mitochondria–ER linker expression in COS-7 cells. Ribosome formation is associated with increased mitochondrial potential, reduced in HT-1080 knockout HT-1080 cells, and induced by Gp78/RibP1-dependent riboMERCs present complex tubular shapes that intercalate between and contact multiple mitochondria. MCS-DETECT of 3D whole-cell super-resolution image volumes, therefore, identifies novel modes of control of tubular riboMERCs, whose formation is dependent on RBBP1 and size modulated by Gp78 E3 ubiquitin ligase activity.

Distinct MERCs in COS-7 and HT-1080 cells

Detecting riboMERCs in HT-1080 cells with MCS-DETECT

Gp78 and RBBP1 are independent regulators of riboMERC expression

Gp78 regulation of riboMERC expression and mitochondrial potential

Figure 1. Quantitative EM analysis of ER mitochondria contacts in HT-1080 and COS-7 cells. (A) Representative EM images of HT-1080 and COS-7 cells, insets show rough ER–mitochondria contact (arrow) in HT-1080 cells (green arrowhead) and smooth ER–mitochondria contacts (red arrow) in HT-1080 and COS-7 cells (red arrowheads). (B) Quantification of contact width, contact length and contact length relative to mitochondrial perimeter, and number of contacts per mitochondria profile are shown for ER–mitochondria contacts in HT-1080 and COS-7 cells. (C) The relative ratio of ER–mitochondria contacts in HT-1080 and COS-7 cells are based on the number of contacts per mitochondria or length of contact. (D) The number of ribosomes per area is plotted versus the length of the contact in nm for HT-1080 and COS-7 cells. ER–mitochondria contacts with five or two ribosomes are shown in red, those with more than five ribosomes are specific to HT-1080 cells and are shown in blue and defined as riboMERCs. Z- stacks images from two independent biological replicates, n=2, ***P<0.001,ibn=2, one-way ANOVA; C: CH2 test. Bar = 500 nm; inset = 200 nm.

MERC identification by differential channel correlation

Figure 2. MERC analysis of sub-pixel resolution contacts. (A) 3D TDE images of HT-1080 and COS-7 showing overlap between mitochondria (magenta) and ER (green), images show TDE sections (65 µm 2 spacing). Bars = 10 µm. (B) Two objects (red and green discs) are shown at corresponding sub-pixel resolutions. Intensity profiles (top row), second derivatives (Laplacian), and Sauvola parameters of the negative part of the Laplacian (bottom row) are shown. Note the Sauvola response overlap and changes consistently with the sub-pixel distance. (C) The detection algorithm (orange) with additional stages that each address a specific confounding factor introduced by the acquisition (blurred) or sample (noise) removed.